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Interactive comment on "Ice Nucleation Activity in the Widespread Soil Fungus Mortierella alpina" *by* J. Fröhlich-Nowoisky et al.

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We thank Referee #2 for constructive comments and suggestions, which are highly appreciated and have been taken into account upon revision of our manuscript. Detailed responses are given below.

Referee #2: The only thing that I am missing is a bit more effort in searching possible evidence for such INP in previous studies. Given the novelty of reported discoveries, no previous study is likely to be found where M. alpine and INP have been investigated together. However, the characteristics of its INP provide clues for signs to look for. As described in the manuscript, they catalyse ice formation within a narrow temperature range, mostly between -5 and -6° C, pass through a 0.1 micron filter, but are larger than 100 kDa, withstand heating to 60°C, but are deactivated by heating to 98°C. This

fits the characteristics of leaf-derived INP studied by Schnell and Vali (1973). Leaf material from temperate regions carried only around 100 INP active at -6°C per gram, whereas leaves from microthermal regions had INP numbers that where 4 to 5 orders of magnitude larger, suggesting the relevance of INP derived from M. alpina, or other fungi producing the same kind of INP, might be limited to microthermal environments, i.e. the continental climates of Eurasia and North America.

Response: The referee is right. We did not explicitly mention the leaf-derived INP as we used always "biological residues" or "biogenic IN" which of course include leaf-derived INP. We compared the characteristics of M. alpina INP with the INP of Fusarium, lichen, pollen and bacterial INP. We now added the leaf-derived INP into this comparison. For the leaf-derived INP it was found that bacteria (P. syringae) are involved in the production (Maki et al., 1974). As the degradation of plant litter is also mediated by fungi, fungi might also contribute to the leaf-derived INP, as suggested for Fusarium by Pouleur et al. (1992). Indeed, M. alpina could be a major source of the prodigious populations of leaf-derived INP recorded in litters of humid microthermal D-type (ie, >50° N latitude) forests by Schnell and Vali (1976).

Maki, L. R., Galyan, E. L., Chang-Chien, M.-M., & Caldwell, D. R. (1974). Ice Nucleation Induced by Pseudomonas syringae. Applied Microbiology, 28(3), 456–459.

Pouleur, S., Richard, C., Martin, J.-G., & Antoun, H. (1992). Ice Nucleation Activity in Fusarium acuminatum and Fusarium avenaceum. Applied and Environmental Microbiology, 58(9), 2960–2964.

R. C. Schnell and Gabor Vali, 1976: Biogenic ice nuclei: part i. terrestrial and marine sources. J. Atmos. Sci., 33, 1554–1564. doi: http://dx.doi.org/10.1175/1520-0469(1976)033<1554:BINPIT>2.0.CO;2

Referee #2: Questions I would like to see addressed in the discussion section are:

a) Is there evidence for M. alpina (or alike) INP in the atmosphere or in precipitation

(e.g. INP active between -5 and -6 degree C and passing through a 0.1 micron filter)?

Response: For M. alpina INP there is no evidence at the moment as the IN activity in M. alpina is a new finding, and several other sources of INP (eg, INA bacteria, INA Fusarium, K-feldspar) are also active at this temperature. We have first to identify the protein and its corresponding gene to be able to detect the protein or the gene in atmospheric and precipitation samples.

When looking for similar fungal INP, it is known that the INP from lichen forming fungi and Fusarium spp. pass through a 0.2 micron filter (see also page 12711, starting line 11 and references therein). In a former study, we have been able to isolate two more ice nucleation active fungal species that were not known as ice nucleation active before from atmospheric samples. Both produce INP that pass through a 0.1 micron filter and one of them is active at -5°C. The results are published in Huffman et al., 2013 and Pummer et al., 2014. However, even if we could detect these fungal INP in atmospheric samples we could not yet assign them to a certain fungal species. Interestingly, fungal or other biological INP that pass through a 0.1 micron filter seem to be ignored in a lot of studies as the cloud water, snow or hail stone samples are usually melted and then either filtered to collect particles >0.2 micron for the freezing assay (e.g., Christner et al. 2008; Hill et al., 2013) or measured without size segregation (e.g., Schnell and Vali, 1976; Garcia et al., 2012; Michaud et al., 2014). We further note that Vali (1966) observed that 25-50% of INP in hail were tiny (<0.01 um).

As it is already written in the manuscript (page 12712, line 25) that: " . . .further studies are necessary for the identification of the IN themselves and the detection and quantification of these fungi and their IN in soil and atmospheric samples.", we will add "precipitation samples" in the text.

Christner, B. C., Morris, C. E., Foreman, C. M., Cai, R., and Sands, D.C.; Ubiquity of Biological Ice Nucleators in Snowfall, Science, 2008: 319 (5867), 1214. [DOI:10.1126/science.1149757]

Garcia, E., T. C. J. Hill, A. J. Prenni, P. J. DeMott, G. D. Franc, and S. M. Kreidenweis (2012), Biogenic ice nuclei in boundary layer air over two U.S. High Plains agricultural regions, J. Geophys. Res., 117, D18209, doi:10.1029/2012JD018343.

Hill, T. C. J., B. F. Moffett, P. J. DeMott, D. G. Georgakopoulos, W. L. Stump, and G. D. Franc (2014), Measurement of ice nucleation-active bacteria on plants and in precipitation by quantitative PCR, Appl. Environ. Microbiol., 80(4), 1256–67, doi:10.1128/AEM.02967-13.

Huffman, J. A., Prenni, A. J., DeMott, P. J., Pöhlker, C., Mason, R. H., Robinson, N. H., Fröhlich-Nowoisky, J., Tobo, Y., Després, V. R., Garcia, E., Gochis, D. J., Harris, E., Müller-Germann, I., Ruzene, C., Schmer, B., Sinha, B., Day, D. A., Andreae, M. O., Jimenez, J. L., Gallagher, M., Kreidenweis, S. M., Bertram, A. K., and Pöschl, U.: High concentrations of biological aerosol particles and ice nuclei during and after rain, Atmos. Chem. Phys., 13, 6151-6164, doi:10.5194/acp-13-6151-2013, 2013.

Michaud, A. B., J. E. Dore, D. Leslie, W. B. Lyons, D. C. Sands, and, J. C. Priscu (2014), Biological ice nucleation initiates hailstone formation, J. Geophys. Res. Atmos., 119, 12,186–12,197, doi:10.1002/2014JD022004.

Pummer, B. G., Budke, C., Augustin-Bauditz, S., Niedermeier, D., Felgitsch, L., Kampf, C. J., Huber, R. G., Liedl, K. R., Loerting, T., Moschen, T., Schauperl, M., Tollinger, M., Morris, C. E., Wex, H., Grothe, H., Pöschl, U., Koop, T., and Fröhlich-Nowoisky, J.: Ice nucleation by water-soluble macromolecules, Atmos. Chem. Phys. Discuss., 14, 24273-24309, doi:10.5194/acpd-14-24273-2014, 2014.

Schnell, R.C. and G. Vali, World-wide source of leaf derived freezing nuclei. Nature, 236, 212-213, 1973.

Vali, G. 1966. Sizes of atmospheric ice nuclei. Nature, 212, 384-385.

b) If so, is this evidence restricted to microthermal environments?

Response: We have been able to isolate two ice nucleating fungal species from atmo-

spheric samples collected in a semi-arid forest in Colorado (Isaria farinosa and Acremonium implicatum, see Huffman et al., 2013). They are not part of this study but we don′t see a restriction as we don′t know the origin and how far the fungal spores traveled before we sampled them. Of course, further studies are necessary to establish relations with climatic zones. Pinus contorta and P. ponderosa forests are restricted to mesothermal or warmer environments, and so we did not test for M. alpina in any forests with a microthermal range. Fig. 8 in Schnell and Vali (1976) suggests that, in rain samples at least, onset of INP activity in the -5 to -6° C range was more common in Canada than in Colorado/Nebraska, and onset of INP activity seemed to be around -6 to -7°C in Colorado rain and hail (Vali 1978). However, INP active at -5 to -6° C were detected in hail and in 12/16 snow samples tested in Hill et al. (2014), and Christner et al. (2008) observed the onset of activity in most Louisiana rain samples to be -5 C to -6°C. So, there is some evidence of INP with warmer activity (ie, with a profile suggestive of M. alpina) in precipitation from microthermal environments, but it is not restricted to these regions.

Christner, B. C., Cai, R., Morris, C. E., McCarter, K. S., Foreman, C. M., Skidmore, M. L., Montross, S. N. and Sands, D. C. 2008. Geographic, seasonal, and precipitation chemistry influence on the abundance and activity of biological ice nucleators in rain and snow. Proceedings of the National Academy of Sciences, 105, 18854-18859.

Vali, G. 1978. Freezing nucleus content of hail and rain in NE Colorado. American Meterological Society Monograph, 38, 93-105.

c) Or, in the absence of other such evidence from soil, atmosphere or precipitation samples, is evidence from environments outside the microthermal regions lacking an indication for M. alpina derived INP?

Response: As we are not able to specifically detect M. alpina INP in soil, atmospheric or precipitation samples this cannot be answered at the moment.
