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Response to JA Huffman (Referee)

To facilitate reading we use different fonts for (1)comments from Referee, (2) our response, *(3) the changes we made to the manuscript.*

Conen et al. submitted a manuscript for review titled "Rainfall drives atmospheric ice nucleating particles in the maritime climate of Southern Norway." The manuscript compares 15 months of measurements of ice nucleating particles (at -8C), two molecular tracers (arabitol and mannitol), and rainfall data to present observations about INP behavior. The authors suggest that INP were likely to have local sources and are linked to rainfall, because of the evidence that INP concentrations correlated with rain. Further, they state that correlations with molecular tracers suggest INP "may consist largely of fungal spores." The manuscript presents interesting environmental data linking warm temperature INP with rainfall and two commonly used molecular tracers. The region sampled is also not over-represented in literature and so provides some atmospheric perspective on this region, including possible parallels with other similar regions of the world, as mentioned. In general, I support the publication of this manuscript, but there is some work that I suggest be done before it is accepted. The analysis and evidence that the observed INP are fungal in nature are both relatively thin and should be improved. I list some suggestions for specific additions below, including some possibilities for added discussion and some suggestions to add to quantitative evidence. These statements are meant to suggest possible areas of improvement, but are not necessarily meant to be comprehensive.

We thank Alex Huffman for having taken the time to read our manuscript. His views on our work and his suggestions to improve it are much appreciated. Thank you also for drawing our attention to additional papers in this field.

The claim about the importance of fungal spores seems not enough supported by data. Also Referee #1 has made this point. We have reflected on this issue, read more papers and came to a more differentiated conclusion.

General comments: Abstract: "INP(-8) correlated positive with the fungal markers arabitol and mannitol, suggesting that INP may consist largely of fungal spores." I think the confidence implied by this conclusions is somewhat over-stated. The evidence shown suggests to me that the INP have a source that is at least correlated with arabitol and mannitol, but this does not necessarily mean that the INP are spores themselves. The observations could also be explained if INP and fungal spores are co-emitted by a similar process or are somehow physically related to one another. A lot of evidence has suggested many fungal spores are not IN-active (e.g. Iannone et al., 2011) while other (i.e. rust spores from the cited Morris et al. paper) are IN-active at high temperatures. There is enough complexity in this conversation, that I think some discussion of these differences should be mentioned and the overall confidence in the implications that fungal spores are the source of INP should be scaled down a bit.

We agree that the issue is more complex than initially portrayed in the manuscript.

Accordingly, we added to the end of the 2nd paragraph of section 3.4: "However, spores found in the atmosphere are not only produced by rusts. For example, Cladosporium species contribute a large proportion of airborne spores (Maninnen et al., 2014) and their onset of freezing is well below -25 °C (Iannone et al., 2011). At the same time, some fungi release INP-8 from their mycelium in form of macromolecules (Fröhlich-Nowoisky et al., 2015), which are unlikely to contain storage products such as arabitol or mannitol." (page 7, lines 29-31).

The statement in the abstract is scaled down to "From mid-May to mid- September, INP-8 correlated positively with the fungal spore markers arabitol and mannitol, suggesting that some fraction of INP-8 during that period may consist of fungal spores." (page 1, lines 15-16). Further, a similar statement in the 1st paragraph of section 3.4 now reads: "These correlations (Fig. 6) support the above-mentioned notion (section 3.3) that FBAP and INP-8 may to some extent be of fungal origin, at least during the warmer part of the year." (page 7, lines 13-14).

The discussion about molecular tracers should also be extended somewhat. For example, arabitol and mannitol are commonly used as tracers for fungal spores, but not without complications. One important thing to mention here is that these specific tracers are typically used as tracers for wet-discharge spores, but only poorly relate to other types of spores (i.e. dry discharge spores like Cladosporium that can be ubiquitous and a large fraction of spore mass). How might this understanding impact the conclusions that are being drawn here? I am aware that the general knowledge linking these tracers with ice activity is low, and so it is unreasonable to require any kind of a quantitative link between known ice fungal ice nucleators and the amount of arabitol or mannitol they release, but I suggest at least mentioning some of the uncertainties that come along with the analysis and assumptions as presented.

Right, arabitol and mannitol are typical tracers for wet discharge spores (e.g. Zhu et al., 2016) and are not always well related to the primary fungal membrane sterol marker ergosterol (Burshtein et al., 2011). Another uncertainty are the dynamics in microbial succession which occur, for example, when leaves are shed and decay in autumn (Purahong et al., 2016). These issues call for an adaptation of the conclusions.

We added to the beginning of section 3.4: "Arabitol and mannitol serve as carbohydrate stores in fungal spores. Their ambient air concentration has been found to correlate well with number concentrations of airborne fungal spores (Bauer et al., 2008), but not necessarily with ergosterol (Burshtein et al., 2011), a dominant sterol in most fungi (Weete et al., 2010). It seems that arabitol and mannitol are specifically associated with spores released under moist conditions, as occur in forests during nighttime (Zhu et al., 2016)." (page 7, lines 6-9).
To the end of the section we added: "Overall, the relative contribution of INP-8 from any type of microorganism might have changed by the end of September as a result of leaves starting to be shed by deciduous trees. Decaying leaves provide the substrate for a highly dynamical succession of interacting fungal and bacterial populations (Purahong et al., 2016)." (page 8, lines 6-9).
We changed the second paragraph of the Conclusions. It now reads: "The assumption of relevant fungal sources is supported during the warmer part of the year by some similarities in the temporal pattern of INP-8 and the fungal spore markers arabitol and mannitol. However, major shifts in microbial community composition occur when leaves are shed in autumn and start to feed a highly dynamical succession of interacting fungal

and bacterial populations. These dynamics likely also affect the strength and composition of the various sources of INP$_{-8}$." (page 8, lines 18-22).

In general, I think Section 3.2 and Figure 3 need more detailed discussion and explanation to help a reader not experienced with this type of analysis. Can you explain what the z-scale implies from this figure and how it relates to the brief observations you make? It's hard to know how much to make of the summary observations reported. How much of this is a function of different averaging times that may lead to random differences? If this is an important piece of evidence, is there some statistical treatment that can be applied here? Flipping back and forth between the comments and the figure I can follow the reasoning of the trends mentioned, but it is hard to know whether the "striking" comment (P5 L14) is stronger than I would have stated – at least having briefly looked at the differences. If the authors are confident of the strong difference, I suggest improving the evidence for that distinction. In contrast to this, however, the last sentence in Section 3.2 essentially says that the authors think the effects are local, which implies to me that Figure 3 should provide evidence *against* long-distance sources, right? This goes back to how Figure 3 should be interpreted as striking differences between high and low INP concentration.

We summarised the source receptor sensitivity (SRS) fields (also called "footprints") for higher (>4 m^{-3}) and lower (<4 m^{-3}) concentrations of INP-8 in order to see whether the higher INP concentrations coincided with "footprints" extending to a region that did not have an influence when concentrations were low (or vice versa). The "footprints" shown in Figure 3 are cumulative, i.e. the sum of all "footprints" for weeks with either high or low concentrations of INP. The analysis here is qualitative in the sense that we look for differences in general patterns. There were no additional areas associated with high INP concentrations which speaks against long distance transport, at least long distance transport from a certain area. The greatest difference is a lack of influence from the north-eastern quadrant when concentrations are low. We had initially looked at "footprints" averaged over one week, where we could also see the lack of influence from the north-eastern quadrant for weeks with INP <4 m^{-3}, but no general pattern in the other quadrants. Summarising the pattern of each concentration category provides for a more direct visual access to this information.

We tried to make the interpretation more accessible by expanding the 2nd sentence in section 3.2: "Higher concentrations of INP$_{-8}$ were not associated with source areas not seen when INP$_{-8}$ were < 4 m^{-3}. Hence, they were not transported to Birkenes from specific strong sources afar. The main difference when INP$_{-8}$ were > 4 m^{-3} was a weaker influence from the northeast …" (page 5, lines 24-26). This re-wording also replaced "strikingly less" with "weaker".
Further, the z-scale is now explained in the Figure legend: "Figure 3: Source receptor sensitivity (SRS) fields for situations with > 4 INP$_{-8}$ m^{-3} (left) and when INP$_{-8}$ were < 4 m^{-3} (right) as derived from FLEXPART. The SRS unit is seconds, which would result in a mass concentration (kg m^{-3}) at the receptor when multiplied with an emission flux (kg m^{-3} s^{-1}) into the model grid cells. Since emission fluxes are not known for INP$_{-8}$, SRS values can be considered as a measure of relative impact that INP emissions from a particular area would have had on INP concentrations at Birkenes. The potential influence was strongest from areas shown in red colour and weakest from those in white and purple colours." (page 14, lines 3-8).

What happens if you do correlations of the traces in Figures 2 and 5? A lot of the observations come down to qualitative comparisons of

these traces, but it is hard to know what this means quantitatively. I think this is one obvious area that could easily improve the manuscript. Without evidence beyond the visual trends presented, it is hard to know how much to make of the possible co-variance. As a simple addition, I would also add the R2 value (or something similar) to Figure 4.

Figure 4 has been adapted as suggested (page 14) and we also added a Figure showing INP_{-8} plotted against the fungal tracers, with different symbols for the warmer and the colder parts of the year (Figure 6, page 15).

Looking at Figure 5, it seems that there is a one-point lag in INP behind arabitol and mannitol during approximately October and November. Do you think this is real? If so, what might be causing this? Or is it just a figure illusion and statistical aberration.

The ups and downs in the concentration of both tracers run in parallel throughout October and November. The impression of a time lag might be caused by the concentration of arabitol having dropped to a very small value (2.6 ng m^{-3}) on 15. November, while that of mannitol had only dropped to an intermediate value (13.0 ng m^{-3}). The following week they both drop again, although mannitol by a much larger fraction. After that they change almost synchronously.

P7 L10: The statement here is that "Since INP were no longer directly related to fungal spore markers during this period, it might be that bacteria contributed more to the total number . . .". Another possibility is that the type of spores being released are of a different variety and are just less efficient at producing IN-active.

We agree that there are alternative explanations for this observation.

The sentence now reads: "Since the time course of INP_{-8} was no longer directly related to that of fungal spore markers during this period, it might be that the fungal composition had changed or that bacteria had become more important sources of INP_{-8}." (page 8, lines 2-3).

Bigg, Soubeyrand, and Morris recently published a paper reporting long-term statistical correlations between ice nuclei and rainfall in Australia (Bigg et al., 2015). I think reference to this work would be appropriate here, probably in the conclusions.

We have added the reference in the Introduction (page 2, line 4).

P6 L1: The authors cite Schumacher et al. as having observed a 2.5 – 3.0 um mode of fluorescent particle during 18-months of study in Finland. That paper also mentions a prominent decrease in fluorescent particles during snow-covered periods, which qualitatively matches some of the observations shown here.

Made reference to this observation on page 6, lines 9-10.

Is snowfall poor at launching INP because of snow-covered vegetation and soil or also because the kinetic velocity at which the drops fall does not kick up material? Some recent papers on rainfall velocity and particle ejection could be cited and discussed here (e.g. P7 L18).

We think that most of the difference comes down to the lower deposition velocity, hence lower kinetic energy, of snowflakes.

Added to the end of the 1st paragraph of section 3.1: "Deposition velocity of snow is in the order of 1 m s^{-1} (Garrett et al., 2012), that of even a small raindrop (1 mm diameter) is already four times as large (Gun and Kinzer, 1949). Hence, for the same mass, the kinetic energy of rain (proportional to the velocity squared) is at least an order of magnitude larger compared to precipitation in form of snow, and so the energy is available for dispersion and aerosolisation of particles." (page 4, lines 24-27).

P7 L23: Are the heat treatment properties of fungal proteins the same as bacterial proteins? I think of spores as relatively robust, and so I wonder if it is possible for some fraction of spore material to remain active, whereas the fraction for bacteria goes to zero? In any case, I think the evidence for these arguments should be stronger.

They are not exactly the same. Evidence for the heat sensitivity of bacterial and fungal INP summarised by Pummer et al. (2015) shows that bacterial INP are deactivated already by moderate heat (40 °C), whereas some fungal INP tolerate temperatures close to 60 °C and few close to 100 °C. All we can say from our observations is that most of the INP seen prior treatment were either of bacterial or fungal origin because >93% of the INP in our samples were deactivated at 90 °C.

Added the reference to Pummer et al. (2015) at the corresponding sentence (page 6, lines 29-30): "The INP of bacteria and most fungi are proteins and denatured at this temperature (Pummer et al., 2015)."

P 6 L12: Another paper by Maninnen et al. (2014) shows seasonal trends in pollen and fungal spores at the boreal Hyttiala site in Finland and they also break the analysis down into PM mass <2.5 um and >10 um. While not at the same land-use type, these measurements may (or may not) be useful for broad comparison here.

Thank you for the reference.

In the revised manuscript it lends support to two statements, one about pollen as an important source of FBAP (page 6, line 22), the other about the large proportion of Cladosporium in airborne spores (page 7, line 29).

Minor technical comments: P1 L10: Move placement of "probably" to "INP were probably aerosolized . . ."

Done.

P1 L12-14: I though this sentence was confusing and could use a revision to make the point clearer.

Changed sentence to: "Further, transport model calculations for large (> 4 m-3) and small (< 4 m-3) numbers of INP-8 revealed greater differences in the likelihood of the potential source regions to provide precipitation to Southern Norway, than in the proportion of land cover or land use type." (page 1, lines 12-14).

P1 L22: snowflake is one word P4 L7 , L8, L10: "Landuse" should be two words P5 L19: Specifically mention that Tenerife is off the W coast of northern Africa.

Done.

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