

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 C. Morris 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.

C. Morris: The interest of these results could be enhanced if more information were provided about the abundance of *Mortierella alpina* in the soils analyzed here. For example, the data presented in Table 2 represent crude counts. This would be more informative if the authors presented estimates of the number of CFU of *Mortierella alpina* per gram of soil - and as a fraction of the total microbial load of the soil. This type of information is important for future estimates of the mass of organic matter contributed by these fungi to organic soil dust.

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Response: We agree that the total number of CFU and of ice nucleation active *M. alpina* per gram of soil is informative. We calculated the values and included them in table 2.

Specific comments, C.Morris:

P 4, L 22: To better understand the procedure used, the title of this section should be “Initial screening for ice nucleation activity”. Somewhere in the text the authors should mention that this initial screening introduced a bias relative to the ability of the fungi to grow to sufficient densities in the liquid medium. If the fungal isolate did not produce sufficient mass to yield at least 1 ice nucleus per 50 l aliquot tested for INA, then the isolate was discarded as negative – right?

Response: The title of the section was changed as suggested. The initial screening was done twice. After the first aliquot was tested, fresh medium was added and after incubation the cultures were tested again (page 12701, line 16). For the initial screening we used aliquots of each culture containing visible mycelia (page 12702, line1). We added the following information in the text: “Out of 489 picked CFU 474 showed growth in the liquid medium and were thus tested for ice nucleation activity.”

P 5, L 2: Please indicate the full species name of the *Fusarium* used in this work.

Response: We added the full species names (*F. acuminatum*).

P 6, L 25 to P 7, L 5: Here the authors indicate that they calculated the number of IN per mass of fungal mycelium. They did not indicate how the mass of the mycelium was determined (use of a precision balance to simply weight the tubes into which the mycelium was placed?). It would also be interesting for the reader to have an idea of the total mass of mycelia that was recovered for these tests (mg? g?, etc.).

Response: As suggested, we added the information of how the mass of the mycelium was determined as well as the range of the total masses used for the tests: “. . .of a fungal culture was harvested by scraping it off the PDA agar surface and transferred it

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into a sterile 15 mL tube which was weighed before and after harvesting. Depending on the individual isolates between 0.1 g and 1.3 g mycelium could be harvested.“

P 7, L 6: It would help the reader if the title of this section indicated that the objective was to determine the mass of the ice nucleation active material. It is confusing because of the mention of mycelial mass in the previous section.

Response: We changed the title into: "Size and mass determination of the IN“

Discussion section: Other points that could be discussed concern propositions for follow-up work to better understand the ecological context of *Mortierella alpine*. Molecular markers could be developed based on the strain collection the authors have established to enlarge the survey beyond the soils studied here so as to map the occurrence of these fungi. Their presence in soil could then be compared with the INA of the associated organic soil dust in the search of correlations to explain the origin of the organic INA material in soil dust.

Response: We added the following statement in the text: " Additionally, studies investigating the occurrence and the distribution of the INA fungi in aerosol samples, samples of fugitive dust, and different agricultural and natural ecosystem soil types could help to estimate their contribution to the organic IN in soil.“

P 13, L 12: The work presented here does not offer any support to this sentence (“The effect of biogenic IN might: : :”). It would be more appropriate to say that the pool of biological and biogenic IN might be larger than currently estimated.

Response: We changed the text as suggested.

Table 1a: It would be useful if the names of the sampling locations were indicated.

Response: We added the names of the sampling locations in table 1a.

Table 4 and Figure 2: Somewhere in the document, and best in this table and figure, information about the behavior of the reference fungi (*Fusarium* spp.) should be

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presented.

Response: As written in the manuscript (section 2.3.) the reference fungi were used as positive controls for the initial screening. *Fusarium* spp. are known to possess ice nucleation activity and several studies characterizing the *Fusarium* IN have already been published (e.g. Hasagawa et al., 1994; Tsumiki 1995). Thus, we decided not to perform the characterization experiments on *Fusarium*. However, we added the information about the number of IN per gram for *Fusarium acuminatum* in the text. “Aliquots of uninoculated DPY broth were used as negative controls. Ice nucleation active *Fusarium acuminatum* cultures (provided courtesy of Linda Hanson, Michigan State University, ≈ 109 IN g⁻¹ mycelium) were used as positive controls.”

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