

## ***Interactive comment on “The adsorption of fungal ice-nucleating proteins on mineral dusts: a terrestrial reservoir of atmospheric ice-nucleating particles” by Daniel O’Sullivan et al.***

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The ability of organic INPs (“leaf-derived nuclei”) to adsorb to and confer enhanced IN activity to minerals (esp. clays) was first proposed and demonstrated by Russ Schnell almost 40 years ago. Recent research has generated an awareness of the potential importance of this process, especially following recent studies showing that organic soil INPs are often very small (ie, would not be detected when bound to mineral particles).

This paper is an important and overdue advancement of this topic. Its main new contribution is that IN proteins are readily adsorbed onto clays while maintaining their activity. Secondly, it shows that ionic concentration (in the range found in soil water) and, to lesser extent, composition are important positive variables enhancing binding, but that

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pH is not. It is also useful and intriguing to be shown that the adsorption is reversible, which to me suggests that the binding process is relatively gentle and, hence, doesn't deform and potentially inactivate the proteins.

I have some suggestion for minor amendments.

P. 3 L. 18: “released” would be better than “lost”.

P. 3 L. 19: date missing for Pouleur.

P. 3 L. 21: no comma.

P. 4 L. 15: “much higher”.

P. 5 L. 4-14: Very nice background to this aspect.

P. 6 L. 22: I would add “(collected in the filtrate)” for those who need reminding of their small size.

P. 8, L. 22: Can you remind us what K is here?

P. 8 Clay-protein interactions in the absence of electrolytes section: In relation to Fig 2., I notice that in Fig. 3 the 1 mM NaCl treatment was also apparently unimpressive after 2 h, but given time was very effective. The same may have held true for the no electrolytes case. If you didn't test these ones for 48 h then you should acknowledge this even though it doesn't change the underlying story, and even though in a soil solution you would seldom encounter such a lack of ions. IN Fig. 2, it would also be nice to know what the underlying kaolinite INP profile was. I assume it's the log-linear line that would exist of the hump starting from -11 C and warmer was removed. Could this be mentioned?

P. 9 L.7: Couldn't it simply be that the ionic strength is just so low that the tendency was for surface ions of any type to remain in the bulk phase, just as K<sup>+</sup> is rapidly stripped off K-feldspar when that is put in DI?

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P. 9 L.11: Surely it's not just the charge, but also the presence of ions in solution to replace any that leave the surface of the clay, in a dynamic equilibrium? It would be useful, too, to provide a typical range for ionic strengths in soil solutions. I see Edmeades et al (1985; Aust J of Soil Res., 23, 151) gives a range of 5-16 mM for NZ grassland soil, which is very relevant to your Fig. 4., supporting the case that under normal conditions the INPs would rapidly bind to the clays.

P. 9 L.17: Date for Yu et al.

P. 9 L.18: Fig. 3 is impressive.

P. 11 L. 9: Also, divalent cations may cause flocculation of both clay particles and proteins, producing clumps of these. The natural pH of the kaolinite soln (5.7) would promote this.

P. 12. L.24: Maybe add the caveat that adsorption to kaolinite did not cause de-activation. During the process of drying, for example, the adsorption/binding may become stronger and so deform and affect the IN activity and propensity to be desorbed.

P. 13 L. 7: Nicely put.

P. 13. L. 14: I don't think all the efficient ones are proteins. Or, at least, it's premature to generalize?

P. 16. L. 16: Fusarium are common in soils yes, but not the most common. They tend to be pathogens. And only a few species are IN active. This sentence is overstating their abundance I think. Since this work would apply equally, I assume, to other IN fungi, such as *Mortierella alpina*, and other as-yet undiscovered IN fungi (the most dominant species, in terms of vegetative biomass, tend to be the Basidiomycetes, which are notoriously difficult to grow in pure culture), this section could be broadened to include adsorption of IN proteins released by many soil organisms.

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