1 Title:

2 Ecological memory and relocation decisions in fungal mycelial networks: responses to

3 quantity and location of new resources

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5 Running title:

6 Intelligence of fungal mycelial networks

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Abstract

Saprotrophic cord-forming basidiomycetes, with their mycelial networks at the soil/litter interface on the forest floor, play a major role in wood decomposition and nutrient cycling/relocation. Many studies have investigated foraging behaviour of their mycelium, but there is no information on their intelligence. Here, we investigate the effects of relative size of inoculum wood and new wood resource (bait) on the decision of a mycelium to remain in, or migrate from, inoculum to bait using *Phanerochaete velutina* as a model. Experiments allowed mycelium to grow from an inoculum across the surface of a soil microcosm where it encountered a new wood bait. After colonisation of the bait, the original inoculum was moved to a tray of fresh soil to determine whether the fungus was still able to grow out. This also allowed us to test the mycelium's memory of growth direction. When inocula were transferred to new soil, there was regrowth from 67% of the inocula, and a threshold bait size acted as a cue for the mycelium's decision to migrate for a final time, rather than a threshold of relative size of inoculum : bait. There was greater regrowth from the side that originally faced

33 the new bait, implying memory of growth direction.

34

35 Introduction

36 Fungi are vital agents for organic matter decomposition, and carbon and nutrient
37 cycling in forest ecosystems by virtue of their huge biomass, enzymatic ability, and
38 efficient translocation of carbon and nutrients by mycelial networks [1]. Cord-forming
39 basidiomycetes are particularly important due to the persistent linear organs that they
40 produce – cords. Cords are aggregations of many parallel-aligned hyphae, which are
41 often differentiated internally, forming large networks at the interface of the litter layer
42 and soil horizon in the forest floor, translocating carbon and nutrients efficiently [2, 3,
43 4]. Cord-forming basidiomycetes colonise, and link together, many different plant litter
44 components within its cord network, from leaf litter and small twigs to large, fallen tree
45 trunks [1]. They are abundant on the forest floor [1], often occupying large areas and
46 being long-lived [5, 6, 7, 8]. A better understanding of developmental cues, nutrient
47 translocation and the mechanisms of network sustainability are essential for
48 understanding cycling and redistribution of carbon and nutrients on the forest floor.

49 The behavior of cord-forming mycelium has been well-studied using soil tray
50 microcosm experiments [9, 10]. When a wood block colonised by a cord-forming
51 basidiomycete is placed as an inoculum on the surface of compressed soil, mycelium
52 grows out from the inoculum onto the soil, colonising any new resources that it
53 encounters. If a newly encountered resource (bait) is sufficiently large compared to the
54 inoculum, connecting cords thicken and non-connected mycelium regresses, resulting in
55 a strong connection between inoculum and bait. Nutrient translocation between
56 resources, via cords, can occur in both directions but extent and timing depends on
57 relative size and decay stage of them, probably reflecting a 'source and sink'
58 relationship [2, 11]. Similar patterns are seen on the forest floor [10], and there is
59 evidence that mycelium sometimes completely abandons small resources [12]. The
60 latter phenomenon has not been investigated experimentally.

61 Foraging and migration behaviour has also been studied using myxomycete
62 plasmodia which, though unicellular, have a superficially similar body design to fungal
63 mycelia, are both heterotrophs, feeding by extracellular digestion, although plasmodia
64 also use phagocytosis [13]. From numerous studies on the model species *Physarum*

65 *polycephalum*, it is known that myxomycete plasmodia can optimise their network
66 structure to connect separately located multiple resources, avoid unfavorable areas [14,
67 15] and solve mazes [16]. They can remember their past activities to avoid previously
68 explored areas, but can decide to enter unfavorable areas if there is no other choice [17],
69 and the time to leave old food sources is determined heuristically [18]. Therefore,
70 plasmodia of myxomycetes are now considered to have intelligence and cognitive
71 abilities even though they have no brain, central nervous system, nor neural networks
72 [18]. If fungal mycelial cord networks have similar intelligence, it will completely
73 change our understanding of carbon and nutrient cycling on the forest floor.

74 The aims of this study were to (1) determine what conditions make a fungal
75 mycelium decide to make its final move from an old inoculum to a new wood resource
76 (bait) wood, and (2) test whether a fungal mycelium within an inoculum remembers the
77 direction of a new resource bait to which it had been connected, if the cord connection
78 between the inoculum and bait was completely destroyed. We hypothesised that relative
79 size of inoculum and bait wood blocks would affect their decision to move and memory
80 of direction of the bait. We used a soil tray microcosm and a saprotrophic cord-forming

basidiomycete *Phanerochaete velutina* (DC.) P. Karst. as a model system. This fungus is common in UK forests [6] and is one of the most well-studied species in the research field of mycelial network behaviour [1, 9, 10].

Materials and methods

Fungal culturing and inoculum preparation

Kiln dried beech (*Fagus sylvatica*) wood was cut into blocks of three sizes: 0.5 x 1 x 1 cm (0.5 cm^3), 2 x 1 x 1 cm (2 cm^3), 2 x 2 x 1 cm (4 cm^3). Blocks were soaked overnight in DH_2O prior to use and then autoclaved at 121˚C for 20 min in double, sealed autoclave bags. The process was repeated three times with 1 day intervals. Sterilised wood blocks were placed onto cultures of *P. velutina* (Cardiff University Collection) which was grown on 0.5% malt extract agar (MEA; 5 g Lab M malt extract, 15 g Lab M agar no. 2) in non-vented 14 cm-diameter Petri dishes (2-cm thick). Plates were sealed with Parafilm®, (Bemis Company Inc., Oshkosh, USA) and incubated in the

97 dark at 20˚C for 3 months before use.

98

99 Microcosm preparation and inoculation

100 Soil was collected from the top 10 cm in a deciduous forest in Tintern
101 Monmouthshire (51.6980 N, 2.6814 W). After sieving on site (10 mm mesh), the soil
102 was air-dried, sieved through a 2-mm mesh and frozen at –18˚C for 48 h. Soil (200 g)
103 was rehydrated with 300 ml DH_2O (to give –0.012 MPa) and transferred to 24 x 24 cm
104 bioassay dishes, smoothed and compacted to about 5 mm depth. A wood block, from
105 which surface mycelia and excess agar had been removed using a razor blade, was
106 placed centrally, 5 cm from one side of each soil tray. When mycelia had extended 6 cm
107 from the inoculum in 50% of the trays, a new beech wood block (bait) prepared and
108 sterilized as described above, but not inoculated with fungi, was placed at the margin of
109 the mycelium towards the middle of the tray. Six sizes of bait wood blocks [0.5 x 1 x 1
110 cm (0.5 cm^3), 1 x 1 x 1 cm (1 cm^3), 2 x 1 x 1 cm (2 cm^3), 2 x 2 x 1 cm (4 cm^3), 4 x 4 x 1
111 cm (16 cm^3), and 6 x 6 x 1 cm (36 cm^3)] were tested in all combinations with the three
112 inoculum sizes (i.e. 18 combinations in total). Ten replicates were made for each

113 combination (i.e. a total of 180 tray microcosms) (Fig. S1).

114

115 Microcosm incubation

116 After set-up, each tray was weighed, and lost water was replaced every week by
117 spraying DH_2O evenly across the soil until each tray reached its original mass. Trays
118 were stacked and sealed in polythene bags to reduce water loss, and incubated at 20˚C
119 and 70% humidity in the dark for 48 days [Period I].

120 After Period I, inoculum wood blocks were retrieved, cleaned of surface mycelia,
121 and placed centrally onto new soil trays freshly prepared as described above. The trays
122 were further incubated at 20˚C and 70% humidity for 8 days [Period II], and the
123 presence and location of outgrowing myclium was recorded. The systems were
124 incubated for a further 8 days and then rerecord, but there was little change in results, so
125 we only analysed the 8^{th} day data. Previous studies on *P. velutina*, and our personal
126 observation in Period I in the present study, showed that there is substantial hyphal
127 growth from inoculum at 2–5 days after placing onto the soil [19, 20], suggesting that 8
128 days are sufficient to check for outgrowth from the inoculum.

129 Trays were randomly repositioned every 3 days during incubation (in both periods I
130 and II) to avoid possible effects of orientation and location within the CT room on the
131 direction of hyphal growth. Trays were photographed when the baits were added to the
132 tray, and at the end of incubation period I and II, using a Nikon Coolpix P80 camera,
133 mounted on a stand at a height of 46 cm, and in the same light conditions to ensure
134 consistency.
135
136 Image analysis
137 Images were analysed using ImageJ (National Institute of Health, USA). A 2-cm
138 calibration line was drawn electronically using a ruler next to each tray. The edge of the
139 soil tray and wood block were removed by windowing, and the resulting image
140 converted to black and white binary with a manually set threshold. The mycelia and soil
141 were indicated by black and white pixels, respectively, allowing hyphal coverage (cm^2)
142 to be determined. Given the difference in bait size, we calculated hyphal density on soil
143 plates by dividing hyphal coverage (i.e. pixel count) by soil area to be compared
144 between different bait size experiments. To compare mycelial growth towards and away

145 from the bait, each image was split into two at the center line of the inoculum wood
146 block (Fig. 1). Hyphal coverage ratio of bait-side and opposite-side were calculated by
147 dividing hyphal coverage of the side closest to the new resource (termed bait-side) and
148 the opposite-side by the hyphal coverage of the whole mycelium.
149
150 Statistical analysis
151 Hyphal density (pixel count per unit soil area) in period I and hyphal coverage in
152 period II were compared across experiments within treatments with the same inoculum
153 size by Tukey's pairwise comparison ($P < 0.05$). Hyphal coverage ratio between
154 bait-side and opposite-side were compared by Wilcoxon rank-sum test.
155 Effects of inoculum size (*Inoc*), bait size (*Bait*), distance between inoculum and bait
156 (*D*), and interaction between inoculum and bait sizes (*Inoc***Bait*) on hyphal regrowth in
157 period II were evaluated using generalised linear models (GLMs). The first model
158 (GLM_1) was applied to explain the presence/absence of regrowth in period II
159 (*GIIcount*). The second model (GLM_2) evaluated the effects of four predictor variables
160 in GLM_1 plus bait-side growth ratio in period I (*GIbait*) on bait-side growth ratio from

inoculum in period II (*GIIbait*). A binomial distribution error was assumed and a logit link function was used in GLM_1, whereas Gaussian distribution was assumed and an identity link function was used in GLM_2. The model descriptions are as follows:

$GIIcount_i \sim$ Binomial (μ_i),

$\text{logit}(\mu_i) = \beta_0 + Inoc_i + Bait_i + D_i + Inoc_i{*}Bait_i$ ——— GLM_1

$GIIbait_i \sim$ Gaussian (μ_i),

$\text{identity}(\mu_i) = \beta_0 + Inoc_i + Bait_i + D_i + Inoc_i{*}Bait_i + GIbait_i$ ——— GLM_2

where β_0 is the intercept and *i* stands for individual soil microcosm. In the present study, it inevitably happens that the distance between inoculum block and bait block differs slightly among the treatments (Fig. S2). We are not interested in distance effects, but included it in the model to check that it has no effect on the results. We included an interaction term between inoculum size and bait size in the models because we hypothesized that relative size of inoculum and bait determines the growth response of

fungi in the microcosm. For model simplicity to keep statistical power, we did not include interaction terms between distance and wood sizes.

For both GLM_1 and GLM_2, the best models were selected based on the Akaike information criterion by backward stepwise selection. The coefficients of the best models were exponentiated to obtain odds ratios (for GLM_1) or risk ratios (for GLM_2). Ratios > 1 indicated that the explanatory variable had a positive effect on the presence/absence of regrowth in period II (GLM_1) or bait-side hyphal coverage in period II (GLM_2), while ratios < 1 indicated negative effects; the difference from one indicated the magnitude of the effect. The level of collinearity between predictor variables was checked by calculating the variance inflation factor (VIF): all VIF values were <3, indicating low levels of multicollinearity in the models.

All statistical analyses were conducted in R 3.5.0 (R core team, 2018) using the DAAG [21] and MASS [22] packages. A power analysis was performed using G*Power software [23], which confirmed that the sample size (n = 180) was sufficient to test the effects of the 5 variables (including interaction terms) on hyphal regrowth in period II.

193

194 Results

195

196 Growth characteristics in Period I

197 There was no significant difference ($P > 0.05$) in hyphal area ratio between
198 mycelium growing out from the inoculum on the bait-side and the opposite-side at the
199 time when the baits were added (Fig. S3), suggesting that there was no preference in
200 hyphal growth direction before baiting. Colonisation of baits by *P. velutina* hyphae
201 occurred in all soil trays (Fig. S4).

202 As the soil area available for mycelial colonization is inherently different in soil trays
203 with different-sized baits, we compared hyphal density on soil in period I across
204 experiments within the same inoculum size. Forty eight days after baiting, hyphal
205 coverage was usually significantly less in mycelial systems with the largest (36 cm^3 and
206 sometimes 16 cm^3) baits than that coupled with the smaller (1 cm^3, 2 cm^3, and 4 cm^3)
207 baits (Fig. 2; Fig. S4). Mycelium on the soil in the area of the inoculum wood block, but
208 not connected to the bait, often died back in systems with largest baits (Fig. S4c), but

209 not in systems with smaller baits (Fig. S4). In most of the combinations, except for 0.5
210 cm^3 inoculum coupled with 4 cm^3 bait, hyphal area ratio on the bait-side was
211 significantly larger than that of the opposite-side (Fig. 3).
212
213 Regrowth in Period II
214 Inocula coupled with 16 cm^3 and 36 cm^3 baits seldom showed regrowth in period II
215 regardless of the inoculum size (Fig. 4). All of the 2 cm^3 and 4 cm^3 inocula whose
216 mycelium joined to small baits (0.5, 1, and 2 cm^3) showed regrowth, although some of
217 the 0.5 cm^3 inocula linked to 0.5 cm^3 and 2 cm^3 baits did not show regrowth. Bait
218 volume had a less predictable effect on regrowth from small inocula than from larger
219 inocula. Among the four variables tested in GLM_1, volumes of inoculum and bait, and
220 their interaction term, were significantly related to occurrence of regrowth, and were
221 selected as factors in the best model (Table 1). The inoculum volume had a strong
222 positive association, and the bait volume had a negative association with occurrence of
223 regrowth from the inoculum.
224 Similar to period I, hyphal coverage in period II was usually significantly less in

225 mycelial systems linked to larger baits than in those linked to smaller baits (Fig. 5).
226 Hyphal area ratio of the bait-side of the inoculum was significantly larger than that of
227 the opposite-side growing from 0.5 cm^3 inocula previously linked to 0.5 cm^3 bait, and
228 from 4 cm^3 inocula previously linked to 1 cm^3 bait (Fig. 6). Among the five variables
229 tested in GLM_2, inoculum volume and bait-side hyphal growth ratio in Period I had
230 significant positive associations with bait-side hyphal growth ratio in Period II (Table 2).
231 Among them, bait-side hyphal growth ratio in Period I had a particularly large risk ratio,
232 indicating a strong effect. Bait volume, interaction between inoculum and bait volume,
233 and distance between inoculum and bait were also selected in the best model, but their
234 associations with bait-side growth in Period II were not significant.
235
236
237 Discussion
238
239 Mycelial decision to migrate
240 We have shown that when mycelia of *P. velutina* grew from inoculum wood blocks

241 and colonised new larger bait wood resources, if the interconnection was subsequently
242 severed, mycelium was often no longer able to grow out of the original inoculum. We
243 did not attempt to reisolate the fungus from the original inoculum, so we cannot be
244 certain whether the fungus had completely lost its viability within the original inoculum.
245 However, the observations certainly suggest more or less complete migration of active
246 mycelial resources from the inoculum to the bait.

247 As predicted, larger baits induced complete migration more frequently than small
248 baits. Interestingly, the threshold volume of the bait that induced dramatic change in
249 frequency of regrowth from original inoculum was somewhere between 4 to 16 cm^3
250 regardless of the inoculum volume (which ranged from 0.5 cm^3 to 4 cm^3). This suggests
251 that the primary factor affecting a mycelial decision to migrate completely to a new
252 resource is actual volume of the new resource rather than the relative size of new
253 resource to original inoculum, at least within the range of wood volume tested in the
254 present study. This may seem counter-intuitive, as the mycelial outgrowth pattern was
255 determined only by the nutritional status of the wood resources, because a larger
256 inoculum contains a larger amount of carbon available to mycelium compared to a

257 smaller inoculum [19, 24]. However, since a mycelium is an integrated system,
258 coordinated resource allocation within a mycelium may explain this behavior. *P.*
259 *velutina* mycelium tends to allocate more phosphorus to large wood resources than to
260 smaller ones [19, 25], suggesting that there is a relatively larger nutritional cost for early
261 colonisation of a larger resource than of a smaller one. This may also explain why
262 mycelial migration from large inocula to the baits is determined by a relatively strict
263 bait volume, whereas this was not the case with migration from small inocula. Given a
264 larger cost to maintain a mycelial presence in large inocula than in smaller inocula, the
265 decision to keep or discard a large inoculum after finding new large resources may be
266 strongly determined by nutritional economy of the mycelium, whereas with small
267 inocula the decision to keep or discard the original inoculum may be more stochastic.
268 Although decay rate of wood blocks were not measured in the present study,
269 size-dependent wood decay rate may also affect the fungal decision of whether or not to
270 migrate. Since decay rate of smaller wood particles is generally faster than larger ones
271 [26], the more rapidly decreasing energy content of smaller wood blocks may cause the
272 mycelium to completely migrate to new resources sooner than from larger ones. Thus,

273 incubation periods longer than 48 days may alter the relationships between migration
274 and wood size.

275 Microbial competitors in soil may also affect the decision of whether or not to
276 migrate. Since the soil used in the microcosm was unsterilised, the focal fungi have to
277 defend their wood blocks from a variety of microbial competitors in soil, which has an
278 energetic cost. Smaller wood territory is more difficult to defend against mycelia
279 occupying larger territory [27, 28], supporting our results showing that *P. velutina*
280 mycelium left the smallest inoculum more often than large baits.

281 It is not clear why the mycelial decision to migrate depended on a certain range of
282 bait size, but not on relative size of bait to inoculum. A possible reason is the limitation
283 in maximum possible size of mycelium in the microcosms regardless of the volume of
284 wood resources within [19, 29]. Since wood is relatively poor in mineral nutrients, e.g.
285 nitrogen and phosphorus [30], most of the nutrients necessary for initial mycelial
286 establishment within new woody resources will be translocated in the foraging
287 mycelium, originating from soil, stored or recycled within resources [4, 19, 20, 25]. To
288 maintain the carbon to nutrient ratio of mycelium, the amount of carbon source (wood

289 block) available for a mycelium is determined by nutrient acquisition, which largely
290 depends on the size of mycelium [4]. In this context, the threshold volume of a bait that
291 would make a mycelium to decide to migrate completely would change according to the
292 size of microcosm, and must be larger in the field where *P. velutina* mycelium colonises
293 larger coarse woody debris [6]. The small size of microcosms may also be the reason
294 why the distance between inoculum and bait wood blocks did not affect the results in
295 the present study. *P. velutina* is known as a 'long-range forager' [10], often forming cord
296 networks extending over many meters [6, 12]. Cords of *P. velutina* can translocate
297 phosphorus at least 75 cm within 5 days in field [31] and probably very much further
298 and faster, given carbon transfer to 18 cm distance from inoculum within 20 min in
299 laboratory microcosms [29]. These effects of microcosm size and incubation time on
300 fungal decisions should be tested in the future. Furthermore, relationships between
301 fungal decision and sizes of inoculum and bait wood blocks should also be tested in
302 more detail using wood blocks with a wider size range and narrower size intervals,
303 because the size range of wood blocks were not evenly distributed in this study.
304 Although the use of unsterilised soil provided a realistic scenario, the systems were

much simplified with various stresses (such as fluctuating temperature and moisture) and disturbance agents (such as soil arthropods) prevented. These may also affect the nutritional economy of the mycelium and thus alter the migration threshold in natural ecosystems.

Mycelial memory of direction of growth

Reallocation of mycelial biomass and mycelial growth in the direction of the bait, as seen in Period I, is in line with previous findings (reviewed in [10]). The completely novel finding is the dominant regrowth, in Period II, from the inoculum side that had originally been joined to the newly colonised resource in Period I. This is a kind of memory of mycelial systems for spatial navigation and is likely to be advantageous for quickly repairing damaged network connections, by regrowing towards self. Directional mycelial growth is also likely to have a considerable advantage in the field, since exploring a new area and avoiding effort in a previously recently explored region, clearly increases the chance of finding new resources. With regard to mechanisms, larger and newer wood baits have a greater flux of nutrients towards them [25, 32, 33],

probably attributable to the large metabolic demand of an invasive mycelium in newly-colonized wood [24]. Previous studies found that destructive disturbance of cord networks of *P. velutina*, removing several baits [34] or severing cords [35], caused polarised growth in the undisturbed direction. Such polarisation may be attributable to undisturbed hyphae forming a 'dominant-sink' for translocation within the mycelial system.

In the present study, it is not appropriate to say that the mycelium remembered the direction of the bait since the effects of bait itself and difference in soil area between bait-side and opposite-side could not be evaluated separately. Further, absence of a second control comprising systems without an added bait wood block did not allow us to completely evaluate the effects of bait wood blocks on the hyphal growth in Period II. However, the design allowed us to confirm that mycelia had no preference in growth direction before addition of bait, and thus we can say that there was memory of the predominant direction in which the mycelium developed. Previous studies have categorised the biotic mechanisms of memory in organisms (or swarms) without (central) nervous systems into two types [36]: (1) external memory, which detects

337 signals deposited in the environment; (2) somatic memory, achieved by storage of both
338 epigenetic and/or non-genetic changes of cell physiology. An example of (1) is foraging
339 plasmodia of slime moulds which avoid areas that have previously been explored by
340 detecting deposited extracellular slime [17]. Foraging ants, on the other hand, use trace
341 pheromones to attract (rather than repel) conspecific individuals to trails which allows
342 sharing information about food, nest or mate location [37]. However, such external
343 memory is not likely to be the case in the present study because the inocula were moved
344 to completely fresh soil trays without any deposits from previous activity. Furthermore,
345 previous disturbance studies without changing the soil tray showed no evidence of
346 positive or negative effects of the area previously covered with mycelium [35].
347 Evidence for the possibility of (2), somatic memory, in fungi was provided in a
348 recent study on *Saccharomyces cerevisiae*, which showed that epigenetic transcriptomic
349 change in mother cells that had experienced environmental change could be transferred
350 to daughter cells, which had not experienced the environmental change [38]. Further,
351 non-genetic changes induced by the environment, such as chemical concentrations and
352 bioelectricity within a single cell [39, 40, 41, 42], or in networks across multiple cells in

353 multi-cellular organisms [43], can also act to maintain memories of polarised growth or
354 habituation if cells were stored after disturbance, dormancy, or regenerations. The third
355 possibility for explaining preferential bait-side regrowth in the present study is a
356 carry-over effect of differential distribution of mycelium within the inoculum wood
357 block, without any physiological change in the mycelium with more mycelium in the
358 inoculum on the side nearest the bait. It is also valid to consider this to be a part of a
359 memory mechanism because the mechanisms of memory in the human brain includes
360 this kind of non-physiological, non-epigenetic phenotypic level change in neuronal
361 network structure [44]. However, we appreciate that there may be semantic conflicts in
362 the concept of non-neuronal memory among scientists as it is a novel and developing
363 study field [36], we believe that recognizing such a carry-over effect as a kind of
364 memory is a first step in the study of non-neuronal intelligence (in the words of Solé et
365 al. [36] 'liquid brain') in a broader sense. Determining which of the above-mentioned
366 mechanisms are involved in a mycelial memory in our system is the next experimental
367 challenge.

368 It is interesting that larger inocula tended to remember their direction of growth

369 better than smaller inocula in the present study. Previous studies on *P. velutina* also
370 reported that mycelium growing from large (8 cm^3) wood blocks showed stronger
371 polarity in nutrient transfer [24] and growth [19] compared to mycelium growing from
372 smaller wood blocks. However, the mechanisms of polarity and memory in fungal
373 mycelium have been poorly explored and are a challenging topic for the future.
374 Whatever the mechanisms involved in the memory of mycelium, the results presented
375 here show that mycelium of *P. velutina* remembered its growth direction after the
376 complete removal of outgrowing hyphae from wood inocula. Recognizing that fungal
377 mycelium has a primitive intelligence with decision-making ability and memory is an
378 important step towards understanding mycelial foraging behaviour, with consequences
379 for carbon and nutrient dynamics on the forest floor.
380
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386

387 Conflict of interest

388 The authors declare that they have no conflict of interest.

389

390 Supplementary information including raw data (datasetS1) is available at the ISME
391 Journal's website.

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513 Figure legends

514

515 Fig. 1. Measurement of hyphal coverage on soil. Original images were converted into
516 binary images by ImageJ (National Institute of Health, colour threshold = 160). To
517 evaluate hyphal growth on the bait-side and opposite-side of the inoculum wood block,
518 each image was split into two parts at the centre line of the inoculum wood block
519 (dashed lines).

520

521 Fig. 2. Hyphal density (cm^2 cm^{-2}) on soil in laboratory microcosms growing from (a)
522 0.5 cm^3, (b) 2 cm^3, and (c) 4 cm^3 inocula 48 days after a new bait resource was added.
523 Different letters on each box show significant ($P < 0.05$) difference across the six bait
524 sizes (Nemenyi-tests, Tukey: $N = 10$). Note that the y axes have different scales.

525

526 Fig. 3. Hyphal coverage ratio of mycelium on the bait-side of (a) 0.5 cm^3, (b) 2 cm^3, and
527 (c) 4 cm^3 inocula 48 days after baiting in Period I. Values in parenthesis are the number
528 of replicates for each experiment, with asterisks indicating a significant difference from

529 0.5 (Wilcoxon rank sum test: *, $P < 0.05$; **, $P < 0.001$).

530

531 Fig. 4. Frequency of (a) 0.5 cm^3, (b) 2 cm^3, and (c) 4 cm^3 inocula with/without hyphal
532 regrowth 8 days after inocula had been moved to new soil trays, depending on the size
533 of bait encountered in Period I.

534

535 Fig. 5. Hyphal coverage (cm^2) of mycelia extending from (a) 0.5 cm^3, (b) 2 cm^3, and (c)
536 4 cm^3 inocula 8 days after they had been moved to new soil trays. Different letters on
537 each box indicate significant ($P < 0.05$) difference across the six bait sizes
538 (Nemenyi-tests, Tukey: $N = 10$). Note that the y axes have different scales.

539

540 Fig. 6. Ratio of the hyphal coverage in Period II, of mycelium extending from the side
541 of the inoculum that had been facing the bait in Period I for (a) 0.5 cm^3, (b) 2 cm^3, and
542 (c) 4 cm^3 inocula 8 days after they had been moved to new soil trays. Values in
543 parenthesis are the number of replicates for each experiment, with asterisks indicating
544 significant difference from 0.5 (Wilcoxon rank sum test: *, $P < 0.05$).