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Potential relevance of Mortierella alpina as a source of ice nucleating particles in soil

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Abstract. Soil organic matter carries ice nucleating particles (INP) of which the origin is hard to define and that are active at slight supercooling. The discovery and characterisation of INP produced by the widespread soil fungus Mortierella alpina permits a more targeted investigation of the likely origin of INP in soils. We searched for INP with characteristics similar to those reported for M. alpina ($INP_{M\text{-}like}$) in 20 soil samples from four areas in the northern midlatitudes and one area in the tropics. In the 15 samples where we could detect $INP_{M\text{-}like}$, they constituted between 1 and 94% (median 11%) of all INP active at -10 °C or warmer associated with soil particles < 5 µm.

1 Introduction

Soils are a relevant source of ice nucleating particles (INP) found in the atmosphere and INP from soils are also found in precipitation (Creamean et al., 2013, 2014) and in rivers (Moffett, 2016; Larsen et al., 2017). Organic matter, or biological residues, associated with soil particles may contribute a major share to atmospheric INP active at temperatures warmer than -10 °C (Conen et al., 2011, O'Sullivan et al., 2014; Conen and Leifeld, 2014; Creamean et al., 2014; Tobo et al., 2014, Hill et al., 2016). A relatively recent discovery in this field of research is that the widespread soil fungus Mortierella alpina produces INP active at -5 to -6 °C which can be washed off the mycelium, are of a small size (< 300 kDa), remain active after heating to 60 °C, but are deactivated by heating to 95 °C and in 6 M guanidinium chloride (Fröhlich-Nowoisky et al., 2015). These findings are based on organisms extracted from soils sampled at an experimental station in Wyoming. Plating and cultivation have allowed to identify M. alpina as the INP-producing organism through DNA sequencing followed by phylogenetic analysis. The discovery of this new INP source raises the question of its more general relevance in soils and possibly in the atmosphere. Trying to identify and count, or determine the mass of M. alpina in soil could be one approach. However, this aproach would not account for the fact that INP produced by the organism can be washed off, may be preserved, accumulate in the soil, and be exported from a watershed during intense rainfall (Larsen et al., 2017). In a first attempt to gauge the potential relevance of INP derived from M. alpina we looked for INP in soils that match the challenge tests (e.g. heating) described above. In the absence of molecular genetic identification of the INP source, and since we

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cannot exclude that also other soil fungi may produce a similar kind of INP, we call these INP "M. alpina alike" (short: $INP_{M\text{-}like}$).

2 Material and methods

We collected grab samples from the surface of arable soils in Novosibirsk, Saskatoon and Colmar, from grasslands in La Brévine, and tropical mountain forests around Ranau (Table 1). Samples were air dried and dry sieved (< 63 µm). From each sample 1 g of dry particles (< 63 µm) was weighed into a 50 ml centrifuge tube containing 20 ml of 0.1% NaCl, was shaken for 2 min by hand and allowed to settle for another 10 min. About 10 ml suspension were withdrawn from the top of the suspension and passed through a syringe filter with 5 µm pore size (sterile cellulose acetate filter; Sterlitech Corporation, Kent, USA), 9 ml of it into a pre-weighed aluminium tray, 1.0 to 1.5 ml into another tube together with the proper amount of 0.1% NaCl to create a 1:20 dilution of the suspension. The tray and its content were dried at 80 °C, re-weighed and the mass of particles < 5 µm determined from the difference to a control tray prepared with only NaCl solution. The tube containing the 1:20 dilution of the suspension was analysed for INP on a freezing nucleation apparatus (Stopelli et al., 2014) in 52 aliquots of 100 µl in 0.5 ml tubes and, if necessary, further diluted to a concentration at which most, but not all of the 52 tubes were frozen at -10 °C. Final concentrations of particles < 5 µm in µg ml^{-1} ranged from 0.02 to 15.5 with a median of 1.0. The remainder of the suspension with the final concentration was then passed through a 0.22 µm syringe filter (same material and supplier as above) and partitioned into three portions. One was analysed for INP without further treatment, the other two were either heated to 60 °C or 95 °C for 15 min in a water bath before being analysed the same way. From the original suspension and a 6 M solution of guanidinium chloride (> 99.5%; Roth GmbH + Co. KG, Karlsruhe, Germany) we prepared a similarly diluted suspension of particles < 0.22 µm and analysed it for INP after 1 to 2 hours of storage at room temperature. Blank samples of 0.1 NaCl solution did not freeze at -10 °C. Our criteria for $INP_{M\text{-}like}$ were an activation temperature of -6.5 °C or warmer that is retained after heating to 60 °C, but which is deactivated by heating to 95 °C and by 6 M guanidinium chloride. For practical reasons (smallest mesh filter size available) we relaxed the size criterion (< 300 kDa) in Fröhlich-Nowoisky et al. (2015) to < 0.22 µm. This may seem generous, but still excludes other potential INP active at -6.5 °C that are associated with cells and are not detached macromolecules. Other potential sources of INP active at -6.5 °C are bacteria and pollen. However, bacterial INP have been found to not withstand heating to 60 °C and pollen derived INP macromolecules are not sensitive to guanidinium chloride or boiling (Pummer et al. 2012, 2014).

3. Results and discussion

We always found $INP_{M\text{-}like}$ in samples with more than 1 INP active at -10 °C µg^{-1} particles < 5 µm (INP_{-10}) (Fig. 1). There might also have been an $INP_{M\text{-}like}$ contribution in samples with less than 1 INP_{-10} µg^{-1}, but it was too small to be detected. Latter applies to all 4 samples from tropical Ranau (INP_{-10} < 0.1 µg^{-1}) and one (of 3) from the wine growing area around Colmar (INP_{-10} = 0.3 µg^{-1}). Guanidinium chloride reduced the number of INP_{-10} in all suspensions of particles < 0.22 µm to below detection limit (to 2% or less of what we found in suspensions prepared with 0.1% NaCl), so did heating to 95 °C.

What we presume are $INP_{M\text{-}like}$ were therefore not derived from pollen (Pummer et al., 2012, 2014). Averaged over all samples 97% (+/- 9%) of $INP_{-6.5}$ associated with particles < 5 µm passed through the 0.22 µm filter and 86% (+/-9%) of those remained active after heating to 60 °C. Consequently, about $5/6^{th}$ (0.97 x 0.86 = 0.83) of all $INP_{-6.5}$ found in soil particles < 5 µm matched characteristics of M. alpina. There might have been a small contribution by Isaria farinosa (Huffman et al., 2013) to the number of $INP_{-6.5}$ determined before heat treatment. However, these INP would have been deactivated after heating to 60 °C (Pummer et al., 2014) and would not have contributed to the number of $INP_{M\text{-}like}$.

$INP_{M\text{-}like}$ made up only $1/20^{th}$ of INP_{-10} around Colmar, but $2/3^{rd}$ of INP_{-10} around Novosibirsk. Regression analysis of the ensemble of 15 samples with detectable $INP_{M\text{-}like}$ from all four areas on three continents (Fig. 1) suggests that a doubling of INP_{-10} may be associated with a tripling of the number of $INP_{M\text{-}like}$ ($2^{1.6} = 3$). This trend not only applies across the different areas investigated, but also within certain areas (Saskatoon, La Brévine). We therefore conclude that M. alpina probably responds more efficiently than other organisms to soil conditions in which the formation of INP may be advantageous. Potential advantages of catalysing 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).

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Table 1: Details of sample origin, including mean annual temperature (MAT) and precipitation (MAP). In each area between 3 and 6 samples (*N*) were collected. A sample consisted of 100 to 300 g of soil collected from the surface within a radius of a few meters. The maximum distance between samples (D_{max}) ranged from 4 to 106 km.

Area	unit	Novosibirsk Southwestern Siberia	Saskatoon Northern Great Plains	La Brévine Jura Mountains	Colmar Upper Rhine Valley	Ranau Borneo
Coordinates	latitude	54°38' to 55°18' N	52°04' to 52°08' N	46°59' N	48°00' to 48°05' N	05°59' to 06°03' N
	longitude	82°44' to 84°23' E	106°29' to 106°37' W	06°36' E	07°19' to 07°23' E	116°42' E
Altitude	(m a.s.l.)	120 - 150	500 - 520	1050	200	450 - 690
Landuse		arable crops	arable crops	grassland	arable crops	mountain forest
MAT	(°C)	1.7	2.6	4.9	10.9	27
MAP	(mm)	448	354	1597	607	2880
Sampling		May, Jun 2013	Oct 2014	Sep 14	Oct, Nov 2014	Mar 2014
N		6	3	4	3	4
D_{max}	(km)	106	12	4	10	8

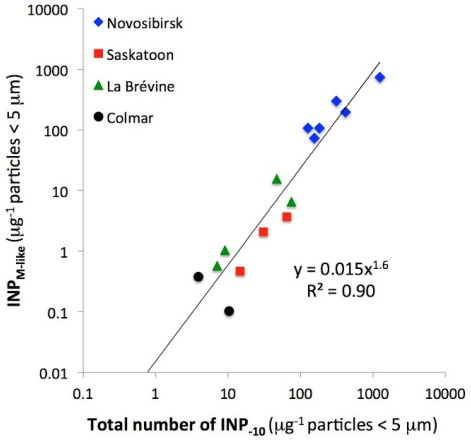

Figure 1: Ice nucleating particles with characteristics of Mortierella alpina ($INP_{M\text{-}like}$) as a function of the total number of INP active at -10 °C in soil particles < 5 µm. The trendline was fitted to all data in the plot. Not plotted are 4 samples from tropical Ranau (INP_{-10} < 0.1 µg^{-1}) and one (of 3) from the wine growing area around Colmar (INP_{-10} = 0.3 µg^{-1}) in which we could not detect any $INP_{M\text{-}like}$.