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Evidence for biological shaping of hair ice

D. Hofmann[1], G. Preuss[2], and C. Mätzler[3]

[1]Institute of Bio- and Geosciences, Forschungszentrum Jülich, Jülich, Germany
[2]Wiedtal-Gymnasium, Friedenstraße, 53577 Neustadt (Wied), Germany
[3]Institute of Applied Physics, University of Bern, Bern, Switzerland

Correspondence to: D. Hofmann (d.hofmann@fz-juelich.de)

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Abstract

An unusual ice type, called hair ice, grows on the surface of dead wood of broad-leaf trees at temperatures slightly below 0 ˚C. We describe this phenomenon and present physical, chemical, and biological investigations to gain insight in the properties and processes related to hair ice. Tests revealed that the biological activity of a winter-active fungus is required in the wood for enabling the growth of hair ice. We confirmed the fungus hypothesis originally suggested by Wegener (1918) by reproducing hair ice on wood samples. Treatment by heat and fungicide, respectively, suppresses the formation of hair ice. Fruiting bodies of Asco- and Basidiomycota are identified on hair-ice carrying wood. One species, *Exidiopsis effusa* (Ee), has been present on all investigated samples.

Both hair-ice producing wood samples and those with killed fungus show essentially the same temperature variation, indicating that the heat produced by fungal metabolism is very small, that the freezing rate is not influenced by the fungus activity and that ice segregation is the common mechanism of ice growth at the wood surface. The fungus plays the role of shaping the ice hairs and to prevent them from recrystallisation. Melted hair ice indicates the presence of organic matter. Chemical analyses show a complex mixture of several thousand CHO(N,S)-compounds similar to fulvic acids in dissolved organic matter (DOM). The evaluation reveals decomposed lignin as the main constituent. Further work is needed to clarify its role in hair-ice growth and to identify the recrystallisation inhibitor.

1 Introduction

1.1 Characteristics of hair ice

One of the most exciting types of ice has the shape of fine hairs (diameter near 0.02 mm, length up to 20 cm). It can be observed in forests, on dead wood, usually

on the ground, and sometimes on still standing trees (Fig. 1). This so-called *hair ice* or *ice wool* grows at the surface of the unfrozen wood body of certain moist and rotten branches of broad-leaf trees. The hairs are smooth, often with a silky shine. They are found in bunches of beautiful structures such as curls and waves, sometimes with clear parting or zoning, but without ramification. Although individual hairs are mostly separate they follow a macroscopic order often with surprising regularity. The hair base is rooted at bark-free positions or where the bark is loose, but never on the bark. The outer end is either free or in contact with an ice crust or with surrounding material, such as bark or leaves. Sometimes hairs loop back to the branch. Bands of parallel hairs can sinter along contacting lines without loosing the original shape until melt onset. In cold, dry air, however, hair ice sublimates within short time. Therefore it can be observed under calm, humid conditions, only, at air temperatures slightly below 0 °C. We observed branches that produced hair ice repeatedly over more than two years. During this time the dry density decreased to about 0.3 g cm^{-3} while the wood was brightening as a consequence of white rot.

Movies of hair-ice growth support visual observations in that the growth is controlled and synchronised over macroscopic parts of the wood. The hair curvature results from lateral gradients in the growth velocity. The primary direction is radial, the hairs being in the prolongation of wood rays (Fig. 2). Indeed, our observations indicate that the hairs are rooted at the mouths of these rays (Wagner and Mätzler, 2009). Furthermore, the hair-ice thickness (Fig. 3) corresponds to the diameter of the cells (Figs. 8 and 9 in Sect. 2) forming the channels in the wood rays (Schweingruber, 1990).

The fine hairs can keep their shape over long time, many hours, even days. This is surprising because recrystallisation of small ice crystals to larger ones is fast, especially at temperatures close to the melting point. The observed stability is an indication that hair ice is doped with a recrystallisation inhibitor. Examples are Anti-Freeze Proteins (AFP), also called Ice-Binding Proteins (IBP), see Griffith and Yaish (2004).

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When the air temperature exceeds 0 °C, hair ice starts to melt. Hairs fixed at both ends are transformed to a type of micro strings covered by small water droplets (Fig. 4) before they decay. Hairs with free ends have been observed to collapse in length. The melted liquid is clear water with a slightly brownish colour indicating the content of organic material.

We received many reports on the observation of hair ice mainly at latitudes between 45 and 55° N from Canada, France, Germany, India, Ireland, Netherlands, Russia, Scotland, Slovenia, Sweden, Switzerland, USA and Wales.

1.2 References to earlier work and the fungus hypothesis

Although the literature on hair ice is not abundant it reaches back by about one century. Alfred Wegener (1918), the founder of the theory on continental drifts, published the most relevant paper of the earlier work. He observed hair ice in Winter 1916/17 in the Vosges Mountains, and in February 1918 in Northern Germany at Rheinsberg in der Mark. He was able to grow hair ice again on the branches that he found. Wegener assumed a relationship between the formation of hair ice and the mycelium visible on the branch surface. His consultant, Arthur Meyer, confirmed that these branches contained fungus mycelia. He assumed a type of Ascomycota, however, he was unable to determine the species. Mühleisen and Lämmle (1975) described the reproduction of hair ice on rotten wood of broad-leaf trees in a climate chamber. They assumed that some type of osmotic pressure is acting. Lenggenhager (1986) observed hair ice in the neighbourhood of Bern, Switzerland from 1979 to 1985. Being aware of these observations, Wagner (2005) supposed the involvement of a fungus activity without the knowledge of Wegener's work. Wagner found that the presence of fungus mycelia in the wood body of hair-ice producing branches is manifested in the microscopic and macroscopic view. Fruiting bodies of fungi were observed as shown by Wagner and Mätzler (2009). They developed a method to repeatedly grow hair-ice on a covered balcony during nights with freezing conditions. Their more specific hypothesis, that the metabolism of these fungi is a prerequisite for the formation of hair ice, was supported

by their tests. A further hypothesis was the assumption that gas pressure, caused by fungal metabolism, ejects water through wood rays to the wood surface. Furthermore they concluded that hair ice is formed from water stored in the wood. Dash et al. (2006) considered hair ice as a frost-heave phenomenon associated with ice segregation. In the present work we recognised signatures of this effect during hair-ice growth.

1.3 Hair ice and ice segregation

Hair ice is growing on a porous substrate containing liquid water. We call this ice type a *basicryogen* in order to distinguish it from ice that grows from atmospheric water. Different basicryogenes grow on different substrates and different ingredients. In common is the co-existence of liquid water, ice and the porous substrate at the ice front. This general phenomenon is called *ice segregation*. The thermodynamic phenomenon of frost heave (Vignes and Dijkema, 1974; Dash, 1989; Ozawa, 1997; Dash et al., 2006) is a common driver for these ice types.

Hair ice is growing at the wood surface while a connected network of water inside the wood remains in the liquid state. Due to molecular interactions at the large specific interface area between the wood cells and the water, the melting temperature inside the wood is reduced as compared to bulk water, known as *Gibbs–Thomson* effect (Ozawa, 1997; Dash et al., 2006). The reduction increases with increasing specific surface, i.e. with decreasing size of capillaries and pores of the wood. The depression can keep the water unfrozen inside the pores while ice is growing outside. The freezing front remains at the wood surface during ice growth. In this way, the ice is segregated from the reservoir of liquid water.

How can we understand this process? The large dipole moment of the water molecule causes charges at the surface of liquid water and ice. Energy is needed to set up the associated electric field. Surface energy can be reduced if a liquid-water film remains between the wood and the ice surface due to the flexibility of the liquid to neutralise the local charges. Under freezing conditions suction forces are set up, caused by the interface energy of the wood-water-ice sandwich, to attract liquid

water from the pores of the wood toward the freezing front. In this way the liquid-water film is maintained. Frost-heave processes in fine-grained soil also act in this way (Ozawa, 1997; Dash et al., 1995, 2006). Although the theory of ice segregation is still incomplete (Dash et al., 2006), empirical insight in the behaviour was found by experimental studies. Osawa and Kinosita (1989) investigated ice growing on the surface of a microporous filter. The thin liquid film, separating the ice from the filter material, was confirmed. The authors observed that the growth rate of ice increases proportionally with the depression $\Delta T_f = T_0 - T_f$ of the temperature T_f at the freezing front, where $T_0 = 0\,°C$, is the melting temperature of bulk ice (ΔT_f values ranging from 0.0 to 0.3 °C). The observations are in agreement with the experiment of Vignes and Dijkema (1974) growing ice at the mouth of a capillary channel that was in contact with a reservoir of supercooled water (ΔT_f values ranging from 0.3 to 0.77 °C). With respect to growth rate and temperature range these experiments agree with our observations of hair-ice growth as will be shown.

1.4 Outline

Advancing the understanding of the hair-ice phenomenon has been the objective of this work. In Sect. 2, we will confirm that a winter-active fungus is needed for hair-ice formation. We will identify the acting fungus. In the physical analysis of Sect. 3 we will study the temperature variation of wood during ice formation. Wood samples with and without the acting fungus will be studied. The results point us to the search for a recrystallisation inhibitor produced by the fungus and thus to the chemical analysis of Sect. 4. Final discussions and conclusions will be given in Sect. 5.

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2   Observations and experiments related to the fungus hypothesis

2.1   Suppression of hair-ice growth by killing the fungus

2.1.1   Experiments with fungicide

In Winter 2010/11 Gerhart Wagner refined the hair-ice experiments of Wagner and Mätzler (2009). A hair-ice active branch was cut into 5 pieces (length 35 cm, diameter 2 cm) and exposed to a commercial fungicide ("Schimmelentferner" MIOCOLOR, Natriumhypochlorit, conc. 4.4 %): Piece No 0 remained untreated, No 1 was exposed for 15 min, No 2 for 30 min, No 3 for 60 min, and No 4 for 120 min. On the first evening (18 December 2010) the air temperature was close to −10 °C. Consequently the growth of hair ice was fast, but ended after one hour at a hair length of 1 cm. The result at 21:30 (LT) is shown in Fig. 5. Hair ice was reduced on pieces with fungicide treatment, but complete suppression was observed for the longest treatment, only.

The following morning all pieces melted at room temperature before they were exposed to the cold again. The air temperature rose from −5 to 0 °C. Two hours later a very similar result was obtained. In the afternoon the experiment was repeated again after wetting the branches in distilled water for a short time. The results showed the expected gradation even more clearly. All results demonstrate that the 15 min treatment causes a significant reduction of hair-ice production, and a treatment for 120 min stopped the production completely. During the following weeks the same branches were repeatedly exposed to the cold. Slow recovery occurred for the fungicide pieces in that they gradually started to grow hair ice again. The pieces with different treatment duration became more and more similar.

2.1.2   Experiments with heat

A hair-ice branch of about 20 cm in length and 2 cm in diameter was cut into four similar pieces on 3 January 2011. Three of them were exposed to hot (90 to 95 °C) water for 1,

2 and 4 min, respectively. After the following frost night the reference was covered with dense hair ice, however, the heated pieces did not show any. The repetition with a thin branch (1 cm) produced the same result. Some days later the heated pieces started to produce traces of hair ice again.

2.1.3   Other treatments

Treating the branches with alcohol (70 %) or with the weak fungicide, IMAVEROL, an imidazole, also called Enilconazolum ($C_{14}H_{14}Cl_2N_2O$), often used in veterinary medicine, did not affect hair-ice growth.

2.1.4   Results

Heat treatment showed the most radical hindrance of hair-ice growth. One minute in hot water was sufficient for a complete suppression. For fungicide the type and duration of the treatment played a role. Whereas our weak fungicide did not have any effect, complete suppression was achieved with the stronger one after treatment for two hours. The results are plausible in view of the fungus hypothesis. At a temperature of at least 90 °C the active cells of the fungus are killed rapidly. Fungicides act much more slowly. Several days or weeks after the treatments, hair-ice growth starts again either due to the recovery of the mycelium or due to the survival of spores.

2.2   Identification of the fungus and microscopic studies on hair-ice producing wood

2.2.1   Methods

From January to March 2012, from December 2012 to April 2013 and November 2013 to March 2014 a total of 78 hair-ice bearing twigs and branches were collected in the forests near Brachbach/Sieg in Germany (northern slope of "Windhahn"). Some of them were kept on a moss-covered shadowy area in the garden of one of the

authors (G. Preuss) to simulate forest-floor conditions or temporarily on a wet towel in a closed plastic box. Additionally 41 hair-ice bearing trees, boles and branches were observed at their natural location in the forest. Mostly the species, or at least the genus of the dead branches, was identified by species-specific characteristics visible in the microscopic wood anatomy (Schoch et al., 2004; Richter and Dallwitz, 2000). Fungi growing on the examined branches and logs were identified if necessary by microscopic characteristics with the help of Gminder (2008), Haller and Probst (1983), Jülich (1984), Krieglsteiner (2000), Laux (2010), Moser (1963), and Rothmaler (1994). Samples of the fruiting bodies were prepared as squash mounts and sometimes coloured by Phloxin B to stain the basidia. Hand-cut sections of hair-ice bearing wood samples were prepared as wet mounts and afterwards coloured by "cotton blue" following the instructions of Bavendamm (1936) according to the description of Riggenbach (1959). This is an approved method to make fungal hyphae visible within wood cells.

2.2.2 Observations and results

The type of examined hair-ice wood varied from fallen twigs, attached and fallen branches to dead standing trees and fallen or felled boles except for one case: a still living hazel tree was bearing hair ice on a dead part of the wood several times.

In the majority of cases the consistency of the dead wood was "rather hard" – indicating an initial stage of decay – but four samples had a "distinctly softened" texture adopting the terms of Heilmann-Clausen and Christensen (2003). The integrity of the bark differed from almost intact to completely lost. If it still existed the bark peeled off from the hair-ice producing wood surface and often got lost during the observation period.

Hair ice was observed on ten different broadleaf tree species belonging to five different plant families (Table 1).

Eleven different fungal fruiting bodies were identified on the wood samples – in some cases up to three species on the same piece of wood. The list is certainly not complete.

One of these fungi, *Exidiopsis effusa* (Ee), deserves closer attention, as it colonised all our hair-ice producing wood samples. In more than half it was the only detectable fungus, but in most cases it was not yet visible macroscopically, when hair ice occurred for the first time in late autumn. Its unspectacular fruiting body appeared some weeks later as a thin whitish coating (Fig. 6) exactly on those areas of the wood surface, where hair ice had grown before (Fig. 6b, d and f) or next to the hair-ice area (Fig. 6d). In the first observation period (winter 2011/12) the coating was present on every observed sample from February on, in the second and third winter the first fruiting bodies were visible in January. The colour was sometimes slightly bluish, greyish or light pink tinted, depending on the humidity and colour of the wood.

Under humid conditions (in nature or in a closed plastic box containing a wet towel) and temperatures above the freezing point small water droplets often appeared on the surface of the fruiting body of Ee over night (Fig. 6f). This might be a case of guttation which means the active excudation of an aqueous solution. The phenomenon is well known from different vascular plants, but also from some fungi (Knoll, 1912; Thielke, 1983). The observation of Sprecher (1959), who describes a reinforcing effect at temperatures near +4 °C on the guttation activity of a fungus, is particularly interesting with regard to the formation of hair ice. Further studies on Ee are necessary to clarify this aspect.

Cross sections of the fruiting body were 30 to 100 µm thick with great variability (Fig. 7a–c). It mainly consists of ovoid to pyriform basidia in a layer of thin, clamped hyphae. The basidia, too, are clamped at their base, 4-septate (Fig. 8a, c and d), longitudinally divided (Fig. 8a), about 10 µm in diameter and circa 15 µm long without the sterigmata. The spores (Fig. 8b) are weakly allantoidal, 13–15 µm long and 4–5 µm in diameter and often contain one big oil drop in the middle. Based on these characteristics the fungus was identified as *Exidiopsis effusa* (Ee) with the help of the manual of Jülich (1984). The descriptions at www.mycobank.org (Robert et al., 2005) and www.mycokey.com (Petersen and Læssøe 2003) confirm the result, while Krieglsteiner (2000) classifies the fungus as *Exidiopsis grisea* var. *effusa*.

Beneath the fruiting body the superficial wood cells are almost filled by hyphae (Fig. 7a and b). The fungus seems to grow along the wood vessels (Fig. 9a) and to intrude into the deeper wood tissue along the wood rays (Figs. 7c and 9b).

Some hair-ice wood samples found in November were examined by microscope, too. At that time of the year, there was no macroscopic sign of a fungal fruiting body on the wood surface. But when microscopic mounts were coloured by "Cotton Blue", they showed, that the wood tissue was pervaded by fungal hyphae running along the wood vessels and rays (Fig. 9b). Except for the density of the hyphae the findings looked exactly like those from the wood samples with a fruiting body of Ee.

As our main result, we unravelled the mystery about the whitish covering described by Wegener (1918) and Wagner (2005) by the identification of Ee. The opinion of Wegener's consultant, A. Meyer, who did not expect the appearance of a fruiting body and thought that the determination of the species would be impossible, has been disproved. Furthermore the result specifies the fungus hypothesis of Wagner and Mätzler (2009).

3 Physics of hair ice

3.1 Thermal effects during hair-ice formation

Insight in the physics of hair ice is found from thermal signatures of hair-ice wood, i.e. the temperature enhancements caused by heat sources associated with the ongoing processes. These include (1) heat generated by fungal metabolism, (2) latent heat of fusion when water freezes in contact with the wood, and (3) heat used or generated by recrystallisation.

Heat generated by these processes diffuses into its environment by radiation, heat conduction, air turbulence, evaporation and sublimation and therefore exact measurements are difficult to achieve. However, by careful design of the experiments, the main heat loss can be reduced to black-body radiation. For heat sources that

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change rapidly with time the temperature changes are damped according to the heat capacity of the wood. On the other hand, under stationary conditions, the heat power P generated in the wood (at temperature T_1) is equal to the net power that the wood looses to its environment (at temperature T_2). Then the temperature difference can be expressed by

$$\Delta T = T_1 - T_2 = \frac{P}{4\sigma A T^3} \tag{1}$$

where $\sigma = 5.673 \times 10^{-8}\,\mathrm{W\,m^{-2}\,K^{-4}}$ is the Stefan–Boltzmann constant, A the branch surface area, and T the mean absolute temperature of T_1 and T_2. The proportionality allows us to express the generated power by temperature differences. Two examples are given below.

3.1.1 Fungal metabolism

For the combustion of nutrients by fungal metabolism we assume a heat of combustion Qb = 15.6 kJ g^{-1}, representative for glucose. A typical branch used in our experiments has a volume of $V = 82\,\mathrm{cm^3}$ and a surface area of $A = 150\,\mathrm{cm^2}$ (length $l = 26\,\mathrm{cm}$, diameter $d = 1.8\,\mathrm{cm}$). Such a branch is able to produce hair ice over about one year. For the active phases of the fungus we assume a duration of 3000 h. Furthermore, assuming 5 g of nutrients, the rate of combustion is on the order of 1.5 mg h^{-1}. Multiplying this value with Qb gives $P = 7\,\mathrm{mW}$, and for $T = 273\,\mathrm{K}$ in Eq. (1) we get $\Delta T = 0.1\,\mathrm{K}$, a value that should be measurable under favourable conditions.

3.1.2 Heat of fusion

Now, assuming for the same branch geometry, that latent heat is generated by freezing 1 g of water per hour, the generated heating power follows from the latent heat of fusion of ice, $L_f = 333\,\mathrm{J\,g^{-1}}$ at $T = 273\,\mathrm{K}$, as $P = 92\,\mathrm{mW}$, and inserting this value in Eq. (1)

gives ΔT = 1.3 K. This temperature difference should be easy to measure. Note that it is proportional to the freezing rate.

3.1.3 Measurement setup

Branches of beech wood with hair ice were collected in a forest at Moosseedorf, Switzerland. The samples were thoroughly wetted in rainwater. Then they were arranged on a plastic socket put on wet snow to stabilise temperature and to keep the humidity at saturation to avoid evaporation and sublimation. The entire stack was put in a plastic tub that was covered and further insulated with a towel to reduce vapour fluxes and heat conduction inside the tub. The tub was put in a garden hut from mid January to early March 2011, and again in January 2012. Hair ice formed on wood samples during cool nights. From time to time wetting was repeated. With this setup we realised conditions for precise temperature measurements and approaching the assumptions that the net heat flux can be represented by Eq. (1). For temperature measurements we used Pt-100 sensors (length 10 mm, diameter 2.6 mm). After calibration with an ice-water mixture at 0.00 °C, the sensors were inserted into the wood with the cylinder axis along the radial wood rays (Fig. 10). Temperatures were registered once per second to monitor changes with sufficient time resolution. The effective temperature of the environment was a mean of T_0 = 273.15 K of the underlying wet snow and of the tub walls cooled from outside. To represent this value of T_2 we either used the air temperature inside the tub or the temperature of a dry, passive tracer (hazel wood in 2011) close to the wetted wood samples.

3.1.4 Experiment of 18–20 January 2011

First measurements occurred in January 2011. During the first night, January 18–19, the temperature stayed above 0 °C (Mätzler et al., 2013). The time-averaged temperature of the hair-ice branches was 1.213 °C whereas the value of the

passive tracer was 1.203 °C. The difference of 0.010 °C is very small, similar to the measurement uncertainty.

First hair-ice growth was monitored from 19 to 20 January 2011 with temperature variations shown in Fig. 11. Before midnight all temperatures decreased linearly with time including the passive tracer. At midnight, sudden increases occurred for all hair-ice branches, indicating ice nucleation and start of latent-heat release by hair-ice growth. The start is almost simultaneous in all branches, with slightly different nucleation temperature (−0.4 to −0.7 °C). Ice was growing until the end of the measurement period in the following morning. During this period the temperature of the hair-ice branches remained nearly constant near −0.2 °C. On the other hand, the passive tracer (8), representing T_2, continued to cool to −1 °C until 05:30 (LT) without any jump. Further experiments confirmed these results. The simultaneity of temperature jumps for different hair-ice woods was observed again, but exceptions occurred as well.

3.1.5 Experiments of 13–15 January 2012

In the second year we tried to find out if the fungus activity is a prerequisite of the observed temperature jumps, in other words, if the jumps are signatures for hair-ice formation. We selected a beech-wood sample with abundant hair-ice growth. After wetting and cutting in half, both pieces still showed abundant hair-ice growth during the night from 13 to 14 January 2012 with jumps similar to Fig. 10. Then one piece (1) was held in hot water for 5 min to kill the fungus. Both pieces were exposed to the cold again during the following night. As shown in Fig. 11, the untreated sample (2) produced dense hair-ice again, but no hair ice was found on the heat-treated sample (1); instead, a thin ice crust covered this wood. The temperature variations are presented in Fig. 12. All sensors started slightly above 0 °C, and all values were near −4.3 °C at 09:00 (LT) on the following morning. Due to colder weather the cooling was faster and reached lower values than in Fig. 11. Both wood samples – with and without hair ice – showed similar and almost simultaneous increases, indicating the onset of freezing at 22:35

(LT). Air temperatures at the centre (3) and edge (4) of the container indicate forced cooling with minor modulations.

3.2 Discussion

Before the onset of freezing the temperature of hair-ice branches cannot be distinguished from the environmental temperature. The small difference ($0.01\,°C$) found during the first night in 2011 is representative for the measurement error. This means that heating by fungal metabolism is much smaller than estimated in Sect. 3.1.1. In other words the combustion rate of nutrients in our test sample is clearly less than $1\,\mathrm{mg\,h^{-1}}$.

Before freezing begins the wood temperatures in Figs. 11 and 12 decrease to supercooled conditions below $0\,°C$. A sudden increase marks the onset of freezing by the release of latent heat. The onset in one branch triggers the onset in near-by branches usually within short time. Ice nucleation appears as an explosive event distributing microscopic ice particles. The temperature increase is similar for samples with hair-ice formation and for samples with killed fungus.

During the phase of ice growth the wood temperature (-0.1 to $-0.4\,°C$) is nearly constant. In addition to the data of Figs. 11 and 12, this range was found in all other measurements that we made (Mätzler et al., 2013). These values are signatures of the Gibbs–Thomson effect and thus of ice segregation. The stabilisation is based on the heat of fusion. With falling ambient temperature the freezing rate increases and thus the transfer of latent heat to the wood increases, too. The associated temperature increase compensates for the decrease of the ambient temperature, keeping the branches close to the melting point. The stabilisation acts as long as there is sufficient liquid water in the wood rays. The continued freezing dehydrates the branch. When the liquid supply is exhausted, ice production comes to an end, the heat production stops, and the wood temperature converges to the ambient one. This transition is shown near the end in Fig. 12.

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The temperature difference between the pieces with and without hair-ice production is small, indicating that the freezing rates are similar. The result means that the fungus activity hardly plays a role for the rate of ice formation. This finding may appear as a surprise when looking at the difference between the branches (1, 2) of Fig. 10. But, note that hair ice is extremely fine, its density being extremely small. On the other hand the ice crust on the heat-treated piece (1) mainly consists of bulk ice. To show that its mass is indeed similar to the hair-ice mass, we compare two situations with the same ice volume, one consisting of N hairs per branch surface area, the other one consisting of an ice crust covering the branch surface with a constant thickness d. If we denote the hair diameter by d_h and the hair length by l_h, it is easy to show that

$$d = \frac{\pi}{4} N l_h d_h^2 \qquad (2)$$

To get an estimate of d we need the parameters on the right-hand side of Eq. (2). From Fig. 3 and similar observations we find: $d_h \approx 0.014\,\text{mm}$, $N \approx 20\,\text{mm}^{-2}$, and for the hair length we choose $l_h = 50\,\text{mm}$ to get $d = 0.15\,\text{mm}$, for the crust thickness. The comparison is consistent with the two branches of Fig. 10.

With regard to Fig. 12, minor differences between Curves (1) and (2) can be noted. Firstly, after freeze onset, Curve (1) shows small oscillations possibly due to recrystallisation. On the other hand, the temperature variation of Curve (2) is very smooth. Secondly, towards the end, when the strongest cooling occurs, the temperature decrease is slower for Curve (2) with hair ice than in Curve (1) due to thermal insulation by the *ice wool*.

The similarity of the temperature and thus of the rate of ice growth means that ice segregation is the common mechanism for ice production at the wood surface. The role of the fungus is in shaping the ice as hairs and to prevent it from recrystallisation. This is the main result from the physics of hair ice. Furthermore it indicates that temperature and pressure enhancements associated with fungal metabolism in hair-ice wood (Wagner and Mätzler, 2009) do not appear to be noticeable.

4 Chemistry of melted hair ice

4.1 Organic carbon

First investigations of non-filtrated and filtrated melt water samples from hair ice by means of Total Organic Carbon analyser (TOC-VCPH, Shimadzu, Japan) show similar and significant amounts of organic carbon ($> 200\,\mathrm{mg\,L^{-1}}$) beside small amounts of (total) nitrogen ($> 10\,\mathrm{mg\,L^{-1}}$). Ion chromatography revealed that the main part (70 %) of nitrogen consists of ammonium ions. We conclude that melting of hair ice provides us with a real organic carbon solution, mostly free of wood and aerosol particles.

4.2 Mass spectrometry

For the elucidation of the unknown components we applied mass spectrometry (MS) aiming at a complete spectrum of organics with regard to molecular size and polarity. Representative screening was achieved by various ionisation techniques in combination with appropriate separation techniques. First, we performed gas chromatography, coupled with electron-impact mass spectrometry (GC-EI-MS) of filtrated aqueous solutions according to Turska et al. (1997), followed by the more sensitive Head Space-GC-EI-MS (Seto, 1994; Snow and Slack, 2002). For small carboxylic acids (known e.g. as plant exsudates) and peptides/proteins (possible degradation products and/or anti-freeze proteins), we used capillary electrophoresis and Electrospray Fourier-Transform Ion-Cyclotron Resonance-Mass Spectrometry (ESI-FT-ICR-MS, Marshall et al., 1998). None of these methods yielded any significant peak.

In the second step, we applied our most sensitive mass spectrometer equipped with an electrospray source coupled to Ultra Performance Liquid Chromatography (UPLC-ESI-MS, Swartz, 2005; Guillarme et al., 2010), resulting in first (non-resolved) chromatograms. The full-scan spectra of positive *as well as* negative mode and from different retention times, respectively, are highly complex – ranging over the whole

mass range, but similar among themselves. Such spectra – gaussian in shape with emerging odd-numbered peaks – look similar to fulvic/humic acids in dissolved organic matter (DOM) of terrestrial/marine water, soil/sediments, peat bog, kerogen up to crude oil (Kujawinski, 2002; Sleighter and Hatcher, 2007; Hertkorn et al., 2008).

Therefore, in the third step, we simultaneously desalted and concentrated hair-ice samples by Solid-Phase Extraction (SPE). The methanolic eluates, mixed with 20 % water for better ionisation yield, were introduced again as flow injection in the ultrahigh-resolving ESI-FT-ICR-MS. In contrast to our former investigation, we arranged the measuring conditions towards complex samples (averaging of 7 spectra of 50 transients each) to improve the statistics. The high-resolution spectrum in the mass range 200–1000 Da (1 Dalton (Da) = 1.660539×10^{-27} kg, corresponding to 1/12 of the mass of a C-12 atom) show a bimodal distribution (maxima at 500 and 610 Da, respectively, not shown). The detailed evaluation of these spectra revealed the typical presence of molecular families containing ions that differ from each other in the number of CH_2 groups, degree of saturation (grey arrows in Fig. 13) and functional group substitution (blue arrows in Fig. 13) with a significantly higher intensity of the odd-numbered masses (description of such spectra in Leinweber et al., 2009). The reason for the latter phenomenon is the mass spectrometric nitrogen rule: under electrospray conditions the ionisation is by proton transfer to $[M-H]^-$ or $[M+H]^+$ ions, therefore nitrogen-free organics like CHO and CHOS compounds are odd numbered, while organics with one (or three or five, respectively) nitrogen atom(s) are even numbered.

In Fig. 13, ions with the mass space of 0.0364 Da (blue arrows) were derived from the mass difference between CH_4 and O, representing the most important formal functional group substitution, giving the typical clusters within a nominal mass. Furthermore, expanded sections of successive, randomly selected nominal masses show that every single (intensive) odd-numbered mass peak has a corresponding lower abundant even-numbered mass peak higher in one m/z – the exact mass space of 1.0034 Da is thereby attributed as its ^{13}C isotopic peak (olive arrows). Still less intense are the mostly one-nitrogen-containing peaks in hair ice. This result shows the same qualitative

tendency like our first organic analyses in terms of organic nitrogen to organic carbon ratio of only approximately $0.014 : 1$ – but under the consideration that complex ESI spectra are never quantitative because of quench effects of easily to poorly ionizing substances.

We developed a post processing to formula assignment of all compounds based on Scilab routines. The resulting mass lists are transformed to Excel Tables for sorting and/or preparation of graphical figures describing the characteristics of DOM. The *van Krevelen diagram* – H/C vs. O/C for all detected compounds – is mostly used to classify the compounds in terms of polarity and aromaticity. Furthermore, Sleighter and Hatcher (2007) and Hockaday et al. (2007) published ranges of biogeochemically important compound classes in such plots. The [O/C, H/C] values found for melted hair ice range between [0.40–0.72,1.52–0.95], correspond to the Sleighter's lignin and tannin ranges, indicated by ellipses in the van Krevelen plot of hair ice and are presented in Fig. 14.

4.3 Discussion

Mass spectrometry has shown that hair-ice water contains fragments of lignin and tannin. Lignin is a main ingredient (20–30 % of dry mass) of wood, stabilising the cells against compression. In contrast to cellulose, it is more difficult to decompose. Lignin is indigestible by animal enzymes; only some fungi (causing white rot) and bacteria are able to secrete lignase and thus to biodegrade the polymer. Lignin is an irregular biopolymer with molecular mass in excess of 10 000 Da, consisting of various types of substructure, that are repeated in a haphazard manner. Similarly irregular are tannins with molecular weights between 500 and 3000 Da and up to 20 000 Da (proanthocyanidins). Lignin and tannin consist of hydrophobic skeletal structures with numerous polar functional groups. The hydrophobic lignin/tannin macromolecules may act as crystallisation nuclei.

The lignin and tannin classification was obtained by atomic ratios, shown in Fig. 14. By the application of electrospray, the most gentle mass spectrometric ionisation

technique, covalent bonds stay intact whereas the weaker ones (Van der Waals and electrostatic, respectively) are destroyed. Consequently, molecules in the mass range between 200 and 800 Da result like in general for fulvic acids. This means that we are unable to distinguish between the original lignin/tannin macromolecules and their (partial) degradation products.

We also detected sulphur- and nitrogen-containing compounds, but with very small intensities in contrast to the CHO peaks and in accordance with our TOC and TN measurements. We assume that we detected marginal leftovers from the total degradation of more easily degradable (and nutritious) wood compounds, like proteins, on their way to mineralization. This would explain the significant ammonium concentration in melted hair ice.

5 Conclusions

Our investigations shed light on the mystery of hair ice that can grow on certain branches of dead wood. The formation is tied to a winter-active fungus. Comparatively dense mycelium was observed in the superficial wood cells of hair-ice bearing sections of the investigated branches. If the fungus activity is stopped, either by a fungicide or by hot water, the production of hair ice ceases as well.

Hair-ice shape and direction are influenced by the geometry at the mouth of the wood rays where the hairs are formed when the water is freezing. Most surprising is the extreme ratio of hair diameter to hair length on the order of $1 : 10\,000$. In spite of the fact that surface tension tries to reduce this ratio, the shape is maintained over many hours, and sometimes several days at temperatures close to the melting point. A recrystallisation inhibitor must be responsible for stabilising the hairs. It may be contained in the thread-forming fibre that appears when hair ice starts to melt, and it could be related to lignin, the main organic component that we found.

With respect to the origin of water, hair ice is a basicryogen, meaning that the ice originates from water in a porous substrate, in our case, the wood. Inside the

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substrate, the melting point is lowered by the Gibbs–Thomson effect. When the external temperature is sufficiently low, water freezes on suitable nuclei at the substrate surface and transfers latent heat of fusion to the substrate. Once ice nuclei have formed, ice segregation starts to extract additional water from the substrate, leading to the growth
5 of ice and to the dehydration of the substrate. We found that this effect occurs under the same conditions with similar freezing rates in wood with and without hair-ice production.

The fungus provides decomposed lignin and tannin as organic materials. That they may act as recrystallisation inhibitors is indicated by properties found for ligninsulfonate (waste of cellulose production) in Sandermann and Dehn (1951) to delay the hardening
10 of cement.

The fungus activity plays a minor role with regard to the rate and amount of ice formation. Hair-ice branches with active and with killed fungus undergo similar temperature curves, indicating that the freezing rates are very similar and that fungal metabolism is too weak to cause a measurable temperature enhancement. The
15 difference must be in shaping the ice. Whereas the untreated branches produce hair ice, the heat-treated ones produce crusty ice sticking to the wood surface. Although the fungus effect is still a mystery, it must be directly related to the hair-ice shape because the suppression of hair-ice growth acts immediately after the fungus activity is stopped.

Our findings not only confirm Wegener's hypothesis that fungal activity plays an
20 important role, but they unravel the mystery about the whitish covering on hair-ice producing wood, described by Wegener (1918) and Wagner (2005). There is clear evidence to suggest a causal relationship between the fungus, *Exidiopsis effusa*, and the growth of hair ice. This fungus is known to cause white rot. Indeed, the brightness of hair-ice wood increases with age, and its dry density decreases more and more,
25 indicating wood decomposition. The chemical analysis confirms and specifies the degradation. Explaining further details of the connection between the fungus and hair ice will be tasks for future research.

Author contributions. C. Mätzler has been working (in collaboration with G. Wagner) since several years on hair ice, its observation in nature, reproduction and the test of a fungus

hypothesis. In the presented paper he is mainly responsible for the physical measurements and interpretations, described in Sect. 3. G. Preuss investigated the fungi and the wood samples by microscopic techniques, described in Sect. 2.2 leading to the identification of Ee. D. Hofmann performed and interpreted the chemical analyses, presented in Sect. 4. The paper is a strong
5   collaboration of scientists different fields to one object with collective discussions.

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Table 1. Hair-ice bearing wood species, range of diameters and identified fungal species. Please explain why is the abbreviation Ee in bold.

Fam.	Species (in brackets, number of finds)	Diameter (cm)	Fungi
Aceraceae	*Acer pseudoplatanus* (4)	1.3–4.8	**Ee**
Adoxaceae	*Sambucus nigra* (3)	1.5–2.5	**Ee**
Betulaceae	*Betula pendula* (3)	0.5–13.0	D, Dd, Dst, E, **Ee**, Hm, Sch
	Carpinus betulus (15)		
	Corylus avellana (16)		
	Alnus glutinosa (14)		
Fagaceae	*Fagus sylvatica* (32)	0.5–18.0	Dd, **Ee**, Ff, Hf, Sch
	Quercus spec. (12, *robur*/*petraea*/bastards)		
Rosaceae	*Prunus avium* (2)	0.6–6.0	Dd, E, **Ee**, Pc, Tm
	Sorbus aucuparia (18)		

D = *Dacrymyces spec.*, Dd = *Diatrype disciforme*, Dst = *Diatrype stigma*, E = *Exidia spec.*, Ee = *Exidiopsis effusa*, Ff = *Fomes fomentaria*, Hf = *Hypoxylon fragiforme*, Hm = *Hypoxylon multiforme*, Sch = *Schizopora paradoxa*, Pc = *Pycnoporus cinnabaria*, Tm = *Tremella mesenterica*.

Figure 1. Hair ice on a stem of dead beech wood (26 December 2009; Moosseedorf), hair length up to 10 cm.

Figure 2. Cross section of hair-ice producing beech branch (ø 22 mm) with radial wood rays.
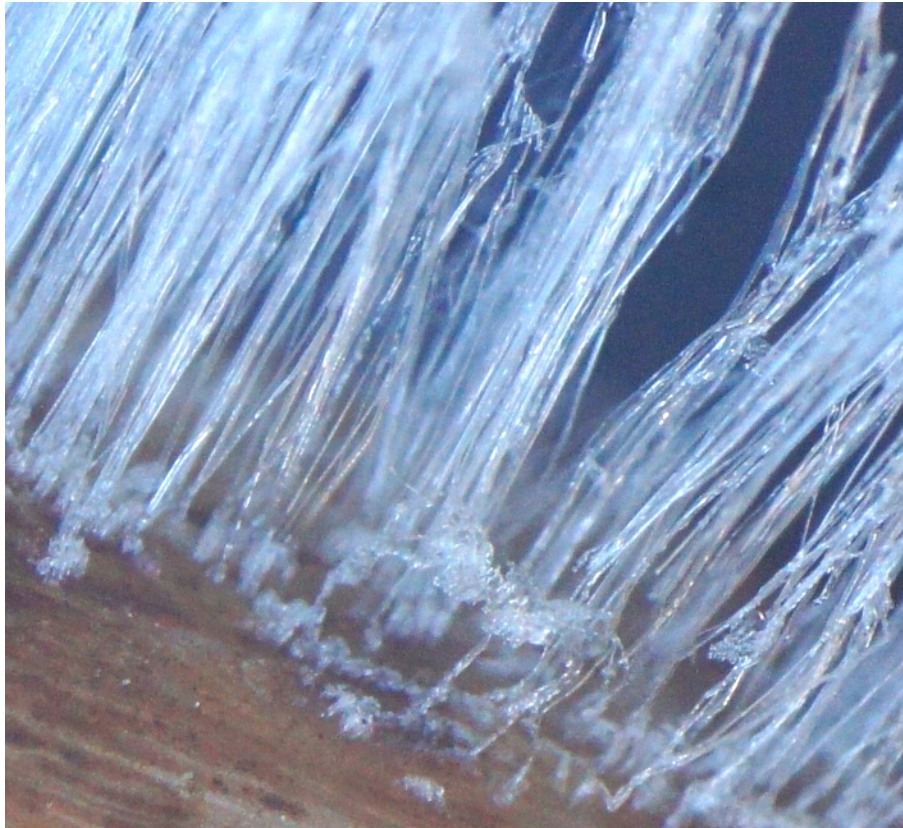
Figure 3. Enlargement of hair ice on beech wood, image width 3.2 mm.

Figure 4. Melting hair ice, showing tiny droplets on hardly visible strings.

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Figure 5. Reduction and suppression of hair-ice growth after fungicide treatment, durations from 0 to 120 min indicated.

Figure 6. Three wood samples, first with hair ice, later with fruiting body of *Exidiopsis effusa* (Ee): *Corylus avellana* (ø 3.8 cm) on 29 January 2012 **(a)** and 15 March 2012 **(b)**, *Carpinus betulus* (ø 3.2 cm) on 25 January 2012 **(c)** and 7 March 2012 **(d)**, *Fagus sylvatica* (ø 4.9 cm) on 3 December 2013 **(e)** and 11 January 2014 (**f**, branch rotated by about 180˚).
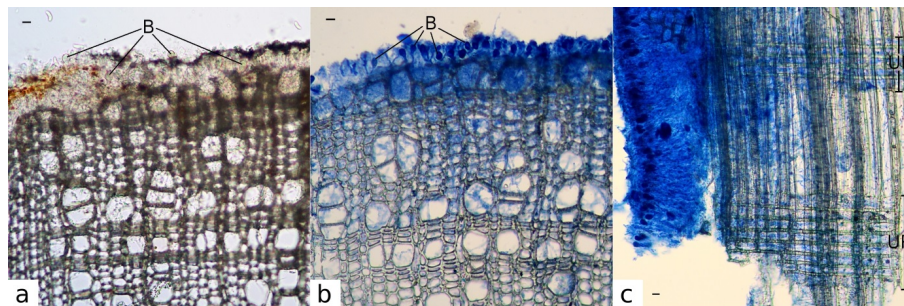
Figure 7. Microscopic views of a hair-ice wood sample (*Alnus glutinosa* twig, ⌀ 9 mm) with the fruiting body of Ee. In pictures **(b)** and **(c)** the fungal hyphae and basidia were coloured by "cotton blue" (scale bar 10 μm, B = basidium, UR = uniseriate ray). **(a)** and **(b)** Cross sections of an *Alnus glutinosa* twig with a thin fruiting body of Ee. The wood vessels are shown in cross section. **(c)** Radial section of the *Alnus glutinosa* twig with a thick fruiting body of Ee showing two uniseriate rays in longitudinal section. The wood vessels are cut longitudinally, too.

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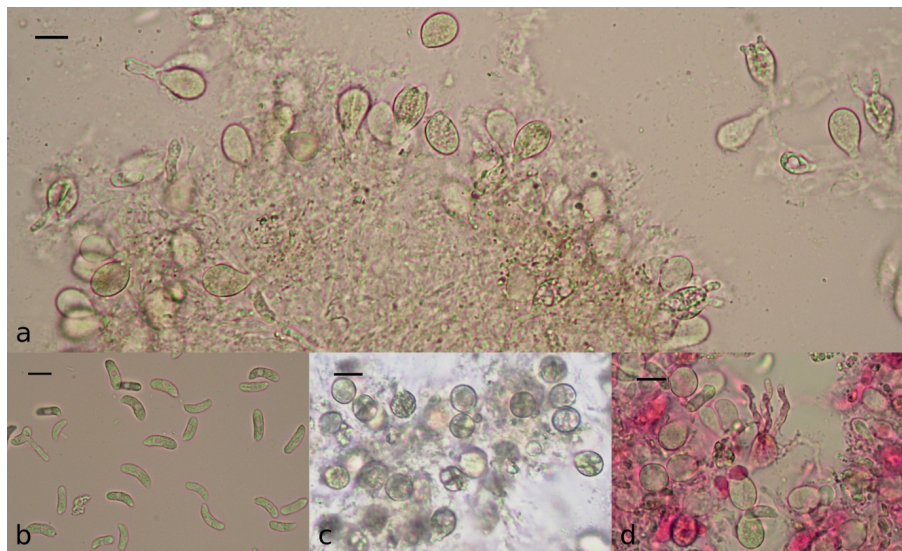
Figure 8. Microscopic characteristics of the fruiting body of Ee (scale bar: 10 μm). **(a)** Fruiting body of Ee showing longitudinal-divided basidia with four sterigmata (squash mount); **(b)** spores of Ee, one is germinating; **(c)** 4-septate basidia in cross-sectional view (sqash mount); **(d)** basidium with four long sterigmata (squash mount, coloured by Phloxin B).

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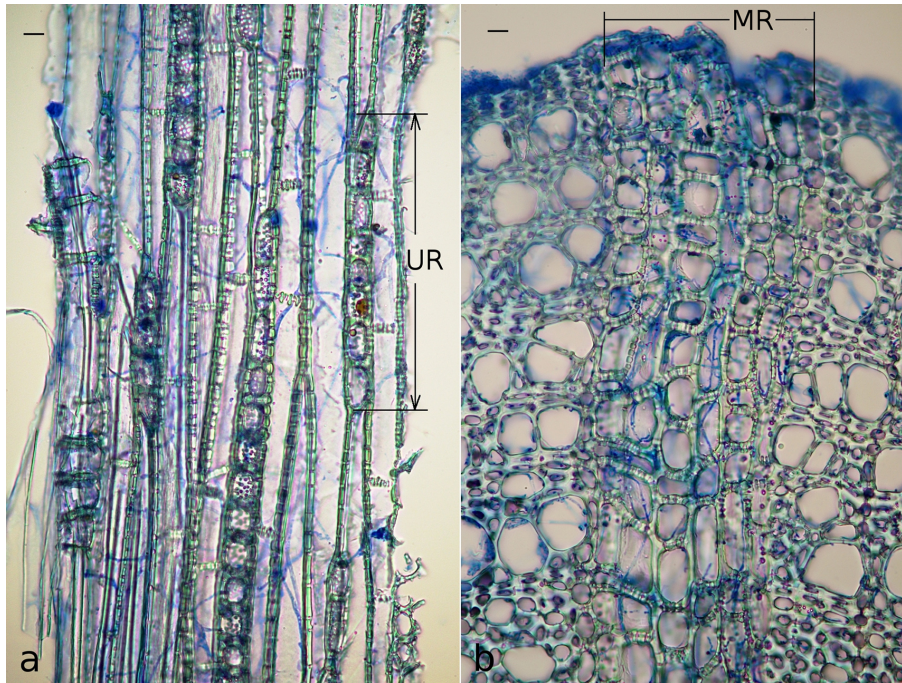
Figure 9. Microscopic view of hair-ice wood showing fungal hyphae coloured by "cotton blue" within the wood tissue. The varying thickness of the fungus hyphae was caused by the colouring procedure (scale bar: 10 µm, UR = uniseriate ray, MR = multiseriate ray). **(a)** Tangential section (similar to the look on the surface) of an *Alnus glutinosa* twig (ø 8 mm), that was partly covered by the fruiting body of Ee. Several uniseriate rays are shown in cross section, the wood vessels are cut longitudinally. **(b)** Cross section of a *Fagus sylvatica* twig (ø 6 mm) bearing hair ice, that had not yet developed the fungal fruiting body of Ee. The wood vessels are shown in cross section, one multiseriate ray is cut longitudinally.

Figure 10. Situation in the morning of 15 January 2012: heat-treated piece (1, bottom right), piece (2, top) with abundant hair ice, air temperature sensor (3, bottom left) with red insulation.
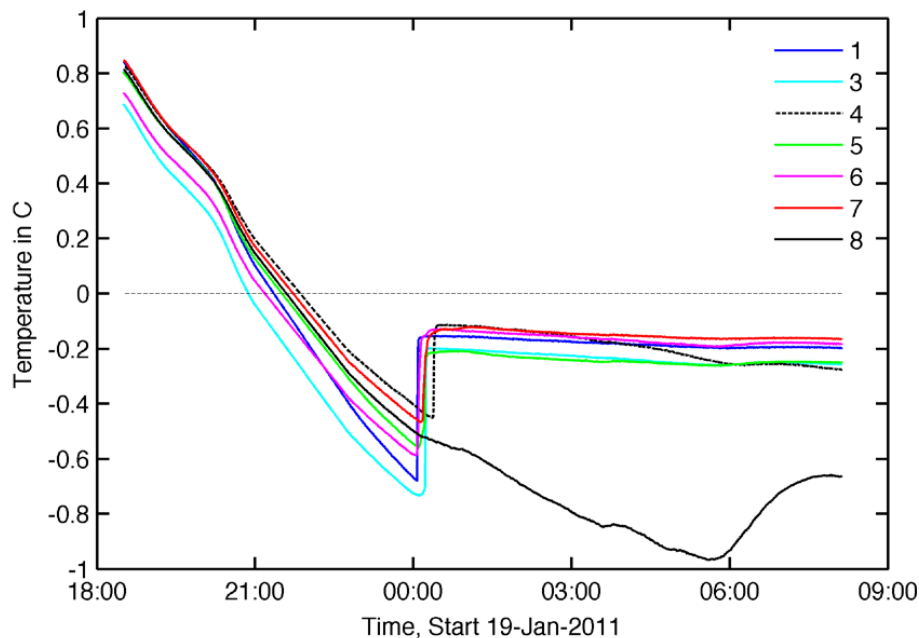
Figure 11. Temperature variation during the first experiment: beech-wood samples (1, 3, 4, 5, 6, 7) with growing hair ice, and dry hazel wood (8) as passive tracer of ambient temperature.
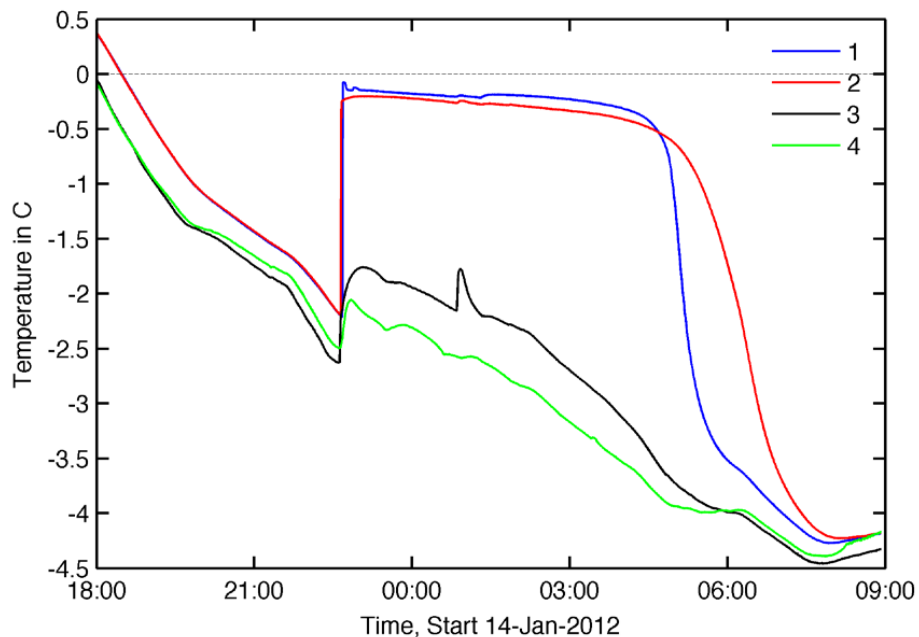
Figure 12. Temperature variation during the second experiment: heat-treated piece (1), untreated piece (2) with hair ice, air temperature close to wood samples (3), and further away (4).

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Figure 13. Detail of a high-resolution mass spectrum of melted hair ice.

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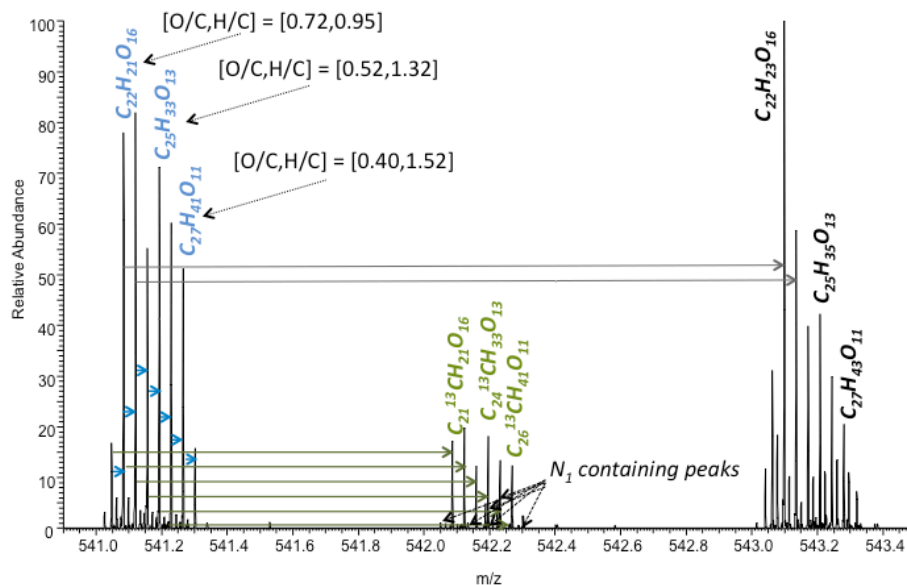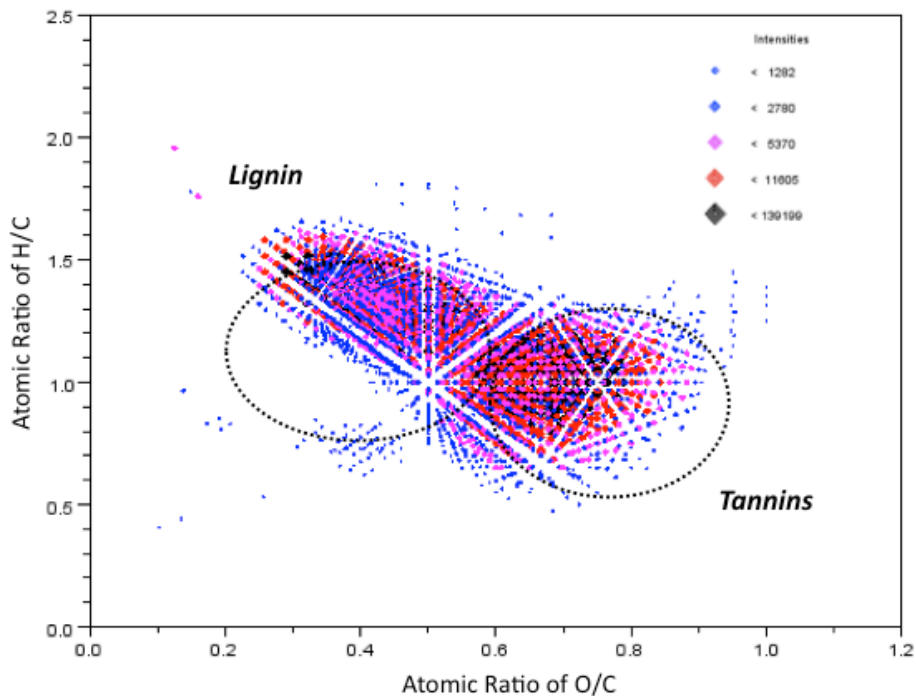Printer-friendly Version

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Figure 14. Van Krevelen Plot of the CHO compounds of hair ice (for peak intensities > 600 counts), underlayed with ellipses, indicating typical ranges selected biopolymer components, adapted from Sleighter and Hatcher (2007).

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