

Links Between Heathland Fungal Biomass Mineralization, Melanization,
and Hydrophobicity

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Abstract:	<p>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</p>	

	<p>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.</p>
<p>Suggested Reviewers:</p>	<p>Bjorn Lindahl bjorn.lindahl@slu.se Pr. Lindahl was the first one to stress the importance of fungal biomass decomposition on soil C cycle especially on heathland-type soils.</p> <p>Christopher W Fernandez cwfernan@umn.edu Pr. Fernandez has been investigating similar hypotheses in mycorrhizal fungal biomass.</p> <p>Marc Buee marc.buee@inra.fr Marc Buee's main area of research is organic matter degradation by fungi in soils.</p>

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1 **Links between heathland fungal biomass mineralization, melanization and hydrophobicity**

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21 Decomposition; Fungal biomass; Heathland; Hydrophobicity; Melanin

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26

27 **Abstract**

28 Comprehending the decomposition process is crucial for our understanding of the mechanisms of
29 carbon (C) sequestration in soils. The decomposition of plant biomass has been extensively studied. It
30 revealed that extrinsic biomass properties, that restrict its access to decomposers, influence more
31 the decomposition than intrinsic ones, that are only related to its chemical structure. Fungal biomass
32 has been much less investigated, even though it contributes to a large extent to soil organic matter,
33 and is characterized by specific biochemical properties. In this study, we investigated to which extent
34 the decomposition of heathland fungal biomass was effected by its hydrophobicity (extrinsic
35 property) and melanin content (intrinsic property). We hypothesized that, as for plant biomass,
36 hydrophobicity would have a higher impact on decomposition than melanin content. Mineralization
37 was determined as mineralization of Soil Organic Carbon (SOC) into CO₂ by headspace-GC/MS after
38 inoculation by a heathland soil microbial community. Results show that decomposition was not
39 affected by hydrophobicity, but was negatively correlated with melanin content. We argue that it
40 may indicate that either melanin content is both an intrinsic and extrinsic property, or that some soil
41 decomposers evolved the ability to use surfactants to access to hydrophobic biomass. In the latter
42 case, biomass hydrophobicity should not be considered as a crucial extrinsic factor. We also explored
43 the ecology of decomposition, melanin content and hydrophobicity among heathland soil fungal
44 guilds. Ascomycete black yeasts had the highest melanin content, and hyaline Basidiomycete yeasts
45 the lowest. Hydrophobicity was an all-or-nothing trait, with most isolates being hydrophobic.

46 **Introduction**

47 Every year, the estimated increase of the atmospheric CO₂ pool is about 3.3 · 10⁹ Tons, to a large
48 extent due to fossil fuel burning and land-use change [1]. Observations and estimations at the global
49 scale indicate that terrestrial ecosystems actually affect this pool in a variable manner, depending on
50 the years, going from a -0.9 sink to a +0.5 · 10⁹ Tons/year source [2]. The outcome depends on the
51 balance between C input rate through Net Primary Productivity (NPP), and C output rate by
52 decomposition through heterotrophic respiration of the dead biomass into CO₂. Therefore, in the last
53 20 years, many investigations were attempting to unravel which factors are regulating NPP and
54 decomposition in terrestrial ecosystems, driving soil biologists to focus on a better understanding of
55 the decomposition process, and, in particular, why is it slower in some ecosystems than others. A
56 recently emerging view is that Soil Organic Carbon (SOC) decomposition is, at equivalent
57 environmental conditions, influenced by its physico-chemical accessibility, and to a lesser extent by
58 its chemical composition *per se* [3]. Indeed, compounds initially thought to be recalcitrant (such as
59 lignin) were shown to have a faster turnover than expected, while the opposite was found for some
60 small, more labile compounds [3-5]. Consequently, decomposition of SOC should be primarily
61 retarded by conditions restricting its access, and only secondarily by its chemical structure.

62 The SOC originates from plant and root litter, root exudates, and microbial biomass. There is
63 increasing evidence that microbes do contribute to a major part of SOC [3-5]. Indeed, fungi produce
64 large amounts of biomass in soils, at the scale of 50 to 1 000 kg/Ha [6-8], for mycorrhizal fungi, and
65 20-80 mg/kg of soil [9] or 1000 kg/Ha for saprophytes [10]. Despite its abundance in soils, however,
66 the fungal biomass decomposition has received much less attention than plant litter, from which it
67 differs by both the nature of structural molecules and physico-chemical accessibility. This is especially
68 the case in heathland ecosystems, where the well-developed soil organic layer has been mostly
69 assumed to be of plant origin, but where fungal biomass is also high [11], and is expected to better
70 resist decomposition [12]. This biomass can contain a fraction of structural compounds known to
71 decompose slowly in soils. The most widespread of these molecules are melanins, which have a
72 polyphenolic structure. Many fungi synthesize melanins to make their biomass resistant to a range of
73 chemical or physical stresses [13]. Melanized fungi are frequent in the heathland ecosystem [12].
74 Consequently, at optimal environmental conditions, melanin content is often negatively correlated
75 with the rate of decomposition. This has already been observed among fungi associated with forest
76 trees [14]. Melanin content may therefore be considered an intrinsic property of the fungal biomass:
77 it is a chemical property that does not affect the influence of environmental factors on
78 decomposition [3].

79 However, some extrinsic properties of the biomass, which define how it interacts with the
80 environment, do govern its stability in soils as well, and this to a much larger extent than chemical
81 structure *per se* [3]. Solubility, for example, is one of the most critical factors limiting decomposition
82 [15]. Indeed, most of the decomposition process relies on hydrolytic enzymes, or on enzymatic
83 reaction steps requiring the substrate to be solubilized. Hence, the higher the hydrophobicity of
84 fungal biomass, the slower should be its decomposition rate. However, this hypothesis has not been
85 verified. The extent to which hydrophobicity influences fungal biomass decomposition has not been
86 compared either with intrinsic properties such as melanin content.

87
88 Our aims were therefore twofold. First, we wanted to investigate how two key properties, one
89 intrinsic (based on molecular structure: melanin content), and the other extrinsic (based on how the
90 biomass interacts with its environment: hydrophobicity), were influencing the decomposition rate of

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

99

100 **Material & Methods**

101 *Sampling site*

102 This study was conducted in a dry heathland in the Nationaal Park Hoge Kempen (Belgium,
103 50°59'0.57"N 5°37'42.9"E). The area has a temperate climate, with an annual mean precipitation of
104 774 mm and a mean air temperature of 9.8°C. The dominant soil types are albic podzols and brunic-
105 dystric arenosols (<https://dov.vlaanderen.be/dovweb/html/index.html>). In autumn 2016, a sampling
106 plot of 50 by 60 meters was established in a dry heathland-dominated area of uniform vegetation
107 and flat topography (50°59'01.9"N 5°37'39.8"E). The vegetation was six to seven years old (30-50cm
108 height) and consisted of about 80 % *Calluna vulgaris*, 15 % *Molinia caerulea*, and 5 % bare soil, with
109 lichens and mosses present under the canopy.

110

111 *Fungal species isolation*

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

115

116 *Fungal species identification*

117 In order to determine melanin content, hydrophobicity and mineralization rate of our isolated soil
118 fungi, we needed to scale down the number of screened isolates to a manageable extent. Hence, we
119 classified the 207 isolates into 26 different groups based on their morphological characteristics
120 (growth rate, color, sporulation, growth pattern). One isolate of each of the 26 morphological groups
121 was selected for this study. We identified the species by sequencing the isolate's ITS region. For this
122 purpose, we collected a plug of actively growing mycelium (5mm diameter, 5mm deep), and
123 inoculated it either into a 12-well plate containing 2ml of liquid Czapek-Dox medium, or a 250ml flask
124 containing 100ml of liquid Czapek-Dox medium, and incubated for three days (fast-growing species)
125 to six weeks (slow-growing). The mycelial balls formed were then ground in liquid nitrogen using a
126 mortar and a pestle, and DNA was extracted on this mycelial powder with the MoBio Powersoil DNA
127 isolation kit. The ITS region was amplified using the ITS1f-ITS4 primers [16]. The PCR reactions were
128 performed in a C1000 Touch Thermal Cycler (BioRad) in a mix composed of 10mM of each primer,
129 2mM MgSO₄, 0.2mM dNTP mix, and 1 unit of Invitrogen Platinum Taq DNA polymerase High Fidelity
130 PCR enzyme (Invitrogen Life Technologies, <http://www.thermofisher.com>). The PCR reactions were
131 done using the following parameters: initial denaturation at 94°C for 3 min, followed by 30 cycles of
132 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
133 extension at 72°C for 10 min. Amplification success was checked in a 1 % agarose electrophoresis gel
134 in 1 % TBE (Tris-Buffer-EDTA) stained with GelRed. When amplification was not successful, we diluted
135 the DNA template 20 times in TE buffer and added 20µl of mM BSA solution to the DNA sample

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

144

145 *Mycelial hydrophobicity*

146 To measure mycelial hydrophobicity, we designed a device consisting of a microscope slide covered
147 by a thin uniform layer of Czapek Dox (CD) agar (45.34 g.l⁻¹ Czapek-Dox medium, 5 g.l⁻¹ Yeast
148 extract), laying in a Petri dish filled with 20ml of water agar medium (to avoid desiccation of the thin
149 CD layer). The device was prepared as follows. First, the microscope slide was sterilized by dipping in
150 96% ethanol and flaming on the Bunsen burner; then 1ml of hot CD agar was poured onto its surface
151 using a 1ml micropipette, let to solidify for five minutes, and transferred to the sterile water agar
152 plate. The 26 fungal isolates were grown for a week on CD agar plates. Then, an actively growing plug
153 of mycelium (0.3mm in diameter) was transferred to the middle of the slide. We prepared four
154 replicates of each isolate (hence 104 devices in total). Devices were then incubated at 23°C in the
155 dark for three weeks, after which slides were covered with at least 1cm² of mycelium. The mycelial
156 hydrophobicity was then assessed by measuring the contact angle of water droplets deposited at the
157 mycelial surface. This was done via sessile drop shape analysis as performed by Chau et al. [17]. We
158 slightly modified this protocol: six water droplets of 2 µl were pipetted from one edge of the slide to
159 the other edge on both sides of the inoculation point. For six isolates (the two *Penicillium velutinum*
160 and the four *Umbelopsis autotrophica* ones), we used 10 µl droplets instead, since smaller ones were
161 repelled by the substrate when being pipetted and ended up falling from the mycelial surface.
162 Analyses were carried out at The Institute for Materials Research (IMO-IMOMEC) of Hasselt
163 University. Contact angles were calculated using ImageJ (<http://rsb.info.nih.gov/ij/>). Measurements
164 of contact angles were obtained by using the Low Bond Axisymmetric Drop Shape Analysis Model
165 (LB_ADSA) plug-in, developed by Stalder et al. [18] (<http://bigwww.epfl.ch/demo/dropanalysis/>).

166

167 *Melanin content*

168 Mycelial melanin content of the isolates was assessed using the protocol of Gadd and Griffiths [19].
169 One actively growing plug (3mm in diameter) of each isolate was placed in a new CD agar plate,
170 covered by a cellophane sheet which was previously sterilized by autoclaving. The 104 Petri dishes
171 (26 isolates times 4 replicates) were then incubated for four to five weeks, depending on the growth
172 rate of each isolate, in order to obtain a sufficient amount of biomass to perform melanin extraction.
173 After incubation, the mycelium was scraped off the cellophane surface with a sterile scalpel and
174 homogenized in liquid nitrogen using a sterile mortar and pestle. The homogenized samples were
175 transferred to 50 ml Falcon tubes, and kept at -72°C. Samples were freeze-dried overnight in a
176 lyophilisator, and transferred to glass tubes. A 5ml solution of absolute ethanol was added to each
177 tube, followed by heating in heating blocks at 60°C for 3 h. Next, samples were vortexed before being
178 transferred to 15 ml Falcon tubes, and subsequently centrifuged for 10 min at 500 G. Supernatant
179 was discarded and samples were again freeze-dried overnight in a lyophilizer, after which 1 ml of
180 distilled water was added to the dried pellets, gently vortexing them before transferring them back

181 to glass tubes. Next, samples were resuspended in 1 ml 6 M HNO₃, and then placed in heating blocks
182 at 75°C for three hours. 5 ml of distilled water was added to each sample. After vortexing gently, the
183 resulting solution was transferred back to 15 ml Falcon tubes. Samples were again centrifuged (10
184 min, 500G), and the supernatant was discarded. Pellets were transferred back to glass tubes as
185 described before. The resulting suspension was heated at 75°C for 20 min in 5 ml of 0.5 M NaOH, and
186 filtered through grade 1 Whatman filter paper (Sigma). Melanin content was quantified by comparing
187 the optical density of samples at 470 nm, with a standard curve generated using serial dilutions (0-40
188 mg/l) of synthetic melanin (Sigma), dissolved in 1 M NaOH.

189

190 *Mineralization of C in fungal biomass*

191 The mineralization of the fungal biomass was assessed by measuring CO₂ production by a soil
192 microbial community growing on a nutrient solution containing mycelial biomass as the sole C
193 source, in a similar way as McDowell et al. [20] in their Method 8, but replacing soil with mycelial
194 biomass. As Fernandez et al. [21] showed that the mineralization rates of ectomycorrhizal fungal
195 biomass also depend on N content, we used a nutrient solution, ensuring that mineralization would
196 be only limited by C quality (adapted from method 8 of McDowell et al. [20]). Briefly, mycelial
197 biomass was prepared as for the measurements of melanin content (growth in CD agar covered by a
198 cellophane sheet and homogenization of the mycelium in liquid nitrogen, then storage of the
199 biomass at -72°C). A soil microbial inoculum was obtained as follows: on January 24th 2017, eight
200 topsoil samples (8cm diameter, 5cm deep) were taken, every 5 m along two transects parallel to the
201 longest side of the plot (four cores per transect). Samples were acclimatized at room temperature for
202 two weeks, due to collection in winter conditions. Afterwards, samples were sieved at 2mm, mixed
203 thoroughly and pooled. One gram of this pooled soil sample was added to a 15ml Falcon tube, and
204 mixed with 10 ml of sterile distilled water. The mixture was centrifuged for five minutes at 2000 rpm,
205 and the supernatant was collected and used as heathland microbial soil inoculum. The nutrient
206 solution contained 1.2 mM KCl, 0.5 mM CaCl₂, 0.5 mM KNO₃, 0.5 mM NH₄Cl, and 0.1 mM K₂HPO₄. A
207 headspace vial was then filled with 5ml of distilled water, 50 µl of the nutrient solution, 50 µl of
208 heathland microbial soil inoculum, and 7.5mg of homogenized, dry mycelial biomass, and sealed with
209 an airtight cap. Negative controls were prepared by preparing three vials containing only distilled
210 water, and three other containing distilled water, nutrient solution and soil inoculum, but no C
211 source. After one week, the CO₂ concentration in the vial gas phase was measured by headspace-
212 GC/MS.

213

214 *Statistics*

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

224

225 **Results**

226 *Mycelial hydrophobicity*

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

239

240 *Melanin content*

241 This experiment was designed to measure the melanin content of all the 26 heathland soil fungal
242 isolates, using the method of Gadd and Griffiths [19]. Since all strains displayed a large range of
243 colorations, from pure white to totally black (Table 1), we expected melanin content to vary in the
244 same proportions. Results showed that indeed there was a wide, two orders of magnitude range of
245 melanin contents among the isolates, ranging from 1 to 170mg/g DW. Black yeasts had the highest
246 melanin content (from 58 to 170 mg/l, Fig.2). One strain of *R. ericae* and one of *U. autotrophica* also
247 displayed high melanin contents (above 50mg/g), while all other strains had low values (below
248 20mg/g). The four *Trichoderma viride* strains were all especially low in melanin (all below 5mg/g).
249 When melanin contents were plotted against hydrophobicity, strains very clearly subdivided into four
250 categories: melanized and hydrophilic (n=16), hyaline and moderately hydrophobic (n=8), hyaline
251 and hydrophobic (n=76) and melanized and hydrophobic (n=4) (Fig.4, supplementary information).
252 Most of the strains were therefore hyaline and hydrophobic. None of the hyaline strains were
253 hydrophilic.

254

255 *Mineralization of C in fungal biomass*

256 The aim of this measurement was to assess the rate at which the C in the biomass of each isolate was
257 decomposed into CO₂ by a local soil microbial community. As for melanin content and
258 hydrophobicity, we expected that biomass mineralization rate would be normally or inversely
259 distributed among soil fungal isolates. We found that all isolates underwent significant degradation
260 within a week of inoculation (Fig.3), since all produced a CO₂ signal at least 300 times higher than the
261 blank without C substrate (12.22 at least in the isolate samples against 0.04 for the blanks, too small
262 to be visible on the Figure). The blanks were filled with ambient air, hence at least 400ppm CO₂ and
263 80% N₂. The negative control (nutrient solution, inoculum but no C source) showed that the inoculum
264 itself was not significant as a C source (peak ratio=0.04). Results showed high diversity, both within
265 and among species and functional groups. The amplitude of the differences was much lower than for
266 melanin contents, though, with a factor 2 only between the slowest and the fastest mineralizing
267 isolates. The isolates with the lowest degradation rate were: *Penidiella sp._100*, *R. ericae_106*,
268 *Saccharicola bicolor_49*, the two *P. velutinum*, as well as *U. autotrophica_101*. In opposite,
269 *Trichoderma viride_72*, *Umbelopsis autotrophica_32*, *Trichoderma viride_9*, *Trichosporon*
270 *porosum_17* and *Trichosporon porosum_15* had the highest mineralization rates. All functional

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

273

274 *Relationship between melanin content, hydrophobicity and C mineralization rates*

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

283

284 **Discussion**

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

291

292 *Relationship between C mineralization rate, melanization and hydrophobicity*

293 Our hypothesis was that hydrophobicity is a parameter that defines how the fungal biomass interacts
294 with the environment, by regulating access of hydrolytic enzymes to their substrate, and therefore
295 should have a larger influence on its decomposition than its melanin content. In fact, our results did
296 show the opposite. The more melanized the biomass, the slower its C was mineralized by a heathland
297 soil microbial community, while hydrophobicity was not correlated with mineralization. Biomass
298 melanization is a widespread character among fungi, and to understand the effects it can have on the
299 mineralization rate, it may be useful to elaborate further on the physiological role of melanin. This
300 polyphenolic compound deposits in the fungal cell walls where it complexes with proteins and
301 carbohydrates [13]. Melanin bears many similarities in its structure with lignin or lignin building
302 blocks; it is therefore itself a stable compound, that can be degraded only by fungal peroxidases
303 produced by white-rot fungi [13]. As for lignin in plants, it can be considered an intrinsic property of
304 fungal biomass. Our observations confirmed the hypothesis that melanization and decomposition
305 rate were negatively correlated. However, this correlation was not always tight: several isolates were
306 melanized but still decomposed fast (*Exophiala sp._96*, *Exophiala sp._94*), others hyaline and
307 recalcitrant (*Saccharicola bicolor_49*). We expected that this variability would be explained by the
308 hydrophobicity of the biomass, another factor that commonly hampers decomposition of organic
309 molecules in soil [23]. Our results show, however, that hydrophobicity did not explain the observed
310 mineralization. Hence, in our experiment, substrate accessibility for hydrolytic enzymes did not play a
311 role in decomposition, while melanin content, an intrinsic property of SOC, significantly did. To
312 explain such unexpected results, one could formulate the hypothesis that melanin is at the same
313 time an intrinsic and extrinsic property of SOC, because it also regulates its accessibility. Indeed,
314 melanin molecules have the property to bind large amounts of water (only 10mg of melanin
315 “granules” -bodies of agglomerated pigments- able to bind 1ml of water [13]). By doing so, they

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

328

329 *Ecology of fungal hydrophobicity and melanization in the heathland ecosystem*

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

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

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

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

365

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

367

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435 filamentous fungi. *Environmental microbiology* 5 (2):85-91

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 CO₂ 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

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

461

462 **Fig.3** Carbon mineralization rate of the biomass of the 26 heathland soil isolates. Carbon
463 mineralization rate was assessed by measuring CO₂ production after one week by a soil microbial
464 community, using 7.5mg of dried fungal biomass as the only C source (and provided with the other
465 nutrients). The biomass of each isolate has been quadruplicated. Bars represent standard deviation
466 between the four replicates

467

468

Figure 1

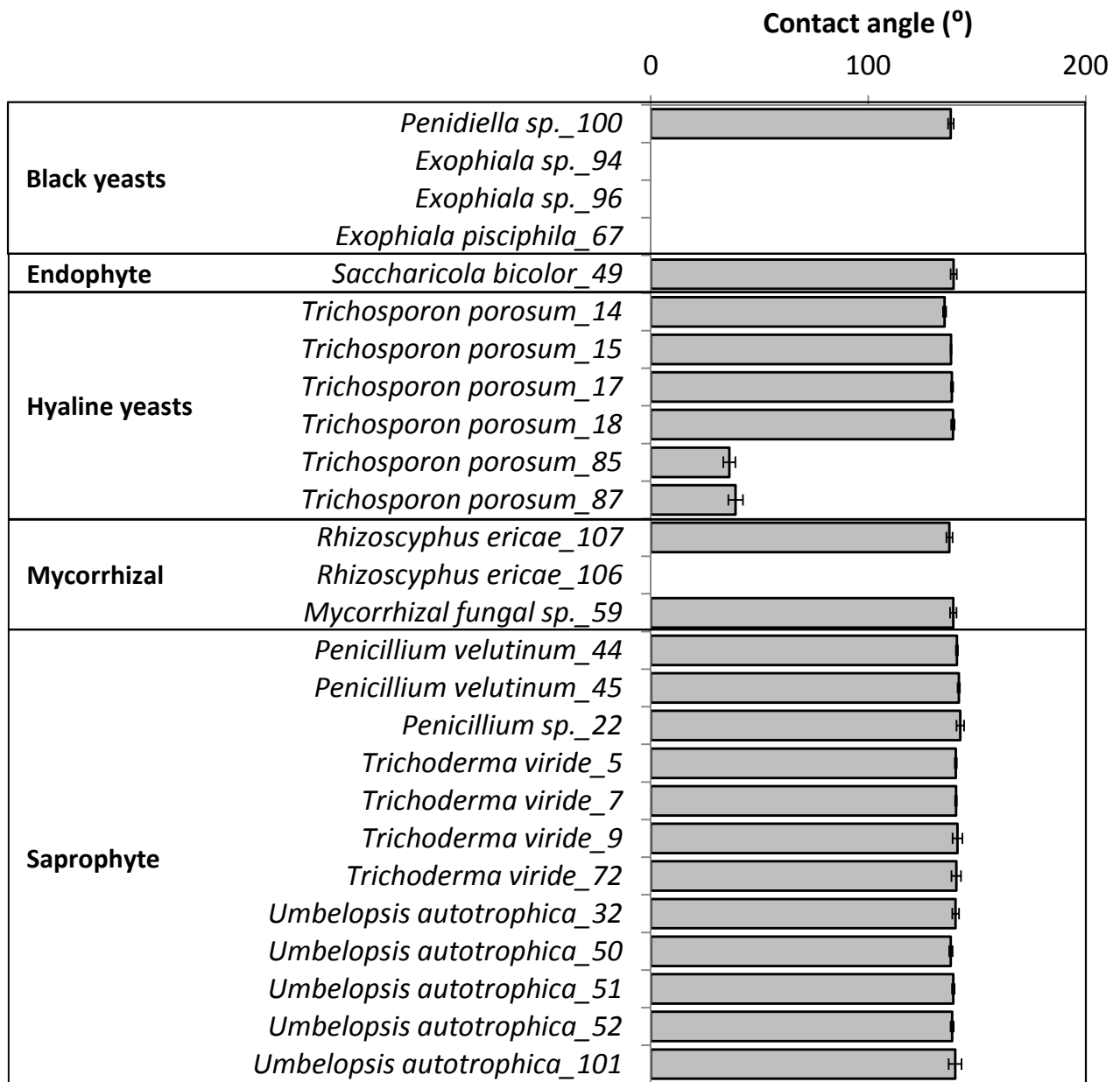


Figure 2

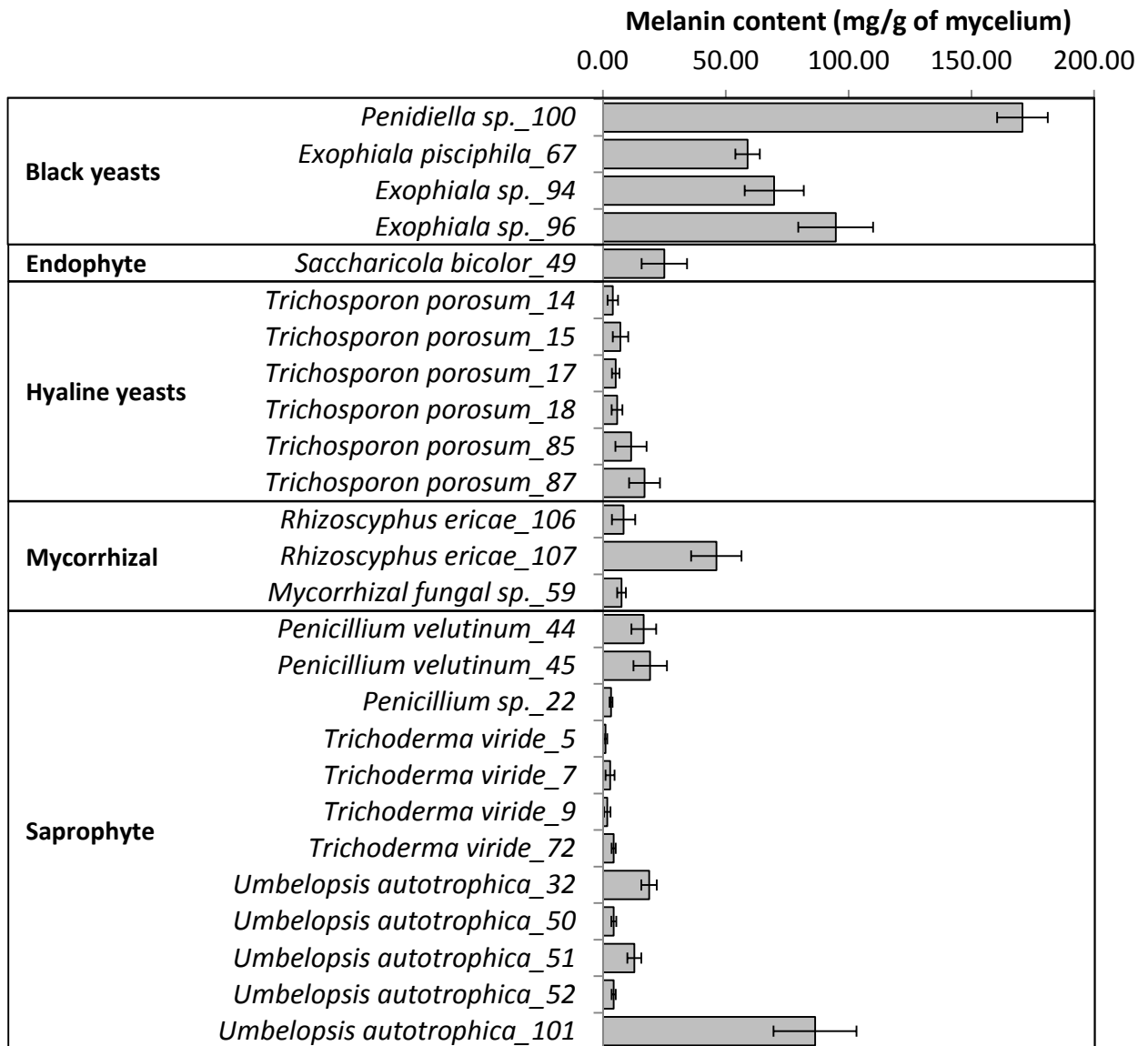


Figure 3

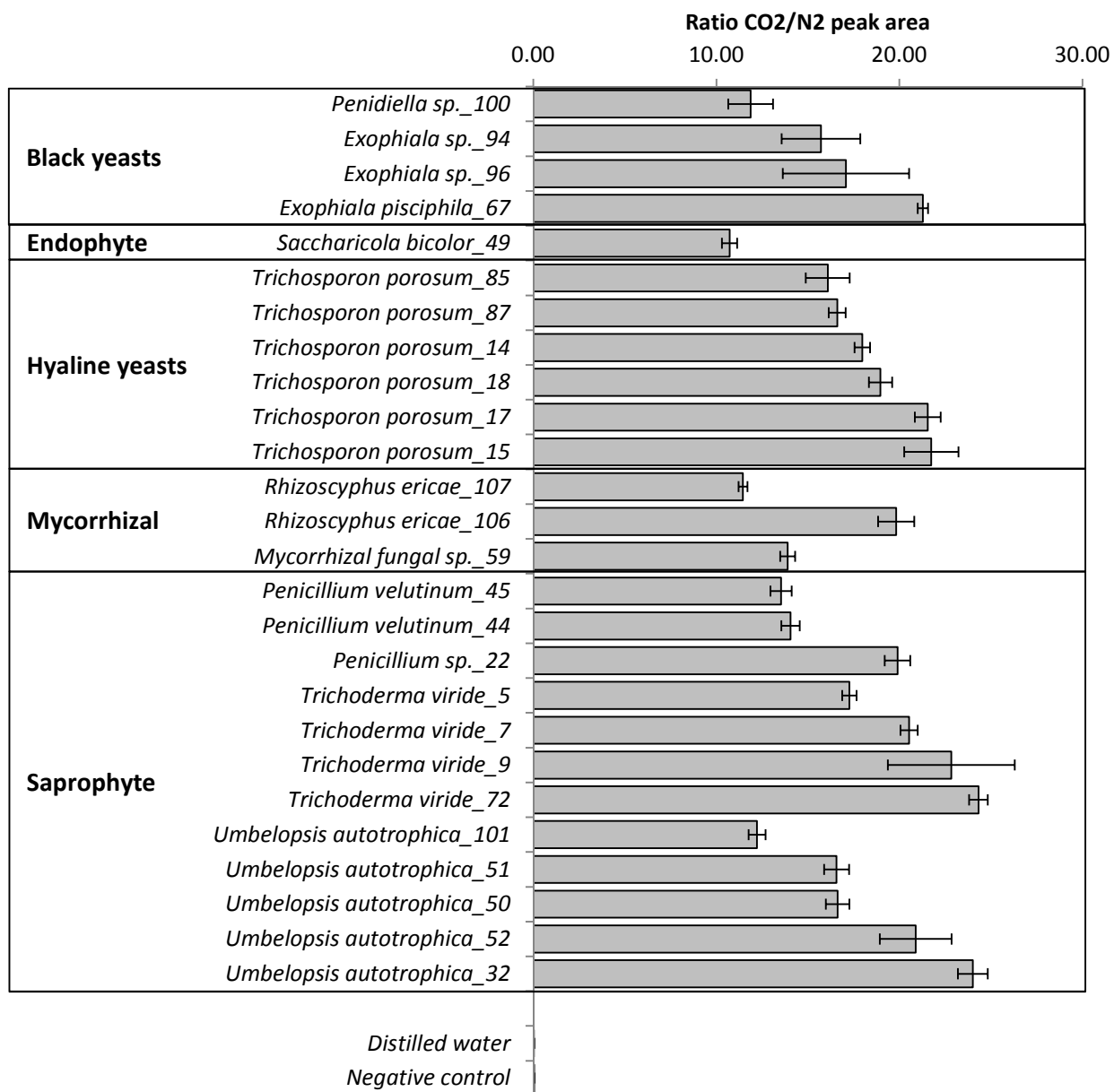


Table 1


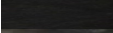

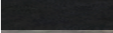

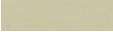






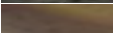











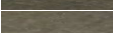

Strain	Species name	Functional group	Color	Growth rate	Taxonomic group	Isolation medium	Isolation method
100	<i>Penidiella sp.</i>	Black yeast		Slow	Ascomycota	MMN	Immersion tube (dilution plate)
94	<i>Exophiala equina</i>	Black yeast		Slow	Ascomycota	Pectin-agar	Immersion tube (dilution plate)
96	<i>Exophiala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



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