

***Interactive comment on* “Potential relevance of *Mortierella alpina* as a source of ice nucleating particles in soil” by Franz Conen and Mikhail V. Yakutin**

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We appreciate your judgement and suggestions, which help us to refrain from interpreting our data with undue bias towards a newly characterised source of INP in soils.

We like the idea of an opinion paper but also think that our study contains new experimental findings going beyond an opinion. The opinionated aspect of the current manuscript is the attribution of the finding to a specific organism. In a revised version we would broaden the current category “INP-M-like” to “cell-free fungal INP” and change the title to “Soils rich in biological ice nucleating particles overproportionately abundant in those resembling macromolecules produced by fungi”.

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To valorize the results, we propose to add the following section at the end of the current manuscript:

4. Inferences from a wider context

4.1. Atmosphere

The frequency of clouds containing ice particles at moderate supercooling is larger above fertile land than downwind a desert (Kanitz et al., 2011). One cause of this difference could be higher concentrations of INP in fertile soils compared to desert soils (Conen et al., 2015). However, regional atmospheric INP concentrations above fertile land do not seem to vary with INP concentration in soils. The one to two orders of magnitude larger concentrations of INP in soils at Novosibirsk, compared to soils in La Brévine and Colmar (Fig. 1), leaves little or no trace in the atmosphere. At Chaumont, a site 30 km to the East of La Brévine and 120 km to the South-west of Colmar, we observed similar concentrations of atmospheric INP active at $-8\text{ }^{\circ}\text{C}$ or warmer as we did in Novosibirsk (Conen et al., 2017). During April and May, when arable soils are prepared and wind erosion is most prominent (Selegey et al., 2011), median values were 4 INP m^{-3} and 7 INP m^{-3} at Chaumont and Novosibirsk, respectively. Thus, soils are unlikely the dominant source of biological INP in the atmosphere above the cropland belt stretching from Western Europe eastward all the way to Novosibirsk. Still, the influence of soils as a source of atmospheric INP might appear unduely small in this comparison because of efficient atmospheric mixing within the latitudinal band. Nevertheless, it is likely that vegetation and leaves decaying at the soil surface make a larger contribution to atmospheric INP ($-10\text{ }^{\circ}\text{C}$) (Schnell and Vali, 1976; Conen et al., 2017). We conclude that cell-free fungal INP associated with soil dust probably have a minor influence on ice formation in supercooled clouds and regional differences between soils are masked by atmospheric mixing with relatively larger contributions of INP from vegetation and decaying leaves.

4.2 Soil

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Postulated potential advantages to an organism able to catalyse ice formation at slight supercooling include the cleavage of structures by ice formation to access otherwise occluded resources (Paul and Ayres, 1991) and the accumulation of water through growing ice from vapour in the surrounding air (Kiefft, 1988). However, there is little experimental evidence to support these ideas in the context of soil. To our knowledge, the most convincing evidence for an accumulation of water in the form of ice was described by Hofmann et al. (2015). Fascinating sculptures of hair ice can form on the surface of dead wood infected by the fungus *Epidiopsis effusa* through the mechanism of ice segregation. This mechanism transports slightly supercooled water from inside the wood to a body of ice growing on the outside of it. The heat released by the phase transition stabilises the front between liquid and ice, as long as water is supplied to the growing ice at a sufficient rate. Although fungal activity is responsible for shaping hair ice, ice segregation proceeds under the same conditions equally without the fungus, but then results in an ice crust. Temperatures recorded by Hofmann et al. (2015) inside and outside of wood samples showed that hair ice formation started when temperatures had decreased to about $-0.5\text{ }^{\circ}\text{C}$ in one experiment, and to $-2.5\text{ }^{\circ}\text{C}$ in another experiment. In both cases temperature inside the wood increased sharply after the onset of ice formation and stabilised near $-0.2\text{ }^{\circ}\text{C}$ through the heat released by ice formation, while it continued to decrease on the outside. In one of the experiments, ice growth stopped when outside temperature had decreased to $-4\text{ }^{\circ}\text{C}$.

The same process of ice segregation as described by Hofmann et al. (2015) may also take place at the surface or within the porous structure of soil, where larger pores are typically air-filled and water is held in finer capillaries, similar to those supplying water to the hair ice growing on wood. Visible phenomena of water accumulating through ice segregation at or near the soil surface include ice needles and ice lenses (Dash et al., 2006). For a soil fungus to benefit from ice segregation, it has to produce INP active as close as possible to $0\text{ }^{\circ}\text{C}$. We think that INP ($-6.5\text{ }^{\circ}\text{C}$) do not provide much of an advantage in this context. Even in a very small volume of soil, pore water is unlikely to supercool to that temperature. Further, the volume of water that might be harvested

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through ice segregation would be irrelevantly small, if there are other INP active at the same temperature nearby, which is definitively the case for all samples shown in Figure 1 (of our current manuscript). It can only be the much rarer INP active closer to 0 °C that potentially provide the advantage of ice segregation to an INP-producing fungus in soil. Pouleur et al. (1992) found about 1 in 10⁴ INP (-6.5 °C) was already active at -2.5 °C. The large numbers of cell-free fungal INP found in our samples may just be a proxy for the soil-ecologically relevant INP active closer to 0 °C. The detection of latter would require larger volumes of soil (e.g. millimetre-size aggregates) tested under conditions where temperature can be controlled with great stability and high precision (e.g. within a dry-block temperature calibrator).

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