Response to Reviewers: *Complex plant-derived organic aerosol as ice-nucleating particles – more than a sum of their parts?*

We would like to thank both reviewers for carefully evaluating our manuscript and for providing valuable feedback. In the following, we want to respond to your overarching comments as well to your technical points.

Response to Reviewer 1

General Comment 1: […] My main criticisms are: a) the effect of micro-organism as an INP is largely not discussed in the interpretation of the retrieved results, although for leaf litter and the agricultural samples, it cannot be excluded that this plays a role […]

In our study, we deliberately focus on organic compounds which can be found in plant tissue, but which are not directly related to microbial activity. Our study should be viewed as a first step towards a better understanding of complex organic particles from biogenic sources, with organic compounds associated with primary biological particles being even more diverse and thus also more variable in their ice nucleation properties. Observed ice nucleation properties of fungi and bacteria vary over roughly six orders of magnitude (see references in Hoose and Moehler (2012)) and recently there have been results questioning the stability of the ice nucleating proteins responsible for the high-temperature ice nucleation efficiency (Polen et al., 2016). Hence, even though we definitely agree with the potential impact of microorganisms, especially at temperatures above 263 K, we have chosen in this study to investigate only a subgroup of the (presumably more stable) organics which play a role in determining the ice nucleation properties of particles derived from leaf litter and soils. These stable organics can be viewed as a lower limit for the ice nucleation activity of more complex particles. We have emphasized the potential role of microbial activity in the Conclusions section (l. 319ff):
"However, the high ice nucleation efficiency of these particles could not be fully explained by the ice nucleation activity of individual organic compounds commonly found in plant tissue, potentially indicating a contribution from primary biological particles or organics associated with microbial activity."

General Comment 2: […] b) the amount of plant derived INP in the atmosphere is derived without properly motivating all the parameters used in the calculation, which, however, will largely influence the atmospheric importance of these respective INP.

We mention in our manuscript that our estimates rely on ground-level particle concentrations and therefore we also caution against over-interpreting our results because of "emission fluxes of organic particles acting as INPs being poorly constrained and more detailed modelling case studies needed" (l. 296f).

To add more detail to the description of INP emissions from agricultural areas we have added the following paragraph:
"Anthropogenic dust sources contribute roughly 25 % to the global dust burden, with regional variations ranging from 7 to 75 % (Ginoux et al., 2012). In areas with intense agricultural land use, e.g. in eastern North America, India, eastern China, and Europe, anthropogenic dust emissions contribute generally more than 60 % to the total dust burden (Huang et al., 2015). Note, however, that there is a substantial uncertainty regarding the number and size of particles emitted from agricultural as well as their transport to cloud altitudes and the resulting atmospheric lifetime. This uncertainty is rooted in a lack of emission flux data above 5-10 m which is the height at which dust

fluxes from agricultural areas are commonly observed, e.g. in the study by Zobeck and Van Pelt (2006)." (lines 284ff)

Similarly, for the leaf litter aerosol we have added more information about the studies that we are referencing:
"Sánchez-Ochoa et al (2007) use cellulose found in aerosol particles as a proxy for plant debris concentrations, relying on observations across 6 European sites for a time span of two years, and with two of the sites being located on mountains. Hildemann et al. (1996) used higher alkanes (e.g. ocurring in plant waxes) to fingerprint plant debris in aerosol particles sampled in the greater Los Angeles Area." (lines 277ff)

Additionally, in Fig. 3 we have now scaled down the INP concentrations from agricultural dusts by a factor 100, respectively by a factor 10 for leaf litter to at least partially account for transport losses (see vertical profiles of dust concentrations in Hoose et al., 2010). The surface area values used to convert mass concentrations into aerosol surface concentrations are taken from the BET measurements conducted as part of this study (see Fig. 2) – this has been clarified in the text now, too (lines 292f).

General Comment 3: More specifically, it has to be said that throughout the text, there is a somewhat strange shortage of mentions of biogenic INP, which, based on their nature, have to be connected to the herein examined plant material samples.

Please see reply to General Comment 1.

General Comment 4: In the absence of any physical or chemical test that could have altered the respective samples (e.g., heating, treatment with H2O2), there is no information about the nature of the INPs, therefore any observed activity in the agricultural sample and the leaf litter may originate from the sample material itself or from micro-organisms connected to it (bacteria, fungi, lichen). The overall text has to be revised in connection to this issue.

Please note that we refer to the potential contribution of primary biological particles in Fig. 2 and the discussion of these results (l. 226ff). We have re-iterated this point in the Conclusions (l. 319ff).

General Comment 5: Also, for all of those samples from plant-related organic compounds, the sources for the cited quantities for agricultural dust and leaf litter have to be explained in more detail. These numbers are used for a very general calculation afterwards, but the reader needs to know if the given concentrations are valid only locally or for a larger area or worldwide and in which altitudes. A mentioning of "occasionally for very strong wind events" (line 256) rather gives the impression that these values are of no general use in this context, and more information is needed.

Please see reply to General Comment 2.

General Comment 6: Also some mentioning of the processes that make particles from dust and leaf litter airborne should be added. These issues and others to be found in my comments below makes a thorough revision of the text necessary before it can be accepted for publication in ACPD.

We have now added another paragraph to the introduction (l. 54ff):
"Agricultural areas may contribute between 7 and 75 % to the regional dust burden (Ginoux et al., 2012) due to emissions driven by wind erosion and land management activities such as tilling and harvesting (Funk et al., 2008). Vegetated areas may be another source for complex organic aerosol particles associated with leaf detritus (Coz et al., 2010)."

Specific comments

line 46: "biological aerosol particles" come a bit out of nowhere, here, and it would be good to shortly mention first, that these, too, can be efficient INP (as done for the mineral dust in the previous sentence).

This sentence now reads:
"Cloud-level concentrations of *potentially very ice-active primary* biological aerosol particles (Hoose and Möhler, 2012) are much lower than background concentrations of mineral dust, with differences of up to 8 orders of magnitude in some cases (Hummel et al., 2018)."

line 66: Your sentence "decaying plant material being one of the major sources of these macromolecules (Hill et al., 2016)," gives a somewhat wrong impression, as (from what I understood from the literature you cite here) these macromolecules may also originate from biological entities. In their abstract, it says: "Organic INPs . . . may originate from decomposing plant material, microbial biomass, and/or the humin component of the SOM."

We eliminated "major".

line 114: It should be added that the identification of an hydrometor as ice is simply based on its size.

We have added some more information regarding this aspect (please also see l. 152 where this had been mentioned, too).
"Ice crystals are discriminated from droplets by choosing a size threshold which is evaluated individually for each experiment." (l. 124)

line 167: These individual plant-related organic compounds likely miss the contribution of micro-organisms, so it is not so astounding that they show a comparably low ice activity ("in comparison to samples from natural environments"). A discussion on this is missing completely from the text.

As mentioned in our reply to General Comment 4, we have acknowledged the potential contribution of primary biological particles, particularly at temperatures above 260 K, in our discussion of Fig. 2. However, now we are also referring to this interpretation at the end of our Conclusions.

line 175: There is only a VERY low number of points for these "complex polysaccarides" at these higher temperatures (one, one, three, for three different ones of these samples (where the one with the three points seems to consist of two different materials, ALG and PEC)). - Particularly the three dots for ALG/PEC seem to be rather a background, as there is no change in the signal over the temperature range from 254 to 259 K, making this really look like a background issue. Interpreting this as a "weaker dependence on temperature" over-interprets what can be learned from these few data-points.

We have now pointed out this caveat explicitly. However, we opted for keeping this observation because we would like to highlight the different behavior compared to cellulose which has been investigated in depth. The paragraph in question now read:
"Our data also indicates that the temperature dependence of the polysaccharides investigated in this study is possibly less pronounced than for cellulose. Note that this finding is based only on a few data points due to the low observed ice nucleation efficiency above 252 K." (l. 187ff)

line 188 ff: The abstract of Conen et al. (2016) says: "Together, both findings suggest that decaying leaves are a strong emission source of IN to the Arctic boundary layer." And they examined litter consisting "of entire leaves and large fragments thereof, mainly from Betula nana and various grasses." – I wonder how this fits to what you wrote: ". . ., leaf litter from the Arctic consisting mainly of grass leaves has been observed to show relatively low ice nucleation efficiencies (Conen et al., 2016), . . ." . Neither seems grass to be the major component of the litter examined by Conen et al.

(2016) (or did I overlook something in the Conen-study?), nor do they claim that this is a low ice nucleation efficiency. Temperature wise, data in Conen et al. (2016) only go down to -15◦C, while the temperature range into which your results fall are generally below that, which has to be acknowledged when comparing values. Please revise this part of the text.

We have now updated this paragraph regarding the sources of leaf litter which also included birch leaves as correctly pointed out by the reviewer. Additionally we have re-phrased this paragraph slightly to further emphasize that we are only comparing ice nucleation efficiencies at 258 K.
"In contrast, at 258 K, leaf litter from the Arctic consisting of birch and grass leaves (Conen et al., 2016) has been observed to show relatively low ice nucleation efficiencies compared to leaf litter in our study based on AIDA results and similar efficiencies when comparing against our droplet freezing assay."

line 208-209: Is there any explanation why for the two samples discussed above large particles were not a problem and then why they might be a problem here? How else does this one sample differ from these two samples above?

For the agricultural dust we observed that the suspension contained larger (presumably dust) particles which appeared to sediment relatively fast even though we tried to keep the suspension as well mixed as possible. We did not observe this behavior for the other samples. Hence, we believe that sedimentation might have played a bigger role for the agricultural dust samples.

212-213: Why should there not also be a contribution from micro-organisms (= biological particles) in the leaf litter sample? After all, originally it was leaves from which Pseudomonas syringae was derived (Maki 1974). While the temperature of the signal's increase does not suggest the influence of a bacteria such as P. syringae, the presence of other micro-organisms could still be responsible for this increase. Testing with e.g., heat or acids or other methods could have increased the understanding.

We did not intend to exclude a contribution from micro-organisms to the observed ice nucleation efficiency of leaf litter. Based on the reviewer's suggestion we have now included a reference to the study by O'Sullivan et al. (2015) which have investigated the ice nucleation abilities of nanofragments associated with the presence of microbial activity:
"In contrast, the reasons for the steep onset observed for the leaf litter sample are a bit more unclear as most studies investigating primary biological particles have observed freezing onsets and high ice nucleation efficiency already at temperatures above 260 K (see references in Hoose and Moehler, 2012). However, one recent study has found indications for macromolecules associated with microbial activity being ice-active at about 258 K (O'Sullivan et al., 2015)." (l. 228ff)

In our study, we have refrained from conducting heat tests as in the case of complex particles they may produce results which are hard to interpret, i.e. leading a reduction in ice nucleation efficiency in some cases (Suski et al., 2018) but also leading to an increase in the observed ice nucleation efficiency for certain mineral dusts (Boose et al., 2019).

line 248/249 and line 254 ff: The explanation on how INP concentrations were obtained, i.e., the atmospheric concentrations assumed for leaf litter and agricultural soil dust, needs to be given first, before referring to the derived INP concentrations shown in Fig. 3. Also, it should be discussed in more detail that these indeed are upper boundaries (you mention "very strong wind erosion events"). And also, please motivate the aerosol surface area (1 m2/g). This would need to be the surface area ascribed to leaf litter or to the dust, exclusively. Is this a reasonable value? This needs to be discussed. Also: the mass concentrations: are they to be expected over larger areas, or were these just measured near sources. This is important if you want to make statements on atmospheric wide INP concentrations and the importance of leaf litter and agricultural soil dust in this context.

As elaborated in our reply to General Comment 2, we have now added more detailed explanations regarding the studies that we used to derive our estimates for ambient plant-related INP concentrations. We hope that it will be now clearer to the reader that these estimates are meant to give order-of-magnitude numbers and that they should be considered as upper limits. Also, we have now added that the assumed surface values come from BET measurements.

line 265: Could it be that this sentence is based on using unrealistically high atmospheric concentrations of materials that were examined and then using this as an argument for the importance of the present study? For enabling your readers to judge that, motivate the 1 m2/g that you used above, and the mass concentrations of leaf litter and dust, as said above.

Please see previous reply.

line 267: The "Section 4: Conclusion" comes a bit abrupt and contains statements that should either be given in a discussion part or otherwise earlier – giving new points of discussion (as the different sources that may emit organic particles or the global extent of the areas that may contribute) is not something that should appear in the conclusions for the first time.

We agree that this might be confusing for the reader and have eliminated one paragraph (l. 306ff) which contains information some of which has now been moved to the introduction.

line 277: Why were INSEKT data not also used to obtain atmospheric INP concentrations? As you say here, the measurements with this instrument were done to get results on a wider temperature range, but then they are not used in the further evaluation. Maybe it could also be seen if AIDA or INSEKT give the more trustworthy data? At least this could be discussed when comparing to the data from literature.

We are unsure to which lines the reviewer is referring here.

Both methods (i.e. AIDA and INSEKT) definitely each have their own value in better understanding ice nucleation, in particular as they allow to investigate different temperature ranges. There are two main aspects which contribute to differences in the observed INAS density values when comparing AIDA experiments against INSEKT droplet freezing studies. First, the method of particle dispersion is different (suspension vs. dry dispersion) and also the surface area used for normalization is different. However, it is not a priori clear how large the differences between the two methods are which is the reason why a direct comparison is still informative to the reader. Secondly, even if we don't look at absolute values when comparing the two methods, INSEKT delivers complimentary information, e.g. by capturing the steep onset at 260 K. In conclusion, both methods are trustworthy, and they deliver individual perspectives on the ice nucleation properties of different samples.

line 282-284: This sentence might have to be revised if the used values for mass concentration and aerosol surface area are revised or cannot be well justified.

Please see reply to General Comment 2 – we have now given more detail regarding the underlying assumptions.

line 284-286: Here it is again not clear why macromolecules from micro-organisms were not considered, as they provide an obvious explanation.

In this study, we have focused our investigation on organic components in plants as a first step. We hope that our study can be a starting point for investigating organic constituents of other complex particles in the future, e.g. macromolecules associated with the microbial degradation of plant material as suggested by the reviewer.

Figure 3: As also already mentioned above, I wonder if you want to imply here that the atmospheric INP mostly come from leaf litter and agricultural dust? There is a lot of literature around that ascribes ice activity at temperatures of roughly < -20◦C to desert dust, so I wonder if you really want to challenge this. If you feel your data is strong enough to do that, come up with a good justification. If you don't trust your derived concentrations so much, make it clearer in the text that you present the absolute possible maximum and that likely values from the lower end of the ranges you give or even blow are more likely.

We would like to emphasize that we have not inferred from our measurements that plant-related aerosols are the most dominant source of INPs. In our study we only wanted to highlight that INPs from vegetation and agricultural areas might be a significant contributor in certain contexts, i.e. certain seasons or regions: "…aerosolized particles from leaf litter and agricultural areas are potentially important contributors to atmospheric INPs." (l. 317)

Technical comments

line 51-52: Check the style of the citations (no brackets should be used in a bracket)

This inconsistency has been corrected.

Table 1: Somehow a "555" shows up at the right side of the table. Probably a line number gone wild?

Unnecessary line numbers have been removed.

Figure 3: "." is missing at the end of the caption

Captions have been updated accordingly.

Response to Reviewer 2

General Comment 1: […] First, ice nucleating active (INA) bacteria were identified decades ago and it is well known that vegetation and leaf litter – depending on type – can host dense populations of these bacteria. This component is discussed in the introductory materials, but not brought up again in comparison with the results for vegetation samples. Why not study P. syringae (as a model for this component) with the same systems and compare to that?

We do agree that a more thorough comparison between various methods to investigate the ice nucleation properties of relevant aerosol species is needed, building on recent intercomparison studies, e.g. for illite and cellulose. For this study, we would like to maintain our focus on plant-related organics, even though we agree that primary biological particles could play an important role for ambient aerosol particles. As discussed later on in this reply, we try to investigate the properties of very "simple" systems as a first step, inviting more complexity in future studies. Also, we would like to highlight that we do compare against the ice nucleation ability of bacteria in Fig. 2.

General Comment 2: Further, the INA component in bacteria is a lipoglycoprotein (with a particular structure that enables its activity), which presumably inspired some of the choices in Table 1. But this is not explained; and in any case, this is also already well known, so it is not clear what was to be accomplished through the selections made for study unless it is implied that other proteins, lipids, etc. might have IN activity as well (if so, why?). The idea of other "unknown" organic constituents being important (e.g., the macromolecules proposed in earlier work by other groups) is certainly raised, but is not explicitly investigated here – except perhaps by ruling out activity from larger particles composed of the selected compounds.

Lipoglycoproteins seem like a new promising avenue for future studies. However, in this study we wanted to investigate organic compounds which are major constituents of plant tissue, deliberately not taking into account the impact from microbial degradation processes, in order to be able to investigate very simple systems which we can then contrast with "real" particles. The organic compounds that we investigated in our study are chosen according to the main components of organic matter found in plant tissue (except for cellulose which has been investigated in great depth already).

General Comment 3: Second, the argument is made that using commercially-available components is preferable because "many of the extraction methods for organic matter may cause significant changes in the physicochemical properties of the extracted organic compounds". Why is this not true also for the commercial products? There is no discussion of how these are manufactured, which seems to be important for the proteins in particular if they are to be considered analogs for natural components.

It is certainly true that industrial extraction processes will also cause structural changes in the organic compounds. However, we preferred the commercial products because we were not sure how reproducible the extraction process would be and using commercial products allowed us to have larger sample amounts to be available, potentially allowing for follow-up studies and more detailed intercomparisons in the future. The samples investigated in our samples should be considered as analogues but the variability, e.g. across different sources of lignin, remains to be investigated.

We have now separated the sentence in question:
"We used commercially available organic compounds as analogues for plant-derived organics. Note that many of the extraction methods for organic matter may cause significant changes in the physicochemical properties of the extracted organic compounds (Kögel-Knabner, 2002)."

General Comment 4: I also have questions regarding the process for generating particles of carnauba wax, which was the only component identified as having significant IN activity: on line 216 it is stated that, "Unfortunately, it was not possible to reliably determine INAS density values for carnauba wax (LIP) due to its very low dispersibility." It is appreciated that generating reproducible particles from solid samples is very difficult, but the uncertainties associated with this should be quantified and carried through the analyses. The results for carnauba wax are noted to be surprising (lines 284-285) but few fully satisfying reasons for this result can be deduced from the present study (some ideas are presented in lines 177-185).

The issue with the carnauba wax was that is consisted mainly of larger particles which was less problematic for dry dispersion (rotating brush generator and cyclone impactors) than for the creation of suspensions where we would have needed to grind the particles, resulting in a substantially different particle distribution and potential surface effects from the grinding procedure.

General Comment 5: A third major point with regard to atmospheric implications is that while soils, leaf litter, harvest debris, etc. can have high densities of INA bacteria or other ice-active components, the mobilization of particles containing those components into the boundary layer, and further, to altitudes where they can impact cloud formation, is a different matter. Limited prior studies suggest there is no direct relationship between surface concentrations and atmospheric concentrations and the atmospheric concentrations become relevant only under conditions where the surface is strongly disturbed (as alluded to in the text). Thus the implications of any findings with respect to atmospheric processes have to be tempered by this consideration. In particular, the concluding sentence of the Abstract, "In contrast, complex biological particles may exhibit ice nucleation activities which are up to two orders of magnitude higher than observed for cellulose, making ambient plant-derived particles a potentially important contributor to the population of ice-nucleating particles in the troposphere" is not a unique conclusion from this work but has been suggested previously, and needs to be modified to acknowledge that the relationship between the surface and ambient concentrations needs to be better understood before quantifying the importance of this source on regional and global scales.

The last sentence of the abstract now reads:
*"*In contrast, complex biological particles may exhibit ice nucleation activities which are up to two orders of magnitude higher than observed for cellulose, making ambient plant-derived particles a potentially important contributor to the population of ice-nucleating particles in the troposphere, *even though major uncertainties regarding their transport to cloud altitude remain*."

We have also adjusted the atmospheric INP concentrations represented in Fig. 2 by applying a scaling factor of 10 for leaf litter emissions and a factor of 100 for the agricultural dust emissions, in order to account for transport losses (see vertical profiles for dust in Hoose et al., 2010). Due to the probably episodic character of these emissions, our estimates still need to be considered as upper limits.

Line 206: I have additional comments for consideration, as follows. It is stated that for some of the tested samples, the AIDA and microdroplet methods agree (lines 206-208). However, there is no overlap between these methods, and the surface area determinations use very different approaches, calling this agreement into question.

For the leaf litter sample, there is an overlap at around 257 K. For the plant protein, there is no overlap but the trajectories are reasonably close, so that we can assume that these trajectories can be virtually extrapolated by 1 K. Also, Fig. 2 intends to highlight that agreements and differences between two ways of inferring INAS densities are strongly dependent on aerosol types.

Line 107: The particle background concentrations for AIDA are stated (line 107) as 100 L-1. Comparing to Figure 3, I'm unclear how this is taken into account; the x-axis scales on Figures 1 and 2 are different, indicating that AIDA is limited at the warmer temperatures, presumably due to this background?

Yes, droplet freezing measurements can be conducted in a way that they are more sensitive at warmer temperatures (i.e. by choosing the weight percentage of suspended particles) and are therefore a great complement to AIDA experiments. For samples like agricultural dust, it can be difficult to reach particle concentrations which allow for ice crystal concentrations above background to be observed during AIDA experiments at very small supercoolings. One of the reasons can be the availability of fine material as we use cyclone impactors to eliminate larger particles and typically the sample amount is limited. For Fig. 3, we only applied the parameterization that we derived from AIDA experiments to prescribed ambient aerosol concentrations. The background limits therefore don't need to be considered.

Line 237: Prior work by Hiranuma et al. (2015b) is cited for data on cellulose for comparison to the present work. The intercomparisons published by Hiranuma et al (2019) are also cited, however, in that study, it is noted that "While the diverse instruments employed in this study agree in that cellulose has the capacity to nucleate ice, their quantitative agreement is poor. Unfortunately, it is not possible yet to say what the cause of this disagreement is." Does this statement apply to the two techniques used in the manuscript? Hiranuma et al. (2019) also call for "comprehensive studies on the ice nucleation activity of other important plant structural materials, such as cellulose polymorphs, lignin materials, lipids, carbohydrates and other macromolecule saccharides", so the present study is a nice follow-on to that recommendation. However, the issue of whether follow-on studies are premature at this point, if there are fundamental questions regarding the measurements and their interpretation, needs to be addressed.

There are differences between the two methods which are challenging to resolve, e.g. the role of soluble material which might lead to differences between wet and dry dispersion. Nevertheless, we want to consent to the point that the reviewer made based on the findings in Hiranuma et al. (2019), calling for more comprehensive studies to better understand differences between methods are dependent on the aerosol type being investigated. We consider our study as a first step highlighting differences between organic components and calling for more detailed studies of this subject.

Line 89: "ambient samples from vegetated environments": my comments above assume these are bulk samples and not obtained by filtering of ambient air. If my interpretation is correct, perhaps the language here needs to be clarified.

The word "ambient" was chosen to clearly distinguish these samples from the individual components and thus refers to samples from the outside environment. We have tried to be a bit more mindful with our choice of words and have therefore adjusted the text by either adding the word "bulk", eliminating the word "ambient" or by replacing "ambient" with "from vegetated and agricultural sources" where appropriate.

Line 148: Brunauer is misspelled. The uncertainties introduced by the different estimates of surface area should be more thoroughly discussed and represented in the figures (how are the uncertainty bars in the figures computed – is this from the variation in the repeat experiments, or does it include other considerations such as surface area?)

Spelling has been corrected.

For the AIDA experiments, there are two factors contributing to the error bars as displayed in Figs. 1 and 2: the uncertainty of the observed ice crystal concentration $\Delta n_{ice}/n_{ice} = 0.2$ and the uncertainty of the aerosol surface area concentration $\Delta A_{aer}/A_{aer} = 0.35$ (see line 152ff). We have now added one more sentence in the Methods section to make it clearer that the aerosol surface area used to derive the AIDA based INAS density values relies on a geometric surface area estimate:

"The combined aerosol size distributions are used to estimate the available aerosol surface based on volume-equivalent sphere diameters which then results in an estimate of the geometric surface area." (line 117ff)

For the INSEKT measurements, the error bars are determined by the statistical uncertainty of the number of frozen droplets (which translates to INP concentrations) and the uncertainty in measuring the BET surface area. However, in most cases the uncertainty of the BET measurements is less than 10 % which is smaller than the statistical uncertainty. We have now slightly updated this paragraph (l. 157ff):

“For our INAS density uncertainty analysis, we considered only the uncertainty of the cumulative INP concentrations *which is based on statistics*. Confidence intervals (at 95 %) have been estimated according to the improved Wald interval which implicitly assumes a normal approximation for binomially distributed measurement errors (Agresti and Coull, 1998). Hence, in our INAS density analysis, we neglected the uncertainties of the BET surface measurements which are in most cases considerably smaller *(i.e. $\Delta A_{aer}/A_{aer} < 0.1$)* than the previously described *statistical* uncertainties of the cumulative INP concentrations (Hiranuma et al., 2015a).”

We have also added one more aspect regarding the sources of differences between INSEKT and AIDA derived INAS densities (l. 167):
“Additionally, suspending particles in water may lead to the desorption and potential redistribution of soluble material. This change in soluble material could also lead differences in the observed ice nucleation properties when comparing cloud chamber experiments with droplet freezing studies.”

Line 174: Desert dust (Ullrich et al., 2017) is mentioned for comparison, but not shown?

We did not want to overload Fig. 2 – in order to make it more obvious to the reader, we have now added a note saying the data from Ullrich et al. (2017) is not displayed in Fig. 2.

Line 204: Is the background for the microdroplet method shown here or in another publication?

Another publication explaining these details is in preparation – pure water droplets commonly start freezing at temperatures below 249 K.

Line 110, 148: could these aerosol size and surface area distributions be shown in the Supplementary Material? This is potentially useful information for other studies that might seek to explore similar science questions with other techniques.

We have now added size distributions for lignin and leaf litter to the Supplementary Material.

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Complex plant-derived organic aerosol as ice-nucleating particles – more than a sum of their parts?

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Abstract

Quantifying the impact of complex organic particles on the formation of ice crystals in clouds remains challenging, mostly due to the vast number of different sources ranging from sea spray to agricultural areas. In particular, there are many open questions regarding the ice nucleation properties of organic particles released from terrestrial sources such as decaying plant material.

In this work, we present results from laboratory studies investigating the immersion freezing properties of individual organic compounds commonly found in plant tissue and complex organic aerosol particles from vegetated environments. To characterize the ice nucleation properties of plant-related aerosol samples for temperatures between 242 and 267 K, we used the Aerosol Interaction and Dynamics in the Atmosphere (AIDA) cloud chamber and the Ice Nucleation Spectrometer of the Karlsruhe Institute of Technology (INSEKT), which is a droplet freezing assay. Individual plant components (polysaccharides, lignin, soy and rice protein) were mostly less or similarly ice-active compared to microcrystalline cellulose, which has been suggested by recent studies as a proxy for quantifying the primary cloud ice formation caused by particles originating from vegetation. In contrast, samples from ambient sources with a complex organic matter composition (agricultural soils, leaf litter) were either similarly ice-active or up to two orders of magnitude more ice-active than cellulose. Of all individual organic plant components, only carnauba wax (i.e. lipids) showed a similarly high ice nucleation activity as the samples from vegetated environments over a temperature range between 245 and 252 K. Hence, based on our experimental results, we suggest to consider cellulose as being representative for the average ice nucleation activity of plant-derived particles, whereas lignin and plant proteins tend to provide a lower limit. In contrast, complex biological particles may exhibit ice nucleation activities which are up to two orders of magnitude higher than observed for cellulose, making ambient plant-derived particles a potentially important contributor to the population of ice-nucleating particles in the troposphere, even though major uncertainties regarding their transport to cloud altitude remain.

1 Introduction

Ice formation in the atmosphere has a significant influence on the microphysical and radiative properties of clouds. At temperatures above 235 K, atmospheric aerosol particles may act as ice-nucleating particles (INPs) (Pruppacher and Klett, 2010; Vali et al., 2015). In mixed-phase clouds, immersion freezing is often the dominant ice nucleation mode (Hande and Hoose, 2017). Immersion freezing refers to a solid particle initiating ice formation inside a supercooled cloud droplet.

Over the past decades, many different particle types initiating freezing in mixed-phase clouds have extensively been studied (Hoose and Möhler, 2012; Murray et al., 2012; Kanji et al., 2017). Mineral dust particles emitted from desert areas have been identified as ubiquitous INPs which initiate ice nucleation in clouds over a wide range of temperature and humidity conditions (Boose et al., 2016; Ullrich et al., 2017). Cloud-level concentrations of potentially very ice-active primary biological aerosol particles (Hoose and Möhler, 2012), ~~in contrast,~~ are much lower than background concentrations of mineral dust, with differences of up to 8 orders of magnitude in some cases (Hummel et al., 2018). Nevertheless, several laboratory studies, remote sensing measurements, and studies characterizing ice crystal residuals have found evidence for the potential impact of these particles and more numerous nanoscale fragments on ice formation in mixed-phase clouds (e.g. Möhler et al., ~~(~~2007~~;),~~ Pratt et al. ~~(~~2009~~);~~ Kanitz et al., ~~(~~2011~~;) and~~ O'Sullivan et al., ~~(~~2015~~)~~). Also, recent studies indicate a missing source of INPs beyond mineral dust, with biological particles from terrestrial environments being a likely candidate for initiating freezing in shallow mixed-phase clouds (O'Sullivan et al., 2018). Agricultural areas may contribute between 7 and 75 % to the regional dust burden (Ginoux et al., 2012) due to emissions driven by wind erosion and land management activities such as tilling and harvesting (Hoffmann et al, 2008; Funk et al., 2008; Iturri et al., 2017). Vegetated areas are another source for complex organic aerosol particles associated with leaf detritus (Coz et al., 2010).

One of the characteristics of biological INPs is that they include a vast variety of different particle types, ranging from primary biological particles such as bacteria, fungi and pollen to complex organic particles carrying different ice-nucleating agents and originating from biogenic sources (Schnell and Vali, 1973; Hoose and Möhler, 2012; Murray et al., 2012; Augustin et al., 2013; O'Sullivan et al., 2014; Tobo et al., 2014; Conen et al., 2016; Steinke et al., 2016). An example for complex organic particles are agricultural soil dust particles where the observed high ice nucleation efficiency can be linked to microbiological activity and the presence of organic macromolecules (O'Sullivan et al., 2014; Tobo et al., 2014; Hill et al., 2016; Steinke et al., 2016; Suski et al., 2018). The expression of bacterial and fungal ice-active proteins is highly variable, also because environmental stress (e.g. a change in temperature) can change the structure of ice-nucleating proteins, resulting in a loss of functionality (Pummer et al., 2012). In contrast, some of the organic macromolecules found in agricultural soils are very inert as they are able to withstand physical and chemical treatments, e.g. with heat or exposure to enzymes (Hill et al., 2016). With decaying plant material being one of the ~~major~~ sources of these macromolecules (Hill et al., 2016), the need arises to better characterize the ice nucleation properties of plant-derived particles as well as their individual organic components.

Lignin and polysaccharides are integral components of plant cell structures and contribute up to 50 % to plant debris (Williams and Gray, 1974). Proteinaceous components of leaf litter (e.g. enzymes, storage proteins or structure proteins) vary considerably but have been found to account for up to 15 % (Williams and Gray, 1974). Lipids contribute up to 10 % to dry leaf mass (Graça et al., 2005). Note that only 50 % of the organic matter is

accessible through chemical degradative techniques which inadvertently impact the structure of the extracted organic matter (Kögel-Knabner, 2002).

In this study, we investigate the immersion freezing properties of commercially available plant-derived organic compounds such as lignin, polysaccharides, plant wax and plant proteins − which are the main components of decaying plant material − as well as ~~ambient~~ ambient bulk samples rich in plant material. We used commercially available organic compounds as analogues for plant-derived organics. Note that ~~because~~ many of the extraction methods for organic matter may cause significant changes in the physicochemical properties of the extracted organic compounds (Kögel-Knabner, 2002). Experiments were conducted at the Aerosol Interactions and Dynamics in the Atmosphere (AIDA) cloud chamber and complemented by drop freezing assay studies using the Ice Nucleation Spectrometer of the Karlsruhe Institute of Technology (INSEKT). From our experimental results we derived temperature dependent parameterizations based on the ice nucleation active surface site (INAS) densities concept (Connolly et al., 2009; Niemand et al., 2012). These parameterizations were then used to estimate upper limits for ambient INP concentrations for complex organic aerosols from vegetated environments.

2 Samples and methods

2.1 Samples

In Table 1 we describe the samples used in this study, which include commercially available plant-derived organic compounds as well as ~~ambient~~ bulk samples from vegetated environments.

Note that the agricultural dust from harvesting machines (bulk sample) contains roughly 90 % of biological material, e.g. partially intact plant cells and similar particles (Fig. S1). The soil dust sample from Wyoming has been investigated in a recent study by Tobo et al. (2014) finding that organics contribute signficantly to the ice nucleation efficiency observed for size-selected particles (d = 600 nm). Representative microscopy images of all other samples used in this study are shown in the supplement (Fig. S2).

2.2 AIDA immersion freezing experiments

Immersion freezing initiated by plant-related particles was investigated in the AIDA cloud chamber (Karlsruhe Institute of Technology, Germany). The AIDA cloud chamber consists of a cylindrical aluminium vessel (volume 84 m^3) which is enclosed by a thermally insulated box. The ascent of cloud parcels is simulated by lowering the pressure from ambient levels (about 1000 hPa) to around 800 mbar, and by that lowering the temperature and increasing the relative humidity in the expanding air of the chamber volume.

A fan at the bottom of the AIDA chamber ensures homogeneous mixing (also with regard to temperature and humidity) across the whole chamber volume, except for transition zones near the chamber walls. The overall uncertainty of the mean gas temperature is about $\Delta T = \pm 0.3$ K (Möhler et al., 2006). The absolute water vapor partial pressure is measured with a tunable diode laser instrument and converted into humidity values by leveraging the saturation pressure formulation given in the review by Murphy and Koop (2005). The relative humidity values can be measured with an accuracy of $\Delta RH_{ice} = \pm 5$ % (Fahey et al., 2014).

Particle background concentrations within the cloud chamber are typically below 0.1 cm^{-3}. For the immersion freezing experiments presented in this work, aerosol samples were injected into the cloud chamber by using a rotating brush generator (RBG-1000, Palas GmbH) for dry dispersion. Additionally, impactor stages were used to eliminate particles larger than 3 to 5 µm. The aerosol size distribution at the beginning of each experimental run was measured by combining data from an Aerodynamic Particle Sizer (APS, TSI, Model 3321) and a

Scanning Mobility Particle Sizer (SMPS, TSI, Model 3076). The combined aerosol size distributions are used to estimate the available aerosol surface based on volume-equivalent sphere diameters which then results in an estimate of the geometric surface area.

Upon reaching water saturation during an expansion experiment, aerosol particles within the cloud chamber are activated to droplets and may freeze subsequently. Ice crystal number concentrations are measured with two optical particle counters (WhitE-Light Aerosol Spectrometer, welas1 and welas2, series 2300 and 2500, PALAS GmbH) with size ranges of 0.7 – 46 and 5 – 240 µm in optical particle diameter, respectively (Wagner and Möhler, 2013). Ice crystals are discriminated from droplets by choosing a size threshold which is evaluated individually for each experiment.

2.2 Droplet freezing assay studies

To investigate the freezing of suspensions created with the bulk samples and hence to account for freezing caused by particles larger than 5 µm, a droplet freezing technique was employed. The Ice Nucleation Spectrometer of the Karlsruhe Institute of Technology (INSEKT) setup (Schiebel, 2017) is based on the droplet freezing assay originally developed at Colorado State University (Hill et al., 2014).

Suspensions were created from bulk samples, combining 2 mg of material with 20 ml of deionized water (resistivity about 18 MΩ) which has been passed through a filter with a pore diameter of 0.1 µm (Whatman Puradisc 25). Suspensions were shaken by hand (about 1 min) and the suspension tube was then submerged in an ultrasonic bath (5 min) to promote dispersion of the particles. In addition to the original suspensions, we also created suspensions with a dilution factor of 15 and 225 by adding filtered deionized water in proportion. Original and diluted suspensions were partitioned into 192 wells (aliquot volume: 50 µL) of a sterile polypropylene polymerase chain reaction (PCR) tray, with 32 wells set aside for blank measurements, i.e. freezing of particle-free filtered deionized water. These blank measurements are used for determining the background which is then subtracted from the observed freezing curves. In this study, droplet freezing was measured at a cooling rate of 0.33 K/min. Cooling is achieved by flowing chilled ethanol through a custom-made aluminium block which encloses the bottom part of the PCR tray. The overall temperature uncertainty is $\Delta T = \pm 0.3$ K (Schiebel, 2017). Exemplary size distributions for leaf litter and lignin are shown in S3.

2.3 Ice nucleation active surface site densities

For all experiments, the ice nucleation efficiency was quantified by calculating the ice nucleation active surface site (INAS) density n_s. The n_s values were derived by scaling the observed ice crystal number concentration n_{ice} with the available aerosol surface A_{aer} (Connolly et al., 2009; Niemand et al., 2012).

For the cloud chamber experiments, the aerosol surface A_{aer} [$\mu m^2/cm^3$] was calculated from the APS and SMPS size distribution data using volume-equivalent sphere diameters (Möhler et al., 2006). In this study, it was assumed that all aerosol particles are activated to droplets upon reaching water saturation. Hence, the full aerosol surface area was considered to be available for immersion freezing. The ice crystal number concentration n_{ice} was derived from particle size distributions measured with the optical particle counters welas1 and welas2, in conjunction with a size threshold above which particles are counted as ice crystals. Based on the measurement uncertainties of the observed ice crystal concentration $\Delta n_{ice}/n_{ice} = 0.2$ and the aerosol surface area concentration $\Delta A_{aer}/A_{aer} = 0.35$, the resulting uncertainty of the INAS density is $\Delta n_s/n_s = 0.4$ (Ullrich et al., 2017).

For the droplet freezing studies, the INAS density values were derived from normalizing the cumulative INP concentration n_{ice} with the specific aerosol surface A_{aer} [m^2/g] derived from Brunauer-Emmett-Teller (BET)

surface measurements. For our INAS density uncertainty analysis, we ~~took into account~~considered only the uncertainty of the cumulative INP concentrations which is based on statistics. Confidence intervals (at 95 %) have been estimated according to the improved Wald interval which implicitly assumes a normal approximation for binomially distributed measurement errors (Agresti and Coull, 1998). Hence, in our INAS density analysis, we neglected the uncertainties of the BET surface measurements which are in most cases considerably smaller (i.e. $\Delta A_{aer}/A_{aer} < 0.1$) than the previously described statistical uncertainties of the cumulative INP concentrations (Hiranuma et al., 2015a). Another source of uncertainty – which is considerably more difficult to quantify – was the contribution of larger particles. These larger particles may sediment quickly within the suspension and were probably under-represented in the sampled aliquots. Thus, the particle surface area available for freezing was most likely overestimated in some cases. This effect seems to be negligible, but should be investigated in more detail in future studies. Additionally, suspending particles in water may lead to the desorption and potential redistribution of soluble material. This change in soluble material could also lead differences in the observed ice nucleation properties when comparing cloud chamber experiments with droplet freezing studies.

3 Results and discussion

In Fig. 1 we present results from AIDA cloud chamber experiments with commercially available plant-related organic compounds and natural samples (see Table 1). For comparison, we show the ice nucleation activity of microcrystalline cellulose (Hiranuma et al., 2015b), which is a prevalent natural polymer deriving from plant fragments, leaf litter, wood fiber, non-wood fiber and/or even microbes (Quiroz-Castañeda and Folch-Mallol, 2013; Vlachou et al., 2018). We also show the ice nucleation efficiency of agricultural soil dusts investigated in a study by Steinke et al. (2016) as well as an estimate for leaf litter from a study by Schnell et al. (1972). The ice nucleation activity of each sample is expressed as the INAS density n_s.

Figure 1 shows that the observed ice nucleation efficiencies of most individual plant-related organic compounds tend to be lower in comparison to samples from natural environments. However, there is a large spread in INAS density values when comparing between different plant-related organic compounds. Particularly noticeable is the low ice nucleation efficiency observed for plant protein for which freezing was observed only below 248 K. In this study, we tested two different types of plant proteins (PROT_R, PROT_SOY), derived from soy or rice (not differentiated in Fig. 1). Only lignin (LIG) shows an ice nucleation activity as low as the plant protein samples. Alginate, pectin, and starch (which mainly consist of highly complex polysaccharides) are similarly ice-active as microcrystalline cellulose (Hiranuma et al., 2015b) and desert dusts (Ullrich et al., 2017 – not shown in Fig. 2). Above 250 K, the complex polysaccharides investigated in this study (ALG, PEC, STAR_P, STAR_C) tend to be more ice-active than cellulose ~~and exhibit a weaker dependence on temperature~~. Our data also indicates that the temperature dependence of the polysaccharides investigated in this study is possibly less pronounced than for cellulose. Note that this finding is based only on a few data points due to the low observed ice nucleation efficiency above 252 K.

Of all plant-related compounds, carnauba wax (LIP) shows the highest ice nucleation efficiency, comparable to decaying leaves and two agricultural samples, i.e. dust from a sugar beet field (AGDUST_WYO) and material collected from harvesting machines (AGDUST_HARV). Carnauba wax is a mixture of hydrocarbons, aliphatic esters and fatty alcohols (Vandenburg and Wilder, 1970) with an average chain length of 50 carbon atoms (Basson and Reynhardt, 1988). Crystalline fatty alcohols (C16 - C18) have been highlighted recently in a study by DeMott et al. (2018) with regard to their ability to nucleate ice at 261 K via condensation freezing. Based on

theoretical considerations, hydrocarbons with long chains are potentially very good at initiating ice formation (Qiu et al., 2017) but conclusive experimental evidence is still missing. Hence, these theoreretical considerations might provide an explanation for the high ice nucleation ability of carnauba wax.

For ~~ambient~~ samples like the agricultural soil dusts and the leaf litter investigated in this study, some studies (e.g Schnell and Vali, ~~(~~1973~~) and;~~ Steinke et al., ~~(~~2016~~)~~) have found similarly high ice nucleation efficiencies. In contrast, at 258 K, leaf litter from the Arctic consisting of birch and grass leaves (Conen et al., 2016) has been observed to show relatively low ice nucleation efficiencies compared to leaf litter in our study based on AIDA results and similar efficiencies when comparing against our droplet freezing assay.

Hence, the high INAS density values observed in our cloud chamber studies can be interpreted as upper limits for the ice nucleation efficiency of ambient plant-related aerosol particles. Note that for our leaf litter samples we did not differentiate between samples collected at different points in time and for different species. Due to the high variability it was not possible to clearly derive a seasonal trend from the observed ice nucleation efficiencies.

In Fig. 2, we show INSEKT-derived INAS density values for selected samples investigated in the previously described AIDA cloud chamber studies. For every sample at least two experimental runs were conducted, using freshly prepared suspensions for each run. The PROT_S sample was investigated to establish the lower boundary of ice nucleation activity observed for plant components whereas the AGDUST_HARV and the LEAF samples were used to represent ambient samples. Note that for the droplet freezing experiments, the INAS densities are evaluated based on the specific surface areas derived from BET measurements rather than the geometric surface areas which were used for analyzing the AIDA experiments. The droplet freezing experiments are complementary to the cloud chamber studies as they deliver insights regarding the freezing properties of the bulk material, in particular with regard to including particles larger than 5 µm which are largely eliminated by impactor stages in our AIDA experiments. Also, observing the freezing of bulk suspensions allows for quantifying the immersion freezing efficiencies at a lower supercooling which are more difficult to quantify in AIDA cloud chamber studies. For leaf litter we observe that INAS density values agree well between INSEKT and AIDA experiments. Similarly for plant protein (PROT_S), the agreement is reasonably. For AGDUST_HARV, there is a difference of approximately more than one order of magnitude which is possibly caused by larger particles being undersampled due to sedimentation within the suspensions.

Figure 2 shows that the hierarchy in ice nucleation activities is similar as observed in the AIDA cloud chamber experiments, with leaf litter and agricultural dust being the most ice-active samples. The steep onset of ice nucleation observed for the agricultural dust at 267 K suggests a contribution from biological particles (Suski et al., 2018). In contrast, the reasons for the steep onset observed for the leaf litter sample ~~in~~ are a bit more unclear~~unclear~~ as most studies investigating primary biological particles have observed freezing onsets and high ice nucleation efficiency already at temperatures above 260 K (see references in Hoose and Möhler, 2012). However, one recent study has found indications for macromolecules associated with microbial activity being ice-active at about 258 K (O'Sullivan et al., 2015). Soy protein particles initiate ice formation at higher temperatures (i.e. already below 258 K) than observed in AIDA cloud chamber experiments, but the overall ice nucleation efficiency is still lower than for the complex organic samples from natural environments. Unfortunately, it was not possible to reliably determine INAS density values for carnauba wax (LIP) due to its very low dispersibility. Figure 2 also shows the INAS density values observed for illite as a proxy for freezing induced by mineral dust.

In conclusion, the results from the droplet freezing studies confirm the trend observed in our AIDA cloud chamber experiments, with particles from ~~ambient~~ vegetated and agricultural environments being highly ice-active, whereas individual organic compounds tend to be lower in their ice nucleation efficiencies. It should be noted that the organic compounds investigated in this study may not fully represent the complexity of real organic compounds in plants which often include mixtures, e.g. ligno-polysaccharide complexes with unknown chemical structures (Kögel-Knabner, 2002). At temperatures above 260 K, the gap between individual plant-related compounds and particles from natural environments may be attributed to primary biological particles (e.g. fungi and bacteria) according to our droplet freezing measurements of harvesting dust. For example, ice nucleation efficiencies observed for particles generated from leaf litter fall within the lower range of values observed for bacteria (Hoose and Möhler, 2012).

There are, however, also differences between the ice nucleation efficiencies derived from AIDA cloud chamber experiments and droplet freezing studies, which strongly dependent on the aerosol type. Some of these differences might be explained by differences in the evaluation of the INAS density values which are either related to the geometric surface or the specific surface area. For illite, normalizing by BET surface area results in INAS density values which are one order of magnitude lower compared to values derived by using geometric surface estimates (Hiranuma et al., 2015). Also, for some samples there are possibly differences in the effective size distribution due to agglomeration or low dispersibility in the suspensions. In contrast, the dry dispersion method (i.e. the rotating brush generator) is more likely to encourage disaggregation of particle agglomerates. Similar differences regarding the freezing of aqueous suspensions in comparison to dry dispersion experiments have been observed in other studies as well (Hiranuma et al., 2015a; Hiranuma et al., 2019).

Our experimental results suggest that the main components of decaying plant material (i.e. cellulose and lignin) are not very good predictors of ice nucleation by ambient plant-related particles. However, the INAS density values observed for leaf litter and agricultural dust may help to constrain the upper limits of their respective ambient INP concentrations. The INAS density values for leaf litter and agricultural dust can be described by temperature-dependent functions, with

$$n_{s,leaf} = \exp\left(-0.246 \cdot T_{leaf} + 84.681\right) \qquad r^2 = 0.70 \qquad (1)$$

and

$$n_{s,agri} = \exp\left(-0.541 \cdot T_{agri} + 157.471\right) \qquad r^2 = 0.84 \qquad (2)$$

Note that these functions are only valid within certain temperature ranges, i.e. T_{leaf} = [243, 258] and T_{agri} = [245, 255], with all temperatures given in [K]. Equations 1 and 2 have been derived from the cloud chamber experiments exclusively and are represented in Fig. 2. Note that based on our droplet freezing experiments, both of these aerosol types may have relatively sharp ice nucleation onsets at 257 K (leaf litter) and 267 K (agricultural dusts).

Figure 3 shows a comparison between ambient INP concentration derived from precipitation samples from several sites in the United States and Europe (Petters and Wright, 2015) and estimates for INP concentrations from leaf litter (eq.1) and agricultural dust (eq.2). Note that ambient INP measurements may scatter significantly more than found in the study by Petters and Wright (2015), with deviations of up to four orders of magnitude between different studies (Kanji et al., 2017).

Ground-based measurements for leaf litter concentrations range between 30 ng/m^3 to 1 µg/m^3 (Hildemann et al., 1996; Sánchez-Ochoa et al., 2007). Sánchez-Ochoa et al (2007) use cellulose found in aerosol particles as a proxy for plant debris concentrations, relying on observations at 6 European sites for a time span of two years, and with two of the sites being located on mountains. Hildemann et al. (1996) used higher alkanes (e.g. ocurring in plant waxes) to fingerprint plant debris in aerosol particles sampled in the greater Los Angeles Area. For agricultural dust, ground-based concentration vary between <10 and 100 µg/m^3, with up to 800 µg/m^3 observed occasionally for very strong wind erosion events (Gillette et al., 1978; Sharratt et al., 2007; Hoffmann and Funk, 2015). Annually averaged boundary layer concentrations for desert dust vary between 0.1 and 30 µg/m^3 (Ginoux et al., 2001) which is comparable to the aforementioned concentrations of complex organic particles. Anthropogenic dust sources contribute roughly 25 % to the global dust burden, with regional variations ranging from 7 to 75 % (Ginoux et al., 2012). In areas with intense agricultural land use, e.g. in eastern North America, India, eastern China, and Europe, anthropogenic dust emissions contribute generally more than 60 % to the total dust burden (Huang et al., 2015). Note, however, that there is a substantial uncertainty regarding the number and size of particles emitted from agricultural as well as their transport to cloud altitudes and the resulting atmospheric lifetime. This uncertainty is rooted in a lack of emission flux data above 5-10 m which is the height at which dust fluxes from agricultural areas are commonly observed, e.g. in the study by Zobeck and Van Pelt (2006). Using eqs. 1 and 2 and assuming an aerosol surface area of 1 and 36 m^2/g as measured by BET analysis, we can derive order-of-magnitude estimates for the expected atmospheric INP contribution from leaf litter and agricultural dust. In Fig. 2, we have scaled down agricultural dust INPs by a factor 100 and leaf litter INPs by a factor of 10 to at least partially account for transport losses.

The estimates presented in this study should be considered as upper limits, with emission fluxes of organic particles acting as INPs being poorly constrained and more detailled modelling case studies needed. We find that plant-derived organic INPs from leaf litter and agricultural areas are within the same order magnitude as INP concentrations derived from precipitation measurements and field campaigns (Petters and Wright, 2015; Kanji et al., 2017). This finding further emphasizes the potential of plant-related sources to contribute to ambient INPs.

Section 4: Conclusions

Complex organic particles are emitted from terrestrial sources, with wind erosion, soil cultivation and harvesting crops as potential main drivers for emissions of organic matter associated with plant debris and decomposed residues ~~(Hoffmann et al., 2008; Iturri et al., 2017)~~(Funk et al., 2008; Hoffmann et al., 2008; Coz et al., 2010; Ginoux et al., 2012). These sources are becoming increasingly important in the global view, as climate change, soil degradation and excessive land use will promote dust emissions from agriculturally used areas. ~~The global extent of the affected areas is comparable with the Sahara, the worldwide largest source of mineral dust, which covers 9.2 million km². Temperate grasslands, susceptible to wind erosion, cover an area of 9 million km², and the total area of grown cereals is 5.6 million km², contributing several times a year to dust emissions by diverse tillage and harvest operations.~~ In this study, we investigated the immersion freezing properties of plant-related organic particles and samples from vegetated environments. We used a combination of AIDA cloud chamber and INSEKT droplet freezing experiments to cover a temperature range between 242 and 267 K. Our experiments show that ~~ambient~~ the samples with a complex organic composition are equally or more ice-active than individual plant-related compounds. Lignin and plant protein samples are inefficient INPs, whereas starches, alginate and pectin show moderate to high ice nucleation efficiencies. Surprisingly, carnauba wax – which is a mixture of aliphatic esters and fatty acids – shows the highest ice nucleation activity of all organic

compounds investigated in this study. INP estimates based on our cloud chamber experiments lend themselves to the hypothesis that aerosolized particles from leaf litter and agricultural areas are potentially important contributors to atmospheric INPs. However, the high ice nucleation efficiency of these particles could not be fully explained by the ice nucleation activity of individual organic compounds commonly found in plant tissue, potentially indicating a contribution from primary biological particles or organics associated with microbial activity. Thus, further future studies are indeed demanded and warranted.

Author contributions

IS and NH designed and conducted the experiments, with contributions from KH, OM and NSU. PGW conducted the BET surface measurements and NT provided the SEM images. IS and NH analyzed the data. IS prepared the manuscript with input from all co-authors.

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Competing interests

The authors declare no competing financial interests.

Data management

All data in this manuscript will be made available as part of a KITopen data repository.

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Tables

Sample name	Acronym	Sample preparation/manufacturer
Ambient bulk samples dominated by decaying plant material		
Leaf litter	LEAF	Dry leaf debris from either spruce or maple trees in Southwestern Germany, dried at 313 K, milled and sieved for particles smaller than 150 µm (collected in spring and autumn in the years 2014, 2015, and 2016)
Agricultural dust	AGDUST_HARV	Dry plant material collected from filters of harvesting machines after rye and wheat harvests in Northwestern Germany, sieved for particles smaller than 63 µm (collected in summer 2016)
Agricultural soil dust	AGDUST_WYO	Top soil samples collected in Wyoming on sugar beet fields (collected in spring 2011)
Alginate	ALG	C.E. Roeper GmbH (article no. NA 4012)
Lignin	LIG	Sigma-Aldrich (article no. 370959 and 471003)
Lipids (Carnaubawax)	LIP	Sigma-Aldrich (article no. 243213)
Plant-related organic compounds		
Pectin	PEC	Herbstreith & Fox KG (article no. AU 015 H I)
Protein (Rice, soy)	PROT_R, PROT_S	Erdschwalbe (article no. 30676 and 30744, food grade quality)
Starch (Potato)	STAR_P	Mueller's Muehle GmbH (food grade quality)
Starch (Corn)	STAR_C	Unilever (food grade quality)

Table 1: Overview of samples used for ice nucleation experiments.

Figures

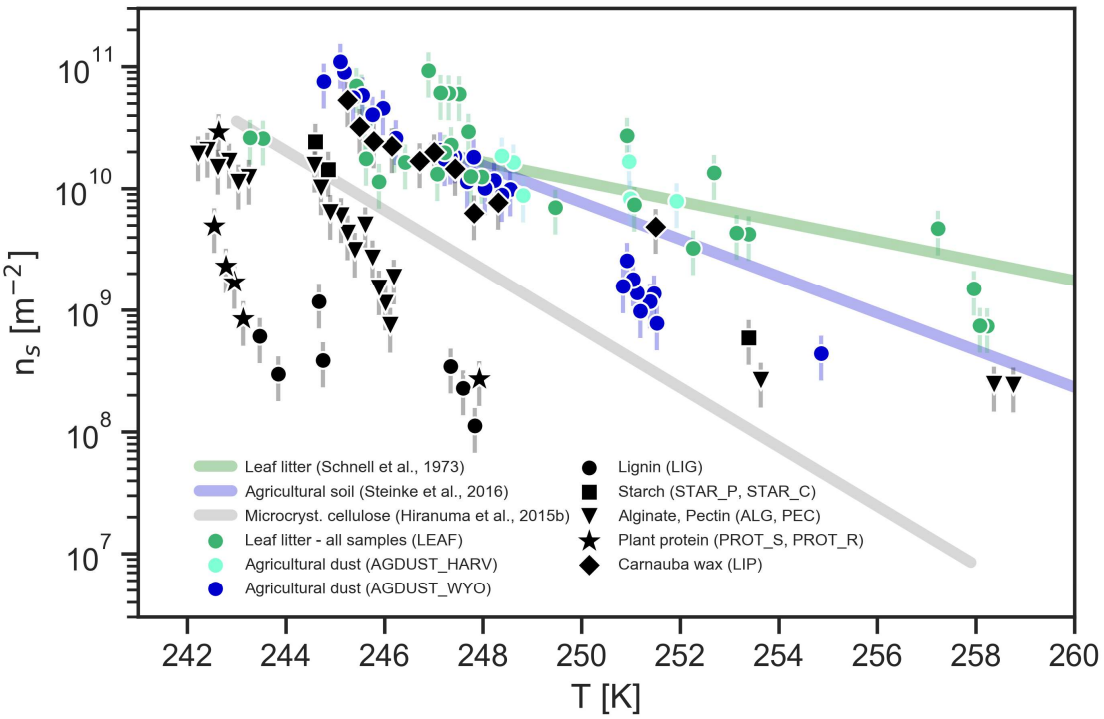

Figure 1: Immersion freezing results for plant-related organic compounds compared to ambient samples – ice nucleation efficiency expressed as INAS density values based on AIDA cloud chamber experiments.

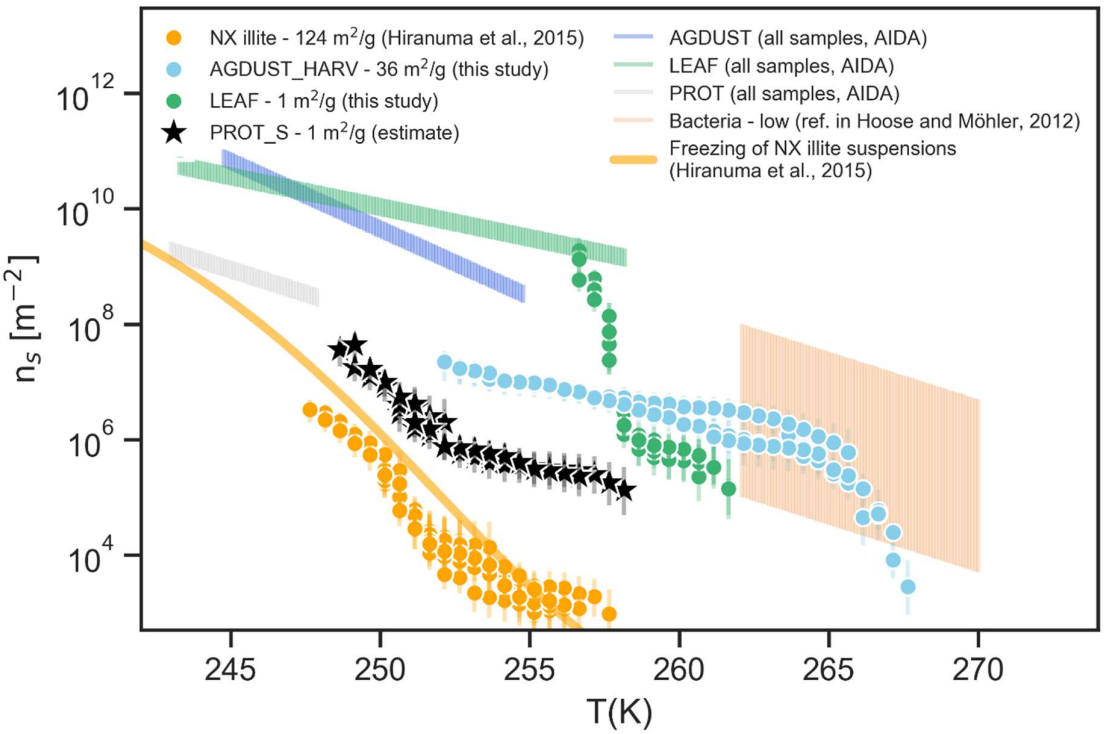

Figure 2: Immersion freezing results for selected plant-related samples and illite, comparing INSEKT-derived INAS density values to results from AIDA experiments (Fig.1) – ice nucleation efficiency expressed as INAS density values based on INSEKT droplet freezing experiments and specific surface areas indicated in the legend.

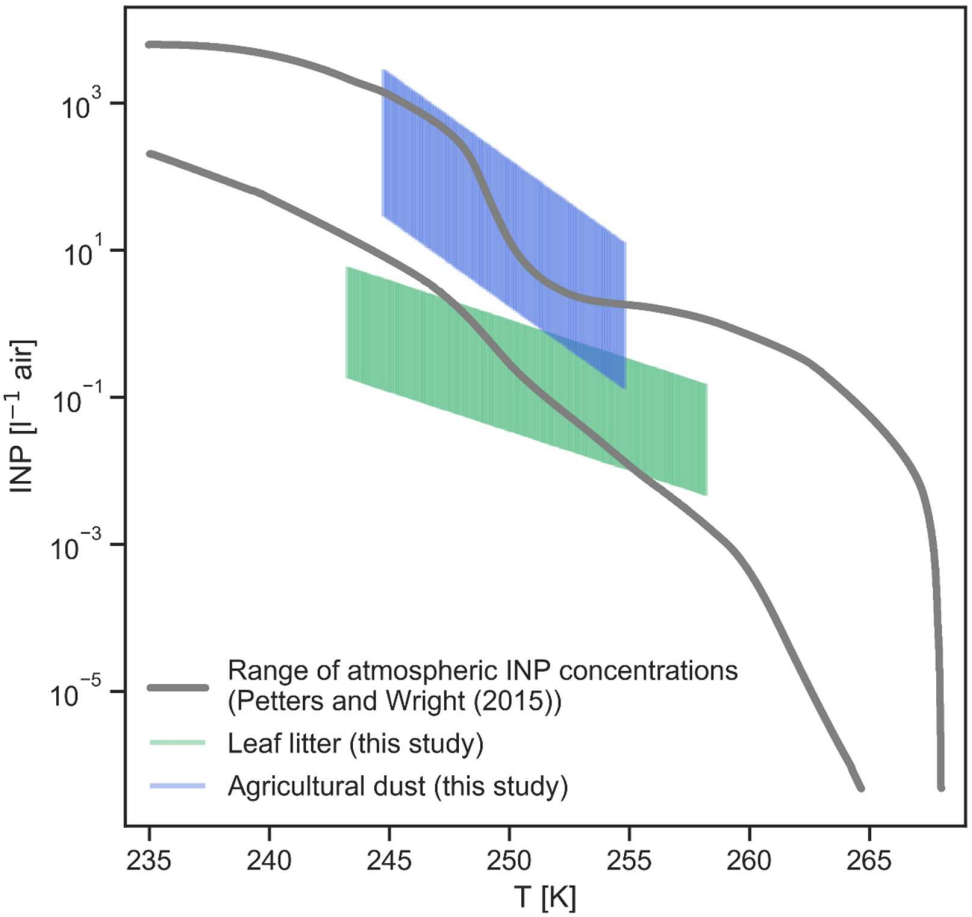

Figure 3: Comparison between atmospheric INP concentrations (Petters and Wright (2015)) and estimates for INPs from leaf litter and agricultural dust based on AIDA cloud chamber experiments.