Links Between Heathland Fungal Biomass Mineralization, Melanization, and Hydrophobicity

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Abstract:	Comprehending the decomposition process is crucial for our understanding of the mechanisms of C sequestration in soils. The decomposition of plant biomass has been extensively studied. It revealed that extrinsic biomass properties, that restrict its access to decomposers, influence more the decomposition than intrinsic ones, that are only related to its chemical structure. Fungal biomass has been much less investigated, even though it contributes to a large extent to soil organic matter, and is characterized by specific biochemical properties. In this study, we investigated to which extent the decomposition of heathland fungal biomass was effected by its hydrophobicity (extrinsic property) and melanin content (intrinsic property). We hypothesized that, as

	for plant biomass, hydrophobicity would have a higher impact on decomposition than melanin content. Mineralization was determined as mineralization of Soil Organic Carbon (SOC) into CO2 by headspace-GC/MS after inoculation by a heathland soil microbial community. Results show that decomposition was not affected by hydrophobicity, but was negatively correlated with melanin content. We argue that it may indicate that either melanin content is both an intrinsic and extrinsic property, or that some soil decomposers evolved the ability to use surfactants to access to hydrophobic biomass. In the latter case, biomass hydrophobicity should not be considered as a crucial extrinsic factor. We also explored the ecology of decomposition, melanin content and hydrophobicity among heathland soil fungal guilds. Ascomycete black yeasts had the highest melanin content, and hyaline Basidiomycete yeasts the lowest. Hydrophobicity was an all-or-nothing trait, with most isolates being hydrophobic.
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	Christopher W Fernandez cwfernan@umn.edu Pr. Fernandez has been investigating similar hypotheses in mycorrhizal fungal biomass.
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Links between heathland fungal biomass mineralization, melanization and hydrophobicity

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Abstract
Comprehending the decomposition process is crucial for our understanding of the mechanisms of carbon (C) sequestration in soils. The decomposition of plant biomass has been extensively studied. It revealed that extrinsic biomass properties, that restrict its access to decomposers, influence more the decomposition than intrinsic ones, that are only related to its chemical structure. Fungal biomass has been much less investigated, even though it contributes to a large extent to soil organic matter, and is characterized by specific biochemical properties. In this study, we investigated to which extent the decomposition of heathland fungal biomass was effected by its hydrophobicity (extrinsic property) and melanin content (intrinsic property). We hypothesized that, as for plant biomass, hydrophobicity would have a higher impact on decomposition than melanin content. Mineralization was determined as mineralization of Soil Organic Carbon (SOC) into CO_2 by headspace-GC/MS after inoculation by a heathland soil microbial community. Results show that decomposition was not affected by hydrophobicity, but was negatively correlated with melanin content. We argue that it may indicate that either melanin content is both an intrinsic and extrinsic property, or that some soil decomposers evolved the ability to use surfactants to access to hydrophobic biomass. In the latter case, biomass hydrophobicity should not be considered as a crucial extrinsic factor. We also explored the ecology of decomposition, melanin content and hydrophobicity among heathland soil fungal guilds. Ascomycete black yeasts had the highest melanin content, and hyaline Basidiomycete yeasts the lowest. Hydrophobicity was an all-or-nothing trait, with most isolates being hydrophobic.

Introduction

Every year, the estimated increase of the atmospheric CO_2 pool is about $3.3 \cdot 10^9$ Tons, to a large extent due to fossil fuel burning and land-use change [1]. Observations and estimations at the global scale indicate that terrestrial ecosystems actually affect this pool in a variable manner, depending on the years, going from a -0.9 sink to a $+0.5 \cdot 10^9$ Tons/year source [2]. The outcome depends on the balance between C input rate through Net Primary Productivity (NPP), and C output rate by decomposition through heterotrophic respiration of the dead biomass into CO_2. Therefore, in the last 20 years, many investigations were attempting to unravel which factors are regulating NPP and decomposition in terrestrial ecosystems, driving soil biologists to focus on a better understanding of the decomposition process, and, in particular, why is it slower in some ecosystems than others. A recently emerging view is that Soil Organic Carbon (SOC) decomposition is, at equivalent environmental conditions, influenced by its physico-chemical accessibility, and to a lesser extent by its chemical composition *per se* [3]. Indeed, compounds initially thought to be recalcitrant (such as lignin) were shown to have a faster turnover than expected, while the opposite was found for some small, more labile compounds [3-5]. Consequently, decomposition of SOC should be primarily retarded by conditions restricting its access, and only secondarily by its chemical structure.

The SOC originates from plant and root litter, root exudates, and microbial biomass. There is increasing evidence that microbes do contribute to a major part of SOC [3-5]. Indeed, fungi produce large amounts of biomass in soils, at the scale of 50 to 1 000 kg/Ha [6-8], for mycorrhizal fungi, and 20-80 mg/kg of soil [9] or 1000 kg/Ha for saprophytes [10]. Despite its abundance in soils, however, the fungal biomass decomposition has received much less attention than plant litter, from which it differs by both the nature of structural molecules and physico-chemical accessibility. This is especially the case in heathland ecosystems, where the well-developed soil organic layer has been mostly assumed to be of plant origin, but where fungal biomass is also high [11], and is expected to better resist decomposition [12]. This biomass can contain a fraction of structural compounds known to decompose slowly in soils. The most widespread of these molecules are melanins, which have a polyphenolic structure. Many fungi synthesize melanins to make their biomass resistant to a range of chemical or physical stresses [13]. Melanized fungi are frequent in the heathland ecosystem [12]. Consequently, at optimal environmental conditions, melanin content is often negatively correlated with the rate of decomposition. This has already been observed among fungi associated with forest trees [14]. Melanin content may therefore be considered an intrinsic property of the fungal biomass: it is a chemical property that does not affect the influence of environmental factors on decomposition [3].

However, some extrinsic properties of the biomass, which define how it interacts with the environment, do govern its stability in soils as well, and this to a much larger extent than chemical structure *per se* [3]. Solubility, for example, is one of the most critical factors limiting decomposition [15]. Indeed, most of the decomposition process relies on hydrolytic enzymes, or on enzymatic reaction steps requiring the substrate to be solubilized. Hence, the higher the hydrophobicity of fungal biomass, the slower should be its decomposition rate. However, this hypothesis has not been verified. The extent to which hydrophobicity influences fungal biomass decomposition has not been compared either with intrinsic properties such as melanin content.

Our aims were therefore twofold. First, we wanted to investigate how two key properties, one intrinsic (based on molecular structure: melanin content), and the other extrinsic (based on how the biomass interacts with its environment: hydrophobicity), were influencing the decomposition rate of

dead fungal biomass. Our hypothesis was that these two properties were both significantly and negatively correlated with decomposition, but that hydrophobicity had more influence than melanin content, because it was restricting the access of decomposers to fungal necromass. We choose to test this hypothesis using fungal strains isolated from a dry heathland soil, where fungal biomass decomposition is poorly characterized while it is likely to be a major contributor to SOC. Second, we wanted to explore further how the properties of fungal biomass varied between different fungal species and functional groups within the same ecosystem; whereby we hypothesized that these properties significantly differ between functional groups.

Material & Methods

Sampling site

This study was conducted in a dry heathland in the Nationaal Park Hoge Kempen (Belgium, 50°59'0.57"N 5°37'42.9"E). The area has a temperate climate, with an annual mean precipitation of 774 mm and a mean air temperature of 9.8^{o}C. The dominant soil types are albic podzols and brunic-dystric arenosols (https://dov.vlaanderen.be/dovweb/html/index.html). In autumn 2016, a sampling plot of 50 by 60 meters was established in a dry heathland-dominated area of uniform vegetation and flat topography (50°59'01.9"N 5°37'39.8"E). The vegetation was six to seven years old (30-50cm height) and consisted of about 80 % *Calluna vulgaris*, 15 % *Molinia caerulea*, and 5 % bare soil, with lichens and mosses present under the canopy.

Fungal species isolation

We tried to isolate as many species as possible from the heathland soil. For this purpose, we used a wide range of isolation methods (see SI). In total, 207 strains were isolated using all these procedures.

Fungal species identification

In order to determine melanin content, hydrophobicity and mineralization rate of our isolated soil fungi, we needed to scale down the number of screened isolates to a manageable extent. Hence, we classified the 207 isolates into 26 different groups based on their morphological characteristics (growth rate, color, sporulation, growth pattern). One isolate of each of the 26 morphological groups was selected for this study. We identified the species by sequencing the isolate's ITS region. For this purpose, we collected a plug of actively growing mycelium (5mm diameter, 5mm deep), and inoculated it either into a 12-well plate containing 2ml of liquid Czapek-Dox medium, or a 250ml flask containing 100ml of liquid Czapek-Dox medium, and incubated for three days (fast-growing species) to six weeks (slow-growing). The mycelial balls formed were then ground in liquid nitrogen using a mortar and a pestle, and DNA was extracted on this mycelial powder with the MoBio Powersoil DNA isolation kit. The ITS region was amplified using the ITS1f-ITS4 primers [16]. The PCR reactions were performed in a C1000 Touch Thermal Cycler (BioRad) in a mix composed of 10mM of each primer, 2mM $MgSO_4$, 0.2mM dNTP mix, and 1 unit of Invitrogen Platinum Taq DNA polymerase High Fidelity PCR enzyme (Invitrogen Life Technologies, http://www.thermofisher.com). The PCR reactions were done using the following parameters: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72 C for 1 min, with a final extension at 72°C for 10 min. Amplification success was checked in a 1 % agarose electrophoresis gel in 1 % TBE (Tris-Buffer-EDTA) stained with GelRed. When amplification was not successful, we diluted the DNA template 20 times in TE buffer and added 20µl of mM BSA solution to the DNA sample

before amplification. Amplicons were then sent to Macrogen for Sanger sequencing. The sequences were trimmed at both their 3' and 5' ends based on the visual inspection of electropherograms (poorly resolved peaks were removed). The sequences were then blasted on the NCBI database (Blastn: https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). The isolate was assigned to the taxon that appeared among the top hits with the highest e-value. In case several taxa had the same top e-value, we assigned the isolate to the one with the longest match. Based on literature, each isolate was then assigned to a group of similar ecology: black yeasts, hyaline yeasts, saprotroph, mycorrhizal fungi or endophytes. Data on species assignment are summarized in Table 1.

Mycelial hydrophobicity

To measure mycelial hydrophobicity, we designed a device consisting of a microscope slide covered by a thin uniform layer of Czapek Dox (CD) agar (45.34 g.l-1 Czapek-Dox medium, 5 g.l-1 Yeast extract), laying in a Petri dish filled with 20ml of water agar medium (to avoid desiccation of the thin CD layer). The device was prepared as follows. First, the microscope slide was sterilized by dipping in 96% ethanol and flaming on the Bunsen burner; then 1ml of hot CD agar was poured onto its surface using a 1ml micropipette, let to solidify for five minutes, and transferred to the sterile water agar plate. The 26 fungal isolates were grown for a week on CD agar plates. Then, an actively growing plug of mycelium (0.3mm in diameter) was transferred to the middle of the slide. We prepared four replicates of each isolate (hence 104 devices in total). Devices were then incubated at 23°C in the dark for three weeks, after which slides were covered with at least 1cm^2 of mycelium. The mycelial hydrophobicity was then assessed by measuring the contact angle of water droplets deposited at the mycelial surface. This was done via sessile drop shape analysis as performed by Chau et al. [17]. We slightly modified this protocol: six water droplets of 2 µl were pipetted from one edge of the slide to the other edge on both sides of the inoculation point. For six isolates (the two *Penicillium velutinum* and the four *Umbelopsis autotrophica* ones), we used 10 µl droplets instead, since smaller ones were repelled by the substrate when being pipetted and ended up falling from the mycelial surface. Analyses were carried out at The Institute for Materials Research (IMO-IMOMEC) of Hasselt University. Contact angles were calculated using ImageJ (http://rsb.info.nih.gov/ij/). Measurements of contact angles were obtained by using the Low Bond Axisymmetric Drop Shape Analysis Model (LB_ADSA) plug-in, developed by Stalder et al. [18] (http://bigwww.epfl.ch/demo/dropanalysis/).

Melanin content

Mycelial melanin content of the isolates was assessed using the protocol of Gadd and Griffiths [19]. One actively growing plug (3mm in diameter) of each isolate was placed in a new CD agar plate, covered by a cellophane sheet which was previously sterilized by autoclaving. The 104 Petri dishes (26 isolates times 4 replicates) were then incubated for four to five weeks, depending on the growth rate of each isolate, in order to obtain a sufficient amount of biomass to perform melanin extraction. After incubation, the mycelium was scraped off the cellophane surface with a sterile scalpel and homogenized in liquid nitrogen using a sterile mortar and pestle. The homogenized samples were transferred to 50 ml Falcon tubes, and kept at -72°C. Samples were freeze-dried overnight in a lyophilisator, and transferred to glass tubes. A 5ml solution of absolute ethanol was added to each tube, followed by heating in heating blocks at 60°C for 3 h. Next, samples were vortexed before being transferred to 15 ml Falcon tubes, and subsequently centrifuged for 10 min at 500 G. Supernatant was discarded and samples were again freeze-dried overnight in a lyophilizer, after which 1 ml of distilled water was added to the dried pellets, gently vortexing them before transferring them back

to glass tubes. Next, samples were resuspended in 1 ml 6 M HNO_3, and then placed in heating blocks at 75°C for three hours. 5 ml of distilled water was added to each sample. After vortexing gently, the resulting solution was transferred back to 15 ml Falcon tubes. Samples were again centrifuged (10 min, 500G), and the supernatant was discarded. Pellets were transferred back to glass tubes as described before. The resulting suspension was heated at 75°C for 20 min in 5 ml of 0.5 M NaOH, and filtered through grade 1 Whatman filter paper (Sigma). Melanin content was quantified by comparing the optical density of samples at 470 nm, with a standard curve generated using serial dilutions (0-40 mg/l) of synthetic melanin (Sigma), dissolved in 1 M NaOH.

Mineralization of C in fungal biomass

The mineralization of the fungal biomass was assessed by measuring CO_2 production by a soil microbial community growing on a nutrient solution containing mycelial biomass as the sole C source, in a similar way as McDowell et al. [20] in their Method 8, but replacing soil with mycelial biomass. As Fernandez et al. [21] showed that the mineralization rates of ectomycorrhizal fungal biomass also depend on N content, we used a nutrient solution, ensuring that mineralization would be only limited by C quality (adapted from method 8 of McDowell et al. [20]). Briefly, mycelial biomass was prepared as for the measurements of melanin content (growth in CD agar covered by a cellophane sheet and homogenization of the mycelium in liquid nitrogen, then storage of the biomass at -72°C). A soil microbial inoculum was obtained as follows: on January 24th 2017, eight topsoil samples (8cm diameter, 5cm deep) were taken, every 5 m along two transects parallel to the longest side of the plot (four cores per transect). Samples were acclimatized at room temperature for two weeks, due to collection in winter conditions. Afterwards, samples were sieved at 2mm, mixed thoroughly and pooled. One gram of this pooled soil sample was added to a 15ml Falcon tube, and mixed with 10 ml of sterile distilled water. The mixture was centrifuged for five minutes at 2000 rpm, and the supernatant was collected and used as heathland microbial soil inoculum. The nutrient solution contained 1.2 mM KCl, 0.5 mM $CaCl_2$, 0.5 mM KNO_3, 0.5 mM NH_4Cl, and 0.1 mM K_2HPO_4. A headspace vial was then filled with 5ml of distilled water, 50 µl of the nutrient solution, 50 µl of heathland microbial soil inoculum, and 7.5mg of homogenized, dry mycelial biomass, and sealed with an airtight cap. Negative controls were prepared by preparing three vials containing only distilled water, and three other containing distilled water, nutrient solution and soil inoculum, but no C source. After one week, the CO_2 concentration in the vial gas phase was measured by headspace-GC/MS.

Statistics

We tested if the mineralization rate could be explained by hydrophobicity or the melanin content of the mycelium as predictor variables using a linear model. The normality of each of the three variables was assessed using a Shapiro test (at a p=0.01 threshold); variables were transformed when possible to fit a normal distribution. The mineralization rate followed a normal distribution, as well as the log-transformed melanin content. Hydrophobicity could not be coerced to a normal distribution, and was instead separated into three categories: hydrophilic (contact angle=0 degrees, 16 data points), moderately hydrophobic (contact angle between 37 and 42 degrees, 8 data points), and hydrophobic (contact angle between 134 and 145 degrees, 101 data points). Statistical analyses were performed and figures were made using R [22].

Results

Mycelial hydrophobicity

In this experiment, we assessed the mycelial surface hydrophobicity of the 26 heathland soil fungal isolates using the sessile drop contact angle measurements. We expected that mycelial hydrophobicity would be, as most functional traits, either normally or inversely distributed. Results showed trimodal values instead, with three types of surfaces of separate hydrophobicity properties (Fig.1). Most isolates had a hydrophobic surface (angle between 135^{o} and 140^{o}). Only 6 of the 26 where hydrophilic to some degree. Two *Trichosporon porosum* isolates had a contact angle of 35-40^{o}. The three *Exophiala spp.* and one *Rhizoscyphus ericae* isolates were extremely hydrophilic, to the extent that a drop of water was immediately spread over the surface of the mycelium, leaving no angle to measure. Hence, we assigned to these measurements a value of 0^{o}. The three *Exophiala spp.* strains showed a differentiated mycelial surface, mostly covered by a smooth, highly hydrophilic basis that immediately absorbed moisture, on top of which sparse hydrophobic patches could be found (Fig.5, supplementary information).

Melanin content

This experiment was designed to measure the melanin content of all the 26 heathland soil fungal isolates, using the method of Gadd and Griffiths [19]. Since all strains displayed a large range of colorations, from pure white to totally black (Table 1), we expected melanin content to vary in the same proportions. Results showed that indeed there was a wide, two orders of magnitude range of melanin contents among the isolates, ranging from 1 to 170mg/g DW. Black yeasts had the highest melanin content (from 58 to 170 mg/l, Fig.2). One strain of *R. ericae* and one of *U. autotrophica* also displayed high melanin contents (above 50mg/g), while all other strains had low values (below 20mg/g). The four *Trichoderma viride* strains were all especially low in melanin (all below 5mg/g). When melanin contents were plotted against hydrophobicity, strains very clearly subdivided into four categories: melanized and hydrophilic (n=16), hyaline and moderately hydrophobic (n=8), hyaline and hydrophobic (n=76) and melanized and hydrophobic (n=4) (Fig.4, supplementary information). Most of the strains were therefore hyaline and hydrophobic. None of the hyaline strains were hydrophilic.

Mineralization of C in fungal biomass

The aim of this measurement was to assess the rate at which the C in the biomass of each isolate was decomposed into CO_2 by a local soil microbial community. As for melanin content and hydrophobicity, we expected that biomass mineralization rate would be normally or inversely distributed among soil fungal isolates. We found that all isolates underwent significant degradation within a week of inoculation (Fig.3), since all produced a CO_2 signal at least 300 times higher than the blank without C substrate (12.22 at least in the isolate samples against 0.04 for the blanks, too small to be visible on the Figure). The blanks were filled with ambient air, hence at least 400ppm CO_2 and 80% N_2. The negative control (nutrient solution, inoculum but no C source) showed that the inoculum itself was not significant as a C source (peak ratio=0.04). Results showed high diversity, both within and among species and functional groups. The amplitude of the differences was much lower than for melanin contents, though, with a factor 2 only between the slowest and the fastest mineralizing isolates. The isolates with the lowest degradation rate were: *Penidiella sp.*_100, *R. ericae*_106, *Saccharicola bicolor*_49, the two *P. velutinum*, as well as *U. autotrophica*_101. In opposite, *Trichoderma viride*_72, *Umbelopsis autotrophica*_32, *Trichoderma viride*_9, *Trichosporon porosum*_17 and *Trichosporon porosum*_15 had the highest mineralization rates. All functional

groups displayed very similar levels of degradability in average, so isolate identity accounted for most of the variance in this variable.

Relationship between melanin content, hydrophobicity and C mineralization rates

The aim of this experiment was to test the hypothesis that fungal biomass mineralization rate depended more on its surface hydrophobicity than on melanin content. Results showed that melanin content predicted decomposition of the fungal biomass, but surface hydrophobicity did not (Table 2, Fig. 6, supplementary information). Within each hydrophobicity category, there was no correlation between mineralization rate and contact angle values (data not shown). Considering the limited number of points, we could not test this relationship between functional groups. However, it was clear that despite high differences in melanin content and hydrophobicity, mineralization rates were similar between functional groups.

Discussion

We investigated to which extent the biomass of heathland soil fungi differed in mineralization rates, and if these rates were best explained by biomass hydrophobicity or melanin content. Results showed that mineralization rates were uninfluenced by hydrophobicity, but negatively correlated with melanin content. We also explored how these three parameters were related to fungal functional groups, and found that mineralization rates varied much more between isolates than between functional groups.

Relationship between C mineralization rate, melanization and hydrophobicity

Our hypothesis was that hydrophobicity is a parameter that defines how the fungal biomass interacts with the environment, by regulating access of hydrolytic enzymes to their substrate, and therefore should have a larger influence on its decomposition than its melanin content. In fact, our results did show the opposite. The more melanized the biomass, the slower its C was mineralized by a heathland soil microbial community, while hydrophobicity was not correlated with mineralization. Biomass melanization is a widespread character among fungi, and to understand the effects it can have on the mineralization rate, it may be useful to elaborate further on the physiological role of melanin. This polyphenolic compound deposits in the fungal cell walls where it complexes with proteins and carbohydrates [13]. Melanin bears many similarities in its structure with lignin or lignin building blocks; it is therefore itself a stable compound, that can be degraded only by fungal peroxidases produced by white-rot fungi [13]. As for lignin in plants, it can be considered an intrinsic property of fungal biomass. Our observations confirmed the hypothesis that melanization and decomposition rate were negatively correlated. However, this correlation was not always tight: several isolates were melanized but still decomposed fast (*Exophiala sp*._96, *Exophiala sp*._94), others hyaline and recalcitrant (*Saccharicola bicolor*_49). We expected that this variability would be explained by the hydrophobicity of the biomass, another factor that commonly hampers decomposition of organic molecules in soil [23]. Our results show, however, that hydrophobicity did not explain the observed mineralization. Hence, in our experiment, substrate accessibility for hydrolytic enzymes did not play a role in decomposition, while melanin content, an intrinsic property of SOC, significantly did. To explain such unexpected results, one could formulate the hypothesis that melanin is at the same time an intrinsic and extrinsic property of SOC, because it also regulates its accessibility. Indeed, melanin molecules have the property to bind large amounts of water (only 10mg of melanin "granules" -bodies of agglomerated pigments- able to bind 1ml of water [13]). By doing so, they

cause the cell wall to swell to a significant extent [21], especially considering that melanins can contribute up to 25% of the fungal dry biomass [21]. A thicker cell wall takes more time to be processed by cell-wall degrading enzymes [24], which retards all biomass decomposition. Moreover, in the same way as other polyphenols such as tannins, melanins can bind to proteins. This includes cell-wall degrading enzymes, where melanin binding potentially inhibits their activity [25]. Hence, melanin content is both an intrinsic and extrinsic parameter of fungal biomass, and its relative influence on decomposition rates may consequently be high. As for hydrophobicity, we cannot rule out either that some degrading organisms developed the ability to produce surfactants to improve their access to hydrophobic organic matter [26]. We did not verify surfactant production in our experiment, and do not know if this trait is widespread among the microflora in heathland soils. In such case, hydrophobicity should not be considered any more a crucial extrinsic factor for biomass decomposition in soils.

Ecology of fungal hydrophobicity and melanization in the heathland ecosystem

Mycelial melanin contents were in line with literature. Fernandez and Koide [21] reported mycelial contents ranging from 39 to 248 mg/g, though this was measured on ectomycorrhizal fungi. In the same paper, the highest values were measured on isolates of the black ascomycete *Cenococcum geophilum*, and the lowest on hyaline basidiomycete isolates. Even though Ascomycetes were more dominant in our soil samples, we found a similar trend. The black yeasts had the highest melanin content, and hyaline yeasts (belonging to the Basidiomycetes) were at the other end of the spectrum.

We found that hydrophobicity was an all-or-nothing trait among our isolates, most of them being very hydrophobic, and a few being very hydrophilic. The dominance of the hydrophobicity trait among isolates was expected, since it is often associated with a better water retention strategy [27], and as stated above this is a crucial trait in dry heathland soils. However, in the literature mycelial hydrophobicity displayed more gradual figures than what we measured [17]. The large proportion of hydrophobic strains probably originates from the fact that the dry heathland environment selects for hydrophobic species. Indeed hydrophobicity may provide better water retention in case of drought, and to some extent better resistance to flooding [27], two common stresses in the well-drained sandy soil of dry heathlands under a rainy Atlantic climate [28]. This does not explain, however, the very few numbers of moderately hydrophobic strains. Mycelial age should not have been a confounding factor in our experiment [29], since it was considered in the experimental setup: water droplets were placed at increasing distance from the colony age, creating an age gradient. Our results demonstrated that age did not affect at all hydrophobicity measurements. However, since the slide was covered with only a thin layer of agar medium, mycelium growing atop had only access to a limited amount of nutrients; most of our strains may therefore have been well within their idiophase growth, which is known to favor aerial hyphae formation [29]. We would therefore turn moderately hydrophobic species into hydrophobic ones because of nutrient starvation during the experiment. In this case, however, it is not clear why fast-growing strains such as *T. porosum* did not show higher hydrophobicity, since they must have experienced nutrient starvation earlier than the slow-growing ones.

Finally, it was striking that most hydrophilic strains were very melanized. This seemed to be due to heterogeneity of mycelial surfaces, with hydrophobic patches surrounded by a very hydrophilic matrix. This latter substrate did not appear to be age-related, nor caused by any stress. It may be an artifact due to culture conditions, but also reveal its natural habitus in the soil. Such heterogeneity is

sometimes observed in other fungal cultures [27]. It has been interpreted as a way for the fungus to balance between substrate exploitation (hydrophilic), nutrient translocation and stress resistance (hydrophobic). In any case, this heterogeneity did not appear to affect mineralization rate of the isolates.

Conflict of Interest: The authors declare that they have no conflict of interest.

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436

437 **Table and Figure legends**

438

439 **Table 1.** Description of the isolates.

440

441 **Table 2.** Results of the linear model describing fungal biomass decomposition depending on surface
442 hydrophobicity and melanin content. Mineralization was expressed as CO_2 production by a heathland
443 soil inoculum growing on the isolate biomass as the sole C source, surface hydrophobicity as contact
444 angle of the isolate mycelium, and melanin content as the amount of melanin per gram of fungal
445 biomass.

446

447 **Fig.1** Hydrophobicity of the mycelial surface of the 26 heathland soil isolates (degrees).
448 Hydrophobicity was measured as water droplet contact angle using the sessile drop analysis. Fungal
449 mycelium was grown for one to four weeks on the surface of a sterile microscope slide covered by a
450 thin layer of agar medium, placed on a water agar surface in a petri dish (to avoid desiccation).
451 Results show the average and SD value of four slides per isolate. In each slide, six (exceptionally four
452 for strain 101, where the mycelium area was too small to put six droplets) drops were measured. The
453 higher the angle, the higher the hydrophobicity. Bars represent standard deviation between the four
454 replicates (slides)

455

456 **Fig.2** Melanin content of the mycelium of the 26 heathland soil isolates. Melanin content is
457 expressed as mg melanin per gram of mycelium DW. All soil isolates were grown on Czapek-Dox agar
458 medium in quadruplicate for four to five weeks, after which mycelium was collected, ground and

freeze-dried, and used for melanin extraction. Bars represent standard deviation between the four replicates

Fig.3 Carbon mineralization rate of the biomass of the 26 heathland soil isolates. Carbon mineralization rate was assessed by measuring CO_2 production after one week by a soil microbial community, using 7.5mg of dried fungal biomass as the only C source (and provided with the other nutrients). The biomass of each isolate has been quadruplicated. Bars represent standard deviation between the four replicates

Figure 1

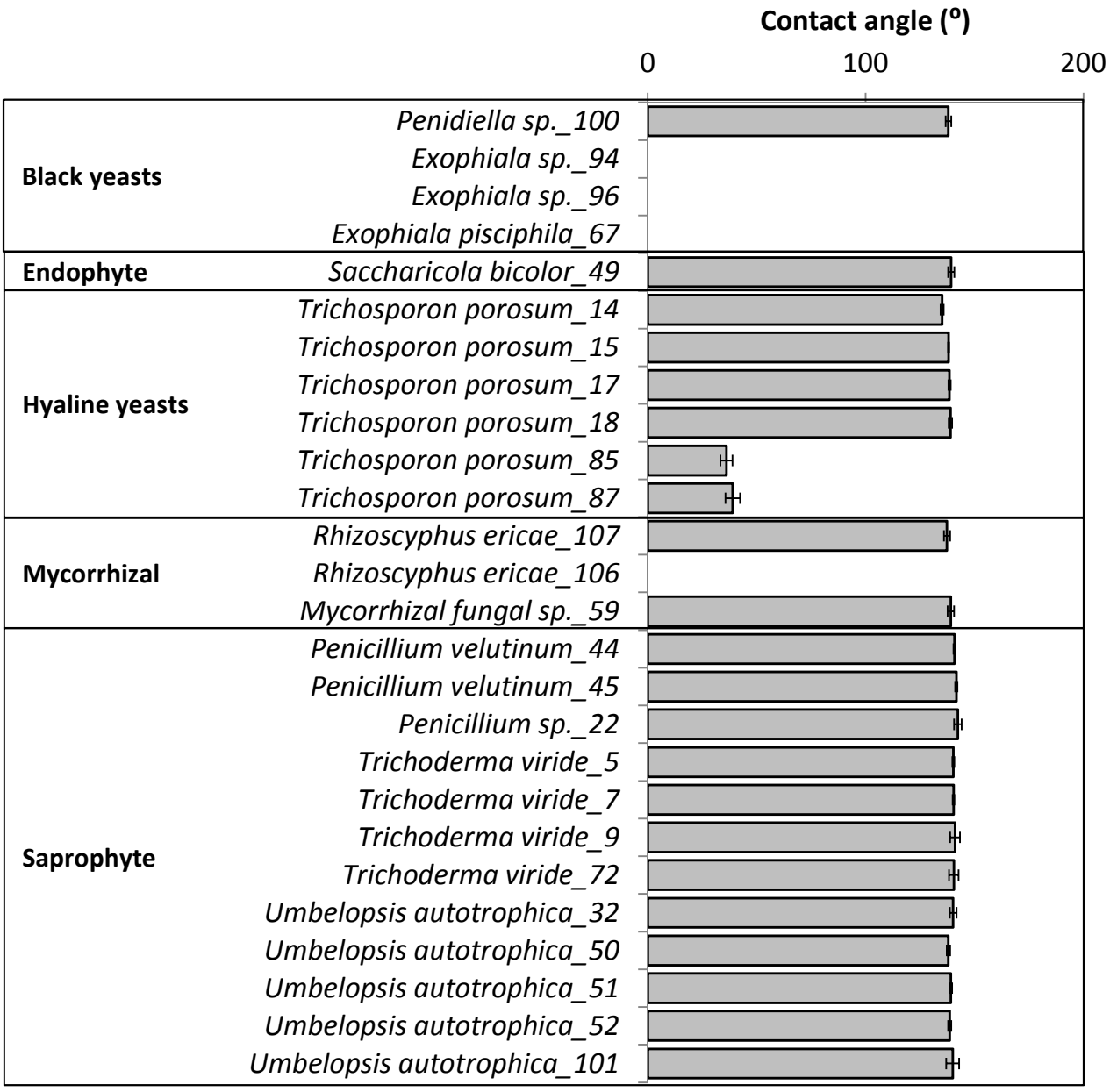
Contact angle (º)
0
100
200
Black yeasts
Penidiella sp._100
Exophiala sp._94
Exophiala sp._96
Exophiala pisciphila_67
Endophyte
Saccharicola bicolor_49
Hyaline yeasts
Trichosporon porosum_14
Trichosporon porosum_15
Trichosporon porosum_17
Trichosporon porosum_18
Trichosporon porosum_85
Trichosporon porosum_87
Mycorrhizal
Rhizoscyphus ericae_107
Rhizoscyphus ericae_106
Mycorrhizal fungal sp._59
Saprophyte
Penicillium velutinum_44
Penicillium velutinum_45
Penicillium sp._22
Trichoderma viride_5
Trichoderma viride_7
Trichoderma viride_9
Trichoderma viride_72
Umbelopsis autotrophica_32
Umbelopsis autotrophica_50
Umbelopsis autotrophica_51
Umbelopsis autotrophica_52
Umbelopsis autotrophica_101

Figure 2

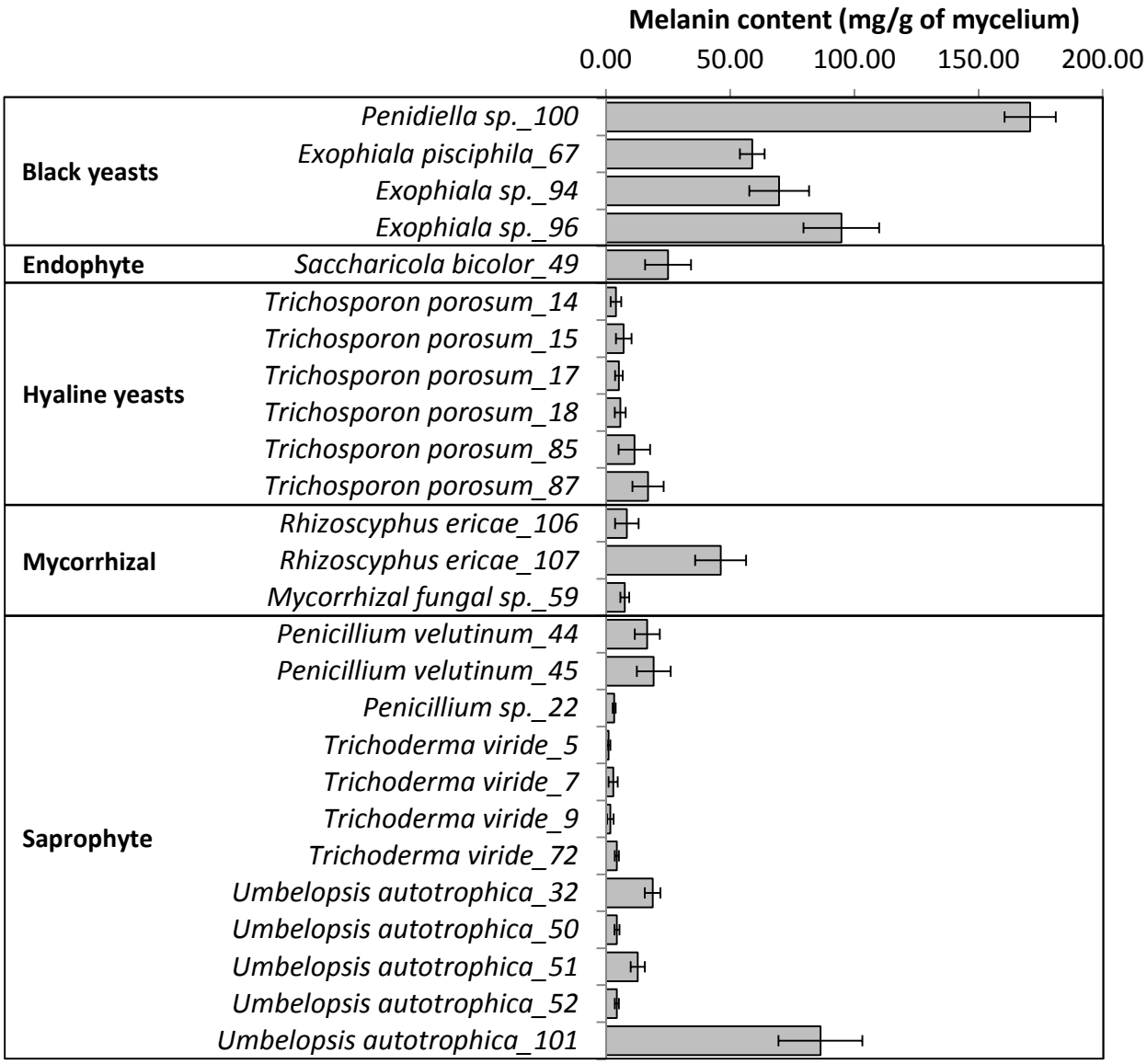
Melanin content (mg/g of mycelium)
0.00
50.00
100.00
150.00
200.00
Black yeasts
Penidiella sp._100
Exophiala pisciphila_67
Exophiala sp._94
Exophiala sp._96
Endophyte
Saccharicola bicolor_49
Hyaline yeasts
Trichosporon porosum_14
Trichosporon porosum_15
Trichosporon porosum_17
Trichosporon porosum_18
Trichosporon porosum_85
Trichosporon porosum_87
Mycorrhizal
Rhizoscyphus ericae_106
Rhizoscyphus ericae_107
Mycorrhizal fungal sp._59
Saprophyte
Penicillium velutinum_44
Penicillium velutinum_45
Penicillium sp._22
Trichoderma viride_5
Trichoderma viride_7
Trichoderma viride_9
Trichoderma viride_72
Umbelopsis autotrophica_32
Umbelopsis autotrophica_50
Umbelopsis autotrophica_51
Umbelopsis autotrophica_52
Umbelopsis autotrophica_101

Figure 3

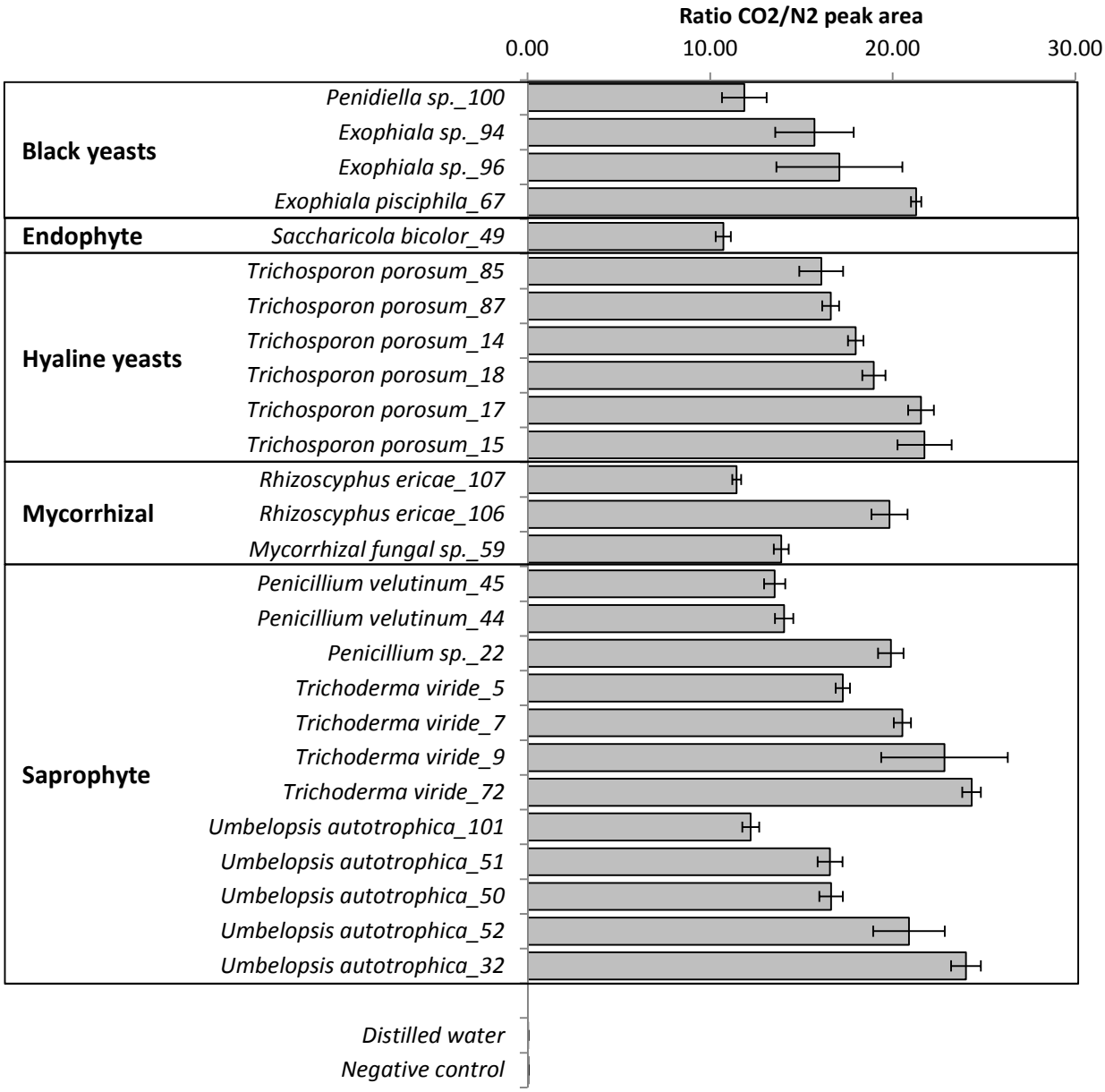
Ratio CO2/N2 peak area
0.00
10.00
20.00
30.00
Black yeasts
Penidiella sp._100
Exophiala sp._94
Exophiala sp._96
Exophiala pisciphila_67
Endophyte
Saccharicola bicolor_49
Hyaline yeasts
Trichosporon porosum_85
Trichosporon porosum_87
Trichosporon porosum_14
Trichosporon porosum_18
Trichosporon porosum_17
Trichosporon porosum_15
Mycorrhizal
Rhizoscyphus ericae_107
Rhizoscyphus ericae_106
Mycorrhizal fungal sp._59
Saprophyte
Penicillium velutinum_45
Penicillium velutinum_44
Penicillium sp._22
Trichoderma viride_5
Trichoderma viride_7
Trichoderma viride_9
Trichoderma viride_72
Umbelopsis autotrophica_101
Umbelopsis autotrophica_51
Umbelopsis autotrophica_50
Umbelopsis autotrophica_52
Umbelopsis autotrophica_32
Distilled water
Negative control

Table 1

Strain	Species name	Functional group	Color	Growth rate	Taxonomic group	Isolation medium	Isolation method
100	*Penidiella sp.*	Black yeast		Slow	Ascomycota	MMN	Immersion tube (dilution plate)
94	*Exophiala equina*	Black yeast		Slow	Ascomycota	Pectin-agar	Immersion tube (dilution plate)
96	*Exophiala equina*	Black yeast		Slow	Ascomycota	Cellulose-agar	Dilution plate
67	*Exophiala pisciphila*	Black yeast		Slow	Ascomycota	Czapek-dox-agar	Dilution plate
49	*Saccharicola bicolor*	Endophyte		Average	Ascomycota	Water-agar	Soil plate
85	*Trichosporon porosum*	Hyaline yeast		Fast	Basidiomycota	Pectin-agar	Immersion tube (dilution plate)
87	*Trichosporon porosum*	Hyaline yeast		Fast	Basidiomycota	MMN-agar	Immersion tube (dilution plate)
14	*Trichosporon porosum*	Hyaline yeast		Fast	Basidiomycota	Pectin-agar	Soil plate
18	*Trichosporon porosum*	Hyaline yeast		Fast	Basidiomycota	Lignin-agar	Dilution plate
17	*Trichosporon porosum*	Hyaline yeast		Fast	Basidiomycota	Water-agar	Dilution plate
15	*Trichosporon porosum*	Hyaline yeast		Fast	Basidiomycota	Czapek-dox-agar	Dilution plate
107	*Hymenoscyphus ericae*	Mycorrhizal		Slow	Ascomycota	Cellulose-agar	Maceration
106	*Hymenoscyphus ericae*	Mycorrhizal		Slow	Ascomycota	MMN	Maceration
22	*Penicilium sp.*	Mold		Fast	Ascomycota	MMN	Immersion tube (dilution plate)
59	*Mycorrhizal fungal sp.*	Mycorrhizal		Slow	?	MMN	Immersion tube (dilution plate)
45	*Penicillium velutinum*	Saprophyte (mold)		Fast	Ascomycota	Czapek-dox-agar	Immersion tube (dilution plate)
44	*Penicillium velutinum*	Saprophyte (mold)		Fast	Ascomycota	Czapek-dox-agar	Dilution plate
5	*Trichoderma viride*	Saprophyte (mold)		Fast	Ascomycota	MMN	Dilution plate
7	*Trichoderma viride*	Saprophyte (mold)		Fast	Ascomycota	Cellulose-agar	Dilution plate
9	*Trichoderma viride*	Saprophyte (mold)		Fast	Ascomycota	MMN	Soil plate
72	*Trichoderma viride*	Saprophyte (mold)		Fast	Ascomycota	Soil	Soil plate
101	*Umbelopsis autotrophica*	Saprophyte (mold)		Fast	Zygomycota	Water-agar	Maceration
51	*Umbelopsis autotrophica*	Saprophyte (mold)		Fast	Zygomycota	Water-agar	Maceration
50	*Umbelopsis autotrophica*	Saprophyte (mold)		Fast	Zygomycota	Czapek-dox-agar	Dilution plate
52	*Umbelopsis autotrophica*	Saprophyte (mold)		Fast	Zygomycota	MMN	Dilution plate
32	*Umbelopsis autotrophica*	Saprophyte (mold)		Fast	Zygomycota	Soil	Dilution plate

Table 2

Variable	Standard error	t value	p-value
Melanin content	-0.04	0.01	2e-4***
Contact angle (hydrophobic)	-0.26	1.15	0.82
Contact angle (moderately hydrophobic)	-2.31	1.7	0.18
Intercept	19.25	1.18	<2e-16***