Not Extremely Plastic: Testing the Limits of Morphological Plasticity in Fungal Mycelia in Response to Soil Grazers

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Conflict of interest

All authors declare no conflict of interest.

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Author contributions

CAAT and MDF conceived the paper. LB provided images of experiments. MDF performed the image analysis, CAAT did the statistical analysis. CAAT wrote the first version of the manuscript. All coauthors contributed in writing the final version of the manuscript.

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Abstract

Phenotypic plasticity is widespread among living organisms, but exceptionally high levels of plasticity are rarely observed in nature. While plasticity can enhance fitness in fluctuating environments, it comes with substantial costs that limit both its expression and evolution. Most of what we know about these limits comes from studies on animals and plants, which typically have fixed body plans. In contrast, modular organisms such as filamentous fungi possess an undetermined, decentralized morphology that allows individuals to adjust their phenotype in response to local environmental conditions. This flexibility has led to speculation that fungi exhibit "extreme phenotypic plasticity," making them an ideal system for studying plasticity mechanisms—yet empirical tests of this assumption remain scarce. To address this gap, we analyzed grazer-induced morphological plasticity in four cord-forming fungal species exposed to soil microfauna with differing feeding behaviors, using a phenotypic trajectory analysis framework and tested alternative hypotheses: One hypothesis is that the flexibility of fungal network development would allow species to converge on a common "grazing-resistant-phenotype". Alternatively, the developmental plasticity for each species might be heavily constrained within species-specific limits. Our results showed that fungal species accounted for most of the total morphological variation, while grazer induced morphologies remained relatively aligned to non-grazed morphological trajectories. Instead of convergence, we found that grazed- phenotypes were more dissimilar across species, reflecting distinct developmental trajectories within species-specific limits. The extent of plasticity varied widely with a maximum of 30% of the morphological variation attributable to grazing pressure. Nevertheless, these findings suggest that morphological plasticity plays a relatively minor role in response to grazing for most species. The dominant species signature and constrained plasticity supports the use of morphological attributes of mycelia as functional traits in ecological studies. Our findings provide new evidence on the extent of plasticity of modular organisms and highlight the need to extend plasticity theories to include organisms with non-determinate, modular body types like fungi.

Introduction

Determining which organisms achieve high levels of phenotypic plasticity has long been a central question in ecology and evolution (Sultan 2003, 2021; West-Eberhard 1989). In fluctuating environments, plasticity—the ability of a single genotype to alter its phenotype in response to environmental change (Pigliucci & Müller 2010)—is crucial for maintaining or enhancing fitness in the new conditions (Forsman 2015; Pfennig 2021). While all living organisms exhibit some degree of plasticity, extreme or "perfect" forms of plasticity are rarely found in nature (Gomulkiewicz & Stinchcombe 2022; Murren *et al.* 2015). This limited occurrence is thought to reflect the inherent costs of plasticity, including the energetic burden of modifying phenotypes and maintaining the regulatory systems needed to sense and respond to environmental variation (Auld *et al.* 2010; Schneider 2022). Yet, theory suggests that modular organisms—those lacking a fixed adult body plan and instead grow by iteration of repeated units or modules—may be less constrained in their plasticity compared to organisms determined body plans like animals (Murren *et al.* 2015). This modularity allows these organisms to adjust development and structure more flexibly in space and time compared to unitary organisms, potentially allowing them to evolve much higher degrees of plasticity. Yet, modular lineages remain underrepresented in studies of plasticity, leaving it unclear whether and how often extreme plasticity evolves in such systems.

Filamentous fungi represent a prime group in which to explore the potential for extreme phenotypic plasticity in modular organisms. This group is a dominant lineage in terrestrial ecosystems (Anthony *et al.* 2023) but have a fundamentally different body plan from that of animals as they grow as networks of interconnected filaments (hyphae) that together form a

80 mycelium (Fricker *et al.* 2017a) without a determined body morphology. Empirical evidence
81 shows that fungi can adjust their morphology in response to environmental variation. In
82 extreme cases, fungi can switch between unicellular (yeast-like) and filamentous (mycelium-
83 like) forms depending on conditions such as temperature, pH, or nutrient availability (Boyce &
84 Andrianopoulos 2015; Gauthier 2015). Within filamentous phenotypes, shifts in space
85 occupancy, colony shape, and pigmentation have been observed in response to resource
86 heterogeneity and environmental stress (Boddy 1999; Slepecky & Starmer 2009; Veresoglou *et*
87 *al.* 2018). This morphological flexibility has led to the proposition that fungi exhibit "extreme
88 phenotypic plasticity"(Klein & Paschke 2004; Slepecky & Starmer 2009; Ugalde & Rodriguez-
89 Urra 2014), and that because of such high levels of plasticity fungi are "the most adaptable
90 phylogenetic lineage in the Tree of Life" (Coleine *et al.* 2022). Yet research quantifying the
91 extent of plasticity in this group has remained scarce (Alster *et al.* 2021; Behm & Kiers 2014).

92 While plasticity can be evaluated in response to any environmental cue, the universe of
93 potential environmental variation leads to a factorial explosion in experimental design.
94 Particularly for fungi, environmental variation includes a plethora of microclimatic conditions,
95 resource heterogeneity and biotic interactions. Variation in resources alone stems from
96 differences in resource type, quantity, accessibility, quality and spatial distribution. However,
97 certain biotic response, such as predator-induced plasticity—where a genotype alters its
98 phenotype in response to the presence of a predator—provides a more tractable experimental
99 system where the extent of plasticity can be probed using a simpler presence-absence
100 experimental design. This approach has been widely used with animals to develop model
101 systems to understand the ecology and evolution of plasticity (Hoverman & Relyea 2007;

102 Tollrian & Harvell 1999). These studies typically involve quantitative comparison between the
103 phenotype of clonal genotypes in the presence and absence of a predator. Classic examples
104 with the model animal *Daphnia* show that exposure to predators induce pronounced changes in
105 its morphological traits (Spitze & Sadler 1996), a pattern that is observed across several other
106 animals including rotifers (Gilbert 2013; Yin *et al.* 2017), dragonflies (Flenner *et al.* 2009), snails
107 (Auld & Relyea 2011) and aphids (Sentis *et al.* 2019). Likewise in plants there is a growing
108 number of studies investigating how the presence of herbivores triggers morphological
109 plasticity (Dorey & Schiestl 2022; Fernández De Bobadilla *et al.* 2022). Furthermore, the
110 changes induced depend on the feeding habits of the herbivore (Lebbink *et al.* 2024).

111 Fungal grazing by soil fauna is analogous to animal predator-prey or plant-herbivore
112 interactions, thus making it a candidate to study plasticity of fungal phenotypes. In a similar
113 manner to experiments conducted in animals, fungal plasticity can be assessed by growing
114 clonal replicates of the same fungal genotype in the presence or absence of naturally occurring
115 grazers, using densities that reflect natural conditions (Crowther *et al.* 2012). There is also
116 evidence that two basic elements for plastic morphological response of fungal morphology to
117 grazing are present in fungi. First, grazing reduces fungal fitness (Tordoff *et al.* 2006, 2008) thus,
118 has the potential to impose evolutionary pressure for the emergence and maintenance of
119 grazer-induced plasticity. Such pressures are imposed by grazing by various different animals,
120 including nematodes, collembola, millipedes and isopods. As each of those grazers have
121 different feeding behaviour, plastic responses may be specific to the behavior of these
122 organisms (Crowther *et al.* 2012).

Second, there is evidence that fungi can detect the presence of grazers. For example, (Schmieder *et al.* 2019) identified sensory pathways activated in the model mushroom *Coprinopsis cinerea* when exposed to a fungivorous nematode. Similarly, the edible oyster mushroom (*Pleurotus ostreatus*) not only detects the presence of nematodes but also releases toxins and develops traps to immobilize and consume them under low-nitrogen conditions (Lee *et al.* 2020).

Until recently one of the main challenges of studying morphological plasticity of fungi has been the difficulty of quantitatively describing the structure of its modular body, that is, their mycelial networks. However, semi-automated pipelines have now been developed that provide multiple metrics describing fungal network morphology and predicted function (Dikec *et al.* 2020; Du *et al.* 2016; Fricker *et al.* 2017b; Heaton *et al.* 2012b; Sten *et al.* 2024; Vidal-Diez De Ulzurrun *et al.* 2015), including mycelia foraging for new resources on the surface of soil microcosms which represent a more realistic environment to measure behavioural responses than homogenous high resource growth media (Aguilar-Trigueros *et al.* 2022). Network-forming fungi do not forage by active movement in the way that animals do, but rather they explore their surrounding environment by growing out of a resource patch even when the surrounding area has no immediate resources available (Bielčik *et al.* 2019). An extreme case of this type of foraging ability is observed for “cord-forming fungi” that form hyphal aggregates termed cords which can be several mm thick and cm to meters long (Boddy 1999), forming macroscopically visible systems often covering large areas in the field (Fricker et al., 2007; Yafetto, 2018). Nutrients and water are transported within these cords between research patches and to and from mycelial fronts (Tlalka *et al.* 2002). Thus these group of fungi provide a tractable model

145 system to investigate morphological plasticity, particularly to grazing, given the numerous
146 studies reporting the impact of grazing on their fitness (reviewed by (Crowther *et al.* 2012).
147 Possible plastic morphological responses of cord-forming mycelia to grazing include the
148 formation of new interconnections providing alternative routes for transport, development of a
149 denser mycelium and thickening of cords to limit damage (Rotheray *et al.* 2008).

150 To determine the extent of grazer-induced plasticity in these fungi, we reanalyzed an existing
151 image dataset of cord-forming mycelia from four fungal species exposed to grazing by a range
152 soil micro-fauna with different feeding behaviour (Crowther *et al.* 2011). The original study
153 quantified changes in radial extension rate, hyphal coverage, fractal dimension, and wood
154 decay rate and detected significant fungal-grazer interactions (Crowther *et al.* 2011). The
155 experimental design parallels reaction norm studies typically used to evaluate predator-induced
156 plasticity (i.e. clones of the same genotype growing with or without grazers) and allowed us to
157 quantify changes in the network architecture of each fungal genotype in response to grazers.
158 Specifically, we measure a suite of mycelial morphological traits that capture the network
159 architecture of the fungal mycelium from time series images (Aguilar-Trigueros et al., 2022;
160 Fricker et al., 2007; Fricker et al., 2017). We used phenotypic trajectory analysis to quantify the
161 extent of developmental plasticity in response to grazing. This method, widely used to study
162 both evolutionary (Adams & Collyer 2009) and developmental plasticity (Metcalfe 2024) using
163 multiple traits (Hoverman & Relyea 2007), has been suggested as a powerful tool for studying
164 fungal plasticity (Behm & Kiers 2014).

165 Given the high degree of morphological plasticity expect in fungal mycelia and the strong
166 fitness pressures imposed by grazing, we tested two alternative hypotheses about how these

167 modular organisms express plasticity in response to environmental stress (Fig. 1). In the first
168 scenario—extreme plasticity—we hypothesized that different fungal species exposed to the
169 same grazing pressure would converge on a common morphological trajectory, producing a
170 shared, potentially optimal "grazing-resistant" phenotype. Under this scenario, plastic
171 responses effectively erase the original phenotypic distinctions between taxa and set their
172 developments into a new path. In contrast, under a constrained plasticity scenario, we
173 expected each species to modify its morphology in response to grazing but remain close to its
174 species-specific developmental trajectory. This scenario would reflect intrinsic limits to
175 plasticity shaped by lineage-specific constraints highlighting evolutionary fixed traits. By
176 disentangling these alternatives, our goal is not only to assess the extent of plasticity in
177 filamentous fungi, but also to uncover the extent that modular growth forms mediate adaptive
178 responses to environmental change.

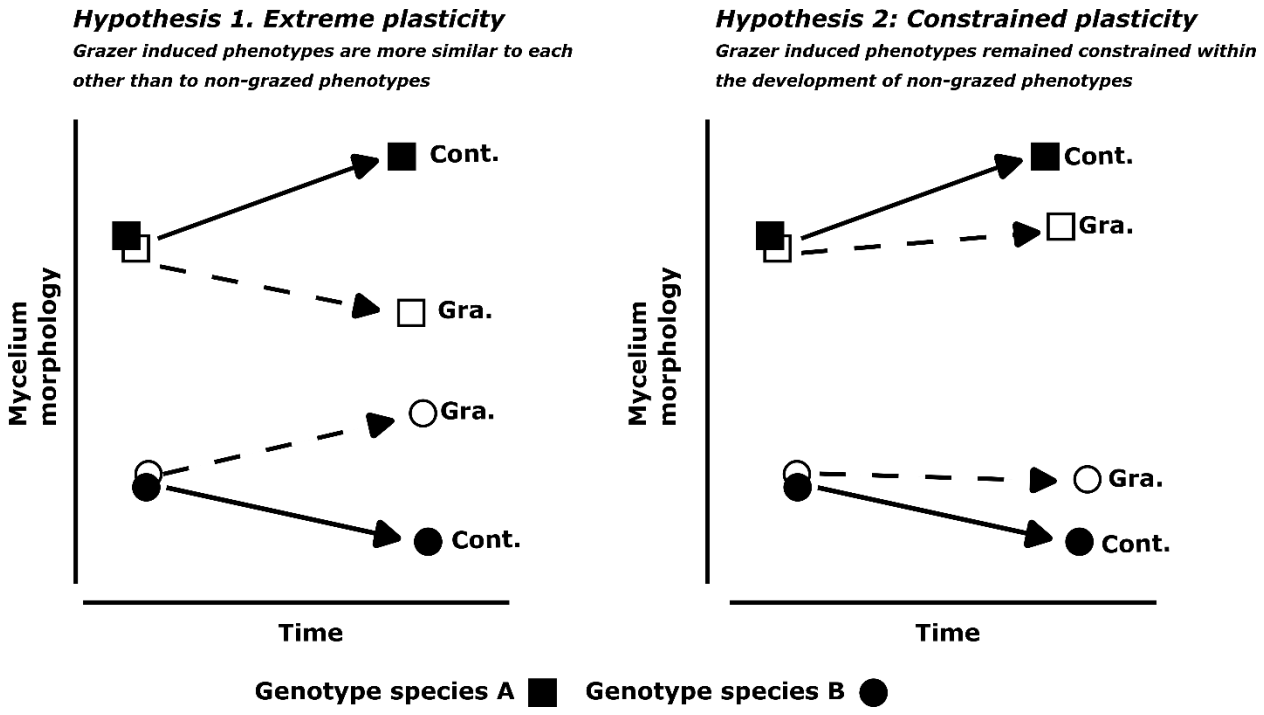

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Figure 1. Conceptual representation of hypotheses predicting fungal morphological plasticity in response to grazing. Under Hypothesis 1 (Extreme plasticity), grazer-induced phenotypes (open symbols, dotted lines) diverge so strongly from their non-grazed counterparts (closed symbols, solid lines) that they resemble grazer-induced phenotypes of other genotypes more than their own non-grazed phenotype. Under Hypothesis 2 (Constrained plasticity), grazer-induced phenotypes still differ from non-grazed ones, but remain within the trajectory defined by their genotype's inherent morphological trajectory.

Materials and methods

Experimental setup

The data for this study were derived from a time-series experiment initially designed to investigate fungal grazing interactions (Crowther *et al.*, 2011). The data set includes isolates from four species of wood-decomposing fungi: *Phallus impudicus*, *Phanerochaete velutina*, *Hypholoma fasiculare*, and *Resinicium bicolor* that were originally isolated from woodlands in the southern UK and are maintained in the Cardiff culture collection (Boddy 1999). Mycelium growth occurs by extension of cords (in length) and by cord thickening. In addition, new cords are created through branching, while some cords fuse with one another to cross-link the network. The resources necessary to sustain growth and metabolism are transported out of the wood block, which provides the only carbon-resource in the microcosm. Differences in morphology arise by changing the rates of cord extension, thickening, branching and fusion. Briefly, for each species, 30 clones from the same genotypes were cultivated from 20 × 20 × 10 mm beech blocks and allowed to grow across compressed soils in lidded 245 mm × 245 mm trays at 18C. The mycelia grew for 8-12 days until at least half of the replicates had reached a

202 diameter of 160-200 mm (see supplementary material for a detailed description of the imaging
203 settings). Five clones per species (i.e. replicates) were then exposed to five types of grazing
204 pressure, along with a set that was maintained grazer free that served as control. Grazing
205 pressure (i.e. grazing treatment) consisted of five grazing levels: collembola (*Folsomia candida*),
206 millipedes (*Blaniulus guttulatus*), woodlice 1 (*Oniscus asellus*), woodlice 2 (*Porcellio scaber*),
207 nematodes (*Panagrellus redivivus*) and a grazer-free control.

208 To monitor mycelial development over time, images were captured at the start of the
209 experiment (day 1, before grazer addition), and subsequently on days 2, 4, 8, 16, and 32. Here
210 we restrict analysis up to the 8-day time-point to ensure the network architecture was not
211 perturbed by physical constraints as some species reached the edge of the petri dish beyond
212 this day. Images were captured with pixel sizes ranging from 80 to 155 µm.

213 *Quantitation of mycelial network architecture*

214 We use a set of image processing algorithms to extract the mycelial morphology/architecture
215 from pictures as described in (Aguilar-Trigueros *et al.* 2022). Briefly, each picture was loaded
216 into a graphical user interface (GUI, Fig. S1) and processed through a standard pipeline that
217 included background correction, network enhancement, segmentation, skeletonization, width
218 estimation and network graph representation using protocols optimized across a range of
219 biological network systems (Fricker, et al., 2017; Obara et al., 2012; Pain et al., 2019; Xu et al.,
220 2021). For these macroscopic networks with cords of different thickness and reflectivity, the
221 mean-phase angle from intensity-independent phase congruency analysis gives marginally
222 better network enhancement performance compared to other standard approaches such as

223 second-order anisotropic Gaussian (Lopez-Molina *et al.* 2015; Vidal-Diez De Ulzurrun *et al.*
224 2015) or Bowler-Hat (Oyarte Galvez *et al.* 2025) algorithms used for networks grown on
225 transparent media, although all are implemented in the software. The software package and
226 complete manual explaining in detail the steps involved is freely available from Zenodo (DOI:
227 10.5281/zenodo.5187932). The output of this pipeline is a network graph representation of the
228 mycelia where the tips, cord branching and cord fusion events are as nodes whilst the cords
229 themselves form 'edges' connecting the nodes. (Fig. S1). The length and width of each edge was
230 automatically measured, whilst the (*x,y*) position and branching angle were recorded for each
231 node. It was not possible to resolve edges within the inoculum, so these are represented as
232 strong connections between each incident edge on the resource and a central node (termed
233 the 'Root' of the network). This information was exported in two tables as .csv and .xlsx files
234 containing the cord (edge) information and the tips, branching and fusion points (node)
235 information. The node and edge tables from the image processing pipeline were imported into
236 R (using the *read_excel* function from the readxl package (Wickham *et al.* 2019) and used to
237 construct a spatially explicit transport network embedded in 2D space given by the Euclidean
238 coordinates of the nodes using the igraph package in R (Csardi G & Nepusz T 2006) (Figure 2.
239 See also details in the code available in the github repo
240 https://github.com/aguilart/Fungal_Networks).

241 *Traits used to define mycelial network morphology*

242 To measure mycelium morphology, we first identified the cords that represent the main routes
243 of the network as the minimum spanning tree (MST) of cords that confer the least transport
244 resistance from the wood block to the foraging tips at the front. Cords that were not part of the

245 MST were recorded as secondary routes that enhance cross-linking between the main cord
246 routes. We measured a set of 22 network metrics that reflect the overall colony morphology,
247 network heterogeneity, space filling and connectivity, predicted transport and predicted
248 robustness to damage (Table 1, S1). Predicted transport in the network was simplified as
249 unidirectional fluid flow from the wood block to the growing front of the network by assuming
250 cords can be represented as a contiguous network of interconnected bundles of cylindrical
251 shape. The predicted resistance to flow scales with cord length and inversely to the square of
252 the radius of the cord (i.e. *resistance* $\mu\ l/r^2$) {Bebber, 2007 #1793;Fricker, 2007 #2029}. Thus,
253 long and thin cords provide higher resistance. This is a simplification of transport dynamics of
254 mycelia but matches well with empirical distributions of radiolabeled nutrient movement in
255 these networks (Heaton *et al.* 2010, 2012a).

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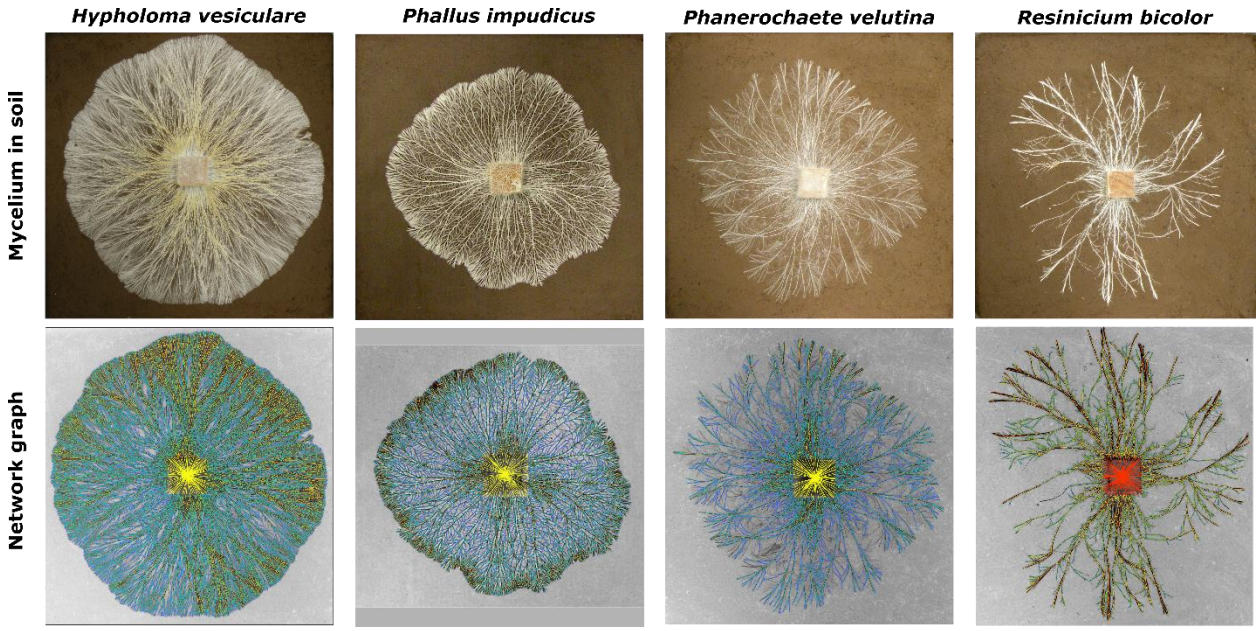


Figure 2. Translating cord-forming fungi into a weighted transport network. *Top row*: Photographs of mycelia from the four fungal species used in this study. The fungi grow out from 2×2x1 cm wood blocks, forming millimeter-thick cords that explore the surrounding environment in search of new resources. Fungi were grown under laboratory conditions on compressed soil in 245 mm × 245 mm trays. *Bottom row*: Network graphs overlaid on inverted grayscale images of the mycelia. These graphs were generated following intensity-independent phase congruency edge enhancement, local adaptive thresholding and skeletonization of the cords and integrated granulometry to measure the cord width. Cords are represented as network edges connected by nodes comprising tips, branching points, and fusion points (anastomoses). Cord width is visualized with color: green for thick cords, blue for thin cords. For visualization purposes, the inoculum is represented as multiple linear edges in yellow, but these are not included in the analysis.

273 **Table 1. Description of the five trait types used in this study**. These traits summarize different

274 patterns of the whole mycelium (see table S1 for full list of 21 metrics measured).

Type	*Biological meaning*	*Trait*
1. Cord and colony morphology	Size and shape of the whole colony and of the cords	Median cord length and width (main and secondary routes), median branching angle, colony circularity.
2. Network heterogeneity	Variation in how fungi build cords within the network	Skewness of cord width and length of main routes.
3. Space filling and connectivity	Area explored for resources and extent of network interconnection	Mycelial density, loop density (meshedness), investment in secondary routes.
4. Predicted transport	Estimated average transport flow through cords and its variation within the network	Expected average transport from inoculum to margin, expected multidirectional transport, skewness of transport from inoculum to margin.
5. Predicted robustness to damage	Expected robustness to removal of cords of the network	Percentage of cords that can be removed before network volume drops below 50%; removal scenarios include random, targeted by cord width, and targeted by cord length.

275

276 *Phenotypic trajectory analysis to measure morphological developmental plasticity*

277 Phenotypic trajectory analysis relies on the use of multivariate ordination methods to

278 summarize the change through time in phenotypes across multiple traits combined using

279 ANOVA-like frameworks for statistical testing (Adams & Collyer 2009; Behm & Kiers 2014). In

280 this study, we used three types of permutational multivariate analyses of variance models

281 (PERMANOVA) to determine level of grazer-induced plasticity across the fungal species using

282 the 22 metrics. In all the models, we controlled for initial differences in inoculum growth at the

283 start of the experiment by using the first two axes from a principal component analysis (PCA) of

284 traits at time 1 (i.e. immediately before the addition of the grazers) as covariate in the
285 PERMANOVA.

286 In the first and more general model, we tested whether induced changes in the development of
287 the morphology (plasticity) converge to a common grazing-resistant -phenotype across all four
288 species (Hypothesis 1, extreme plasticity) or whether plastic response remained constrained
289 close to the trajectories of non-grazed phenotype per species. In this model, time, fungal
290 species identity, and grazer type were used as fixed factors and PC1 and PC2 of the traits used
291 at the start of the experiment to control for initial conditions (see table 2 for the full formula
292 used in this model). If fungal species converge to a common phenotype, we expect to detect a
293 strong (and significant) main effect of grazer together with an interaction between grazer and
294 time. That is, strong interaction grazer x time suggests more deviation of the development of
295 the morphology of grazer-exposed clones compared to the grazer-free clones, while strong
296 main grazer effect suggest similarity of morphology of the grazed phenotype while.

297 In the second model, we determine which grazers hand the strongest effect in inducing a
298 common phenotype at the end of the experiment. We ran five PERMANOA models (i.e. one for
299 each grazer) where the morphological traits of all four fungal species were used as response
300 variables while the only fixed factor was the grazer treatment with two levels (presence or
301 absence). Convergence would be shown by a significant, large effect size of grazer identity. To
302 further visualize and validate the output of these models, we complemented this model by
303 testing whether the grazers increase the similarities among fungal species compared to the
304 control. This test was done by measuring the distances between each fungal species across the
305 respective grazer treatments to their multivariate mean (centroids) followed by a permutation

306 based-test for multivariate homogeneity of group dispersions (implemented with the function
307 *betadisper* from the package *vegan* in R). If grazers increase the similarity (or decrease it) it
308 would be shown as a strong and significant reduction in the distances between each species
309 and their respective centroids.

310 The third model tested which fungal species had the most plastic response to grazers. We ran
311 PERMANOVA models for each fungal species. Thus, this test measured the extent of
312 development plasticity of fungal species irrespective convergence to a common phenotype.
313 Finally, for the fungal species showing the largest plastic responses, we ran a set of models with
314 each grazer separately to identify which trait drove the plastic responses.

315 In all PERMANOVA models, we used a forward model selection approach to determine which
316 fixed factors should be retained. We used Principal Component Analysis (PCA) to visualize the
317 changes in the phenotype through time. Analysis of the distances to each centroid were done
318 using Euclidean distances. Effect sizes were measured as the proportion of explained variance
319 of the fixed factors. PERMANOVA, PCA, analysis of distances to centroids, model selection and
320 effect sizes were calculated using functions from the vegan package (Oksanen *et al.* 2007). All
321 statistical analysis were conducted in R (R Core Team 2013).

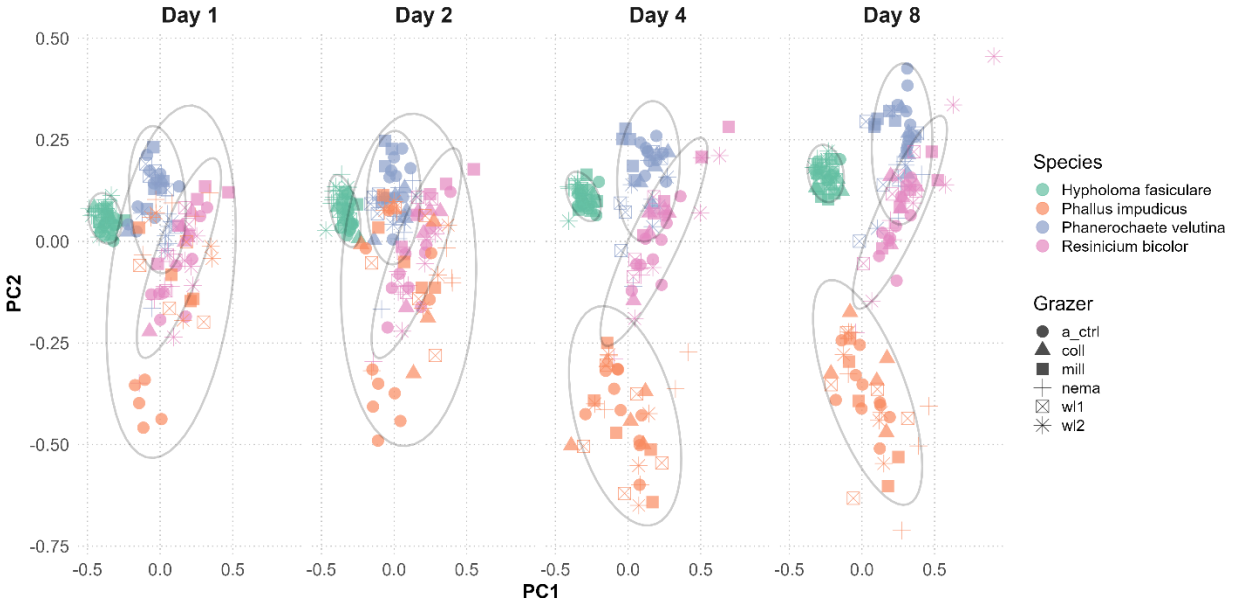
322 **Results**

323 *Support for constrained plastic response rather than extreme plasticity*

324 Species identity had the strongest effect, explaining up to 90% of the variation in morphology
325 across all treatments and time points using model 1 (Fig. 3). We found significant effects of

326 grazer identity and an interaction with time, but neither effect was larger than 5% of the total

327 variation (Table 2).



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329 Figure 3. **Figure 3. PCA showing the temporal development of mycelial morphology in four**

330 **fungal species.** At the start of the experiment, species show similar mycelial traits, but as time

331 progresses, distinct species- (genotype-) specific morphologies emerge. While the presence of

332 grazers alters these developmental trajectories (visible as a spread in the data), grazer-induced

333 phenotypes remain constrained within the morphological space of each genotype (ellipses

334 around the phenotypes per genotype/species).

335 Table 2. PERMANOVA results with effect sizes for the first PERMANOVA model that includes fungal
336 species, grazer identity and developmental time. The variation in the initial condition for each colony
337 was corrected using PC1 and PC2 co-ordinates for day 1 as co-variates.

SOURCE	DF	VARIANCE	F	R2	PR(>F)
SPECIES	3	8.5397	208.4704	0.4314	<0.001
GRAZER	5	0.5294	7.7541	0.0267	<0.001
DAY	1	0.8777	64.2807	0.0443	<0.001
SPECIES:DAY	3	1.1389	27.8028	0.0575	<0.001

SPECIES:GRAZER	15	1.2921	6.3084	0.0653	<0.001
GRAZER:DAY	5	0.1542	2.2581	0.0078	<0.001
SPECIES:GRAZER:DAY	15	0.5309	2.5923	0.0268	<0.001
RESIDUAL	493	6.7317			

In the second set of models that focus on the effect of each grazer separately, we did not find support for convergence to a common phenotype. Instead, we found the opposite, that is, we found a significant effect for increasing dispersion among genotypes in grazer-induced morphology (except for Collembola for which the effect was not significant) (Fig. 4). Effect size varied from 5 to 10% and was strongest for woodlice 1 (Oniscus), followed by millipede, woodlice 2 (Porcellio) and nematode treatment. Taken together, these results show first that grazer-induced plasticity was detectable across fungal species, but it was constrained within the trajectory of each genotype and second, that instead of converging to a common phenotype it increased the dissimilarity of the phenotypes.

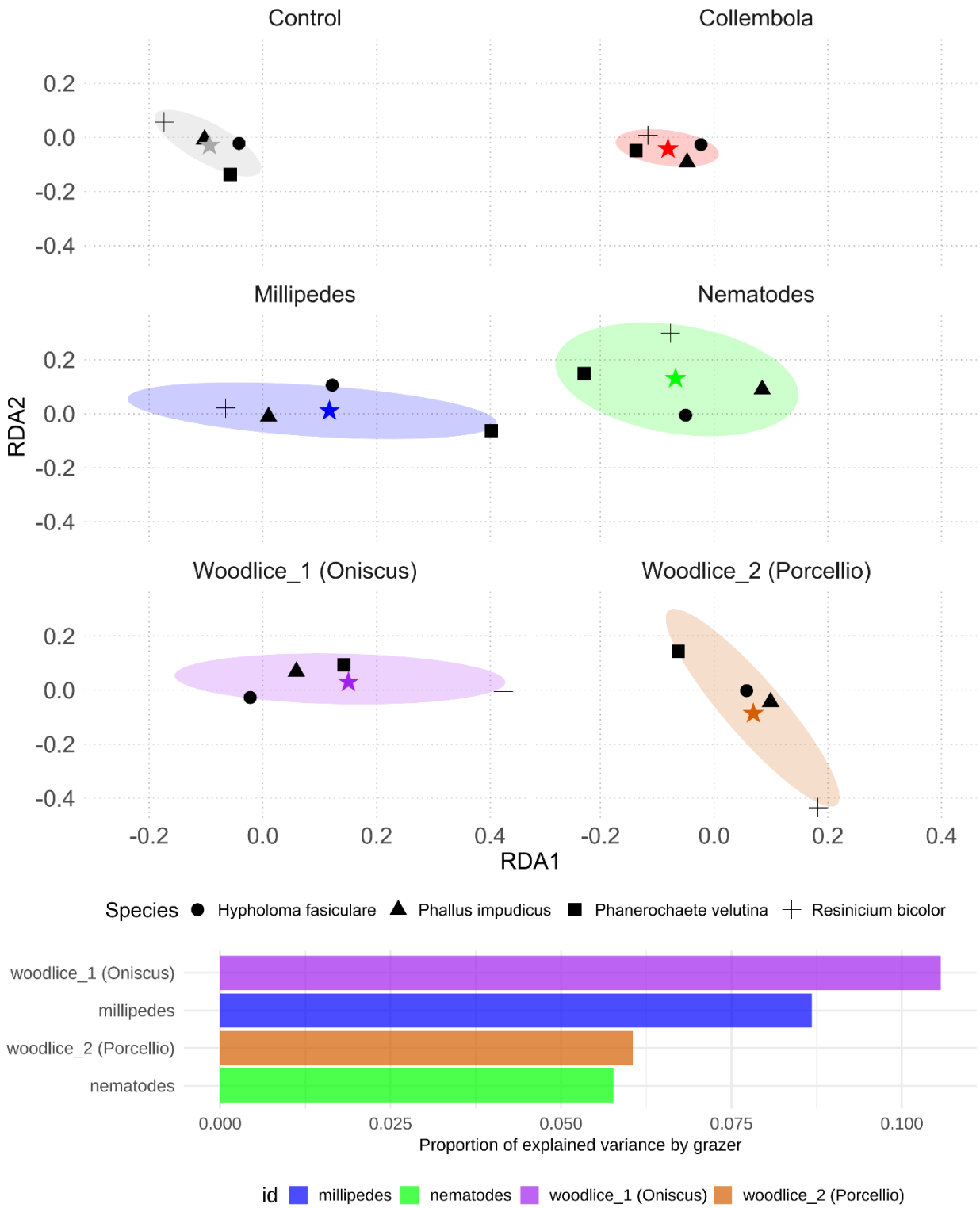


Figure 4. Testing for convergence or divergence in fungal phenotypes under grazer pressure. Each ordination plot shows the average mycelial morphotype of each genotype per grazing treatment (circle = *H. fasciculare*, triangle = *P. impudicus*, square = *P. velutina*, cross = *R.*

bicolor). The across-genotype average per grazer treatment is shown as a star. Dispersion around this centroid is visualized with shaded ellipses. With the exception of collembola, where no significant effect was detected, all grazer treatments increased morphological dispersion. Bar plots at the bottom indicate the effect size of grazer-induced changes in dispersion for significant treatments.

Trajectories of grazer-induced plasticity differed in magnitude and direction among fungal species

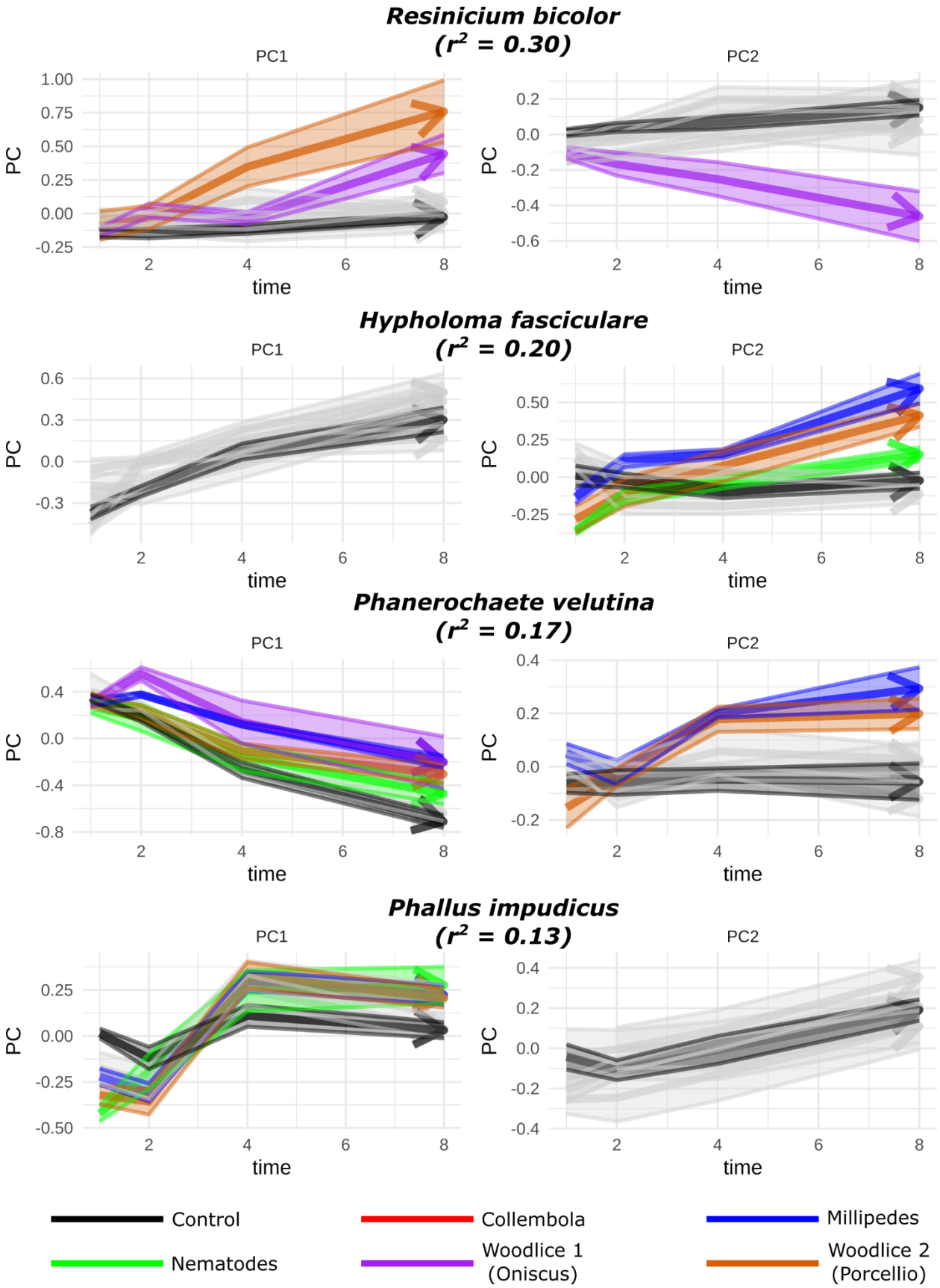
We found large variation in the level of plasticity among the fungal species tested across the grazers (Fig. 5). *R. bicolor* exhibited the highest morphological plasticity in response to grazers, with up to 30% of the variation in morphology attributable to grazing ($p < 0.01$), particularly in response to the two woodlice species (Figs. 6). In both cases, RDA plots revealed clear separation between grazed and ungrazed genotypes of this species (Fig. S2). Multiple traits contributed to this divergence—indicating a broadly distributed response rather than dominance by a single or few traits (Fig. 6). Among the two woodlice, woodlouse 1 (*Oniscus*) had the stronger effect. The overall colony shape remained stable when exposed to this grazer, but the cord morphology shifted to shorter, thinner cords with straighter branching angles. A more prominent change was the increase in cord density, although loop density was unaffected. Despite these structural changes, the predicted transport dynamics remained stable, and robustness even improved in this treatment, particularly against random attacks ortargeted attacks on main cords.

Colonies exposed to Woodlice 2 (*Porcellio*) became more circular and exhibited similar changes in cord morphology—short, thin cords with straight branching angles. Cord heterogeneity also shifted: cord lengths became more even, while width distribution became more uneven, suggesting the emergence of a few dominant thick cords. In this treatment, the number of

loops decreased while density remained stable. These changes were associated with reduced predicted transport efficiency, especially for multidirectional transport, and increased variability in robustness to random attacks. Importantly, robustness to attacks on thick cords declined, indicating these few thick cords were critical for network connectivity and vulnerable to disruption.

H. fasiculare showed the second-highest response with ca. 20% ($p < 0.05$) of morphological variation explained by the presence of the grazer (Fig. 5). This effect was primarily driven by exposure to millipedes with up to 23% explained variation attributed to this grazer. In this case, only a subset of traits shifted, including the development of a more irregular colony shape and a marked decrease in loop number. These structural changes were associated with a reduction in predicted transport efficiency and a sharp increase in the heterogeneity of main transport cords. However, predicted robustness remained largely unaffected, with only a slight increase in robustness to attacks on thin cords. Responses to Woodlice 2 (*Porcellio*) and nematodes were weaker (with around 9% of explained variation attributed to the presence of these grazers (Fig. 6)) and more variable across replicates, as reflected in less consistent RDA separation (Fig. S2).

P. velutinae and *P. impudicus* exhibited the lowest morphological plasticity, with only 17% and 13% of trait variation attributed to grazer effects, respectively Fig. 5. Although these effects were statistically significant, RDA plots showed inconsistent separation between treatment groups (Fig. S2), suggesting limited and variable responses across replicates.


Resinicium bicolor
(r² = 0.30)
PC1
PC2
Hypholoma fasciculare
(r² = 0.20)
PC1
PC2
Phanerochaete velutina
(r² = 0.17)
PC1
PC2
Phallus impudicus
(r² = 0.13)
PC1
PC2
PC
time
Control
Collembola
Millipedes
Nematodes
Woodlice 1 (Oniscus)
Woodlice 2 (Porcellio)

399

Figure 5. Species-specific trajectories of mycelial morphological development over time and deviations caused by grazer presence. The greatest grazer-induced deviation was observed in *R. bicolor*, where grazers explained 30% of the variation in morphology through time, followed by *H. fasciculare*, *P. velutina*, and *P. impudicus*. Trajectories of grazer-induced morphologies that differ by more than twice the joint standard error from the non-grazed phenotypes are color-coded to highlight these contrasts.

.

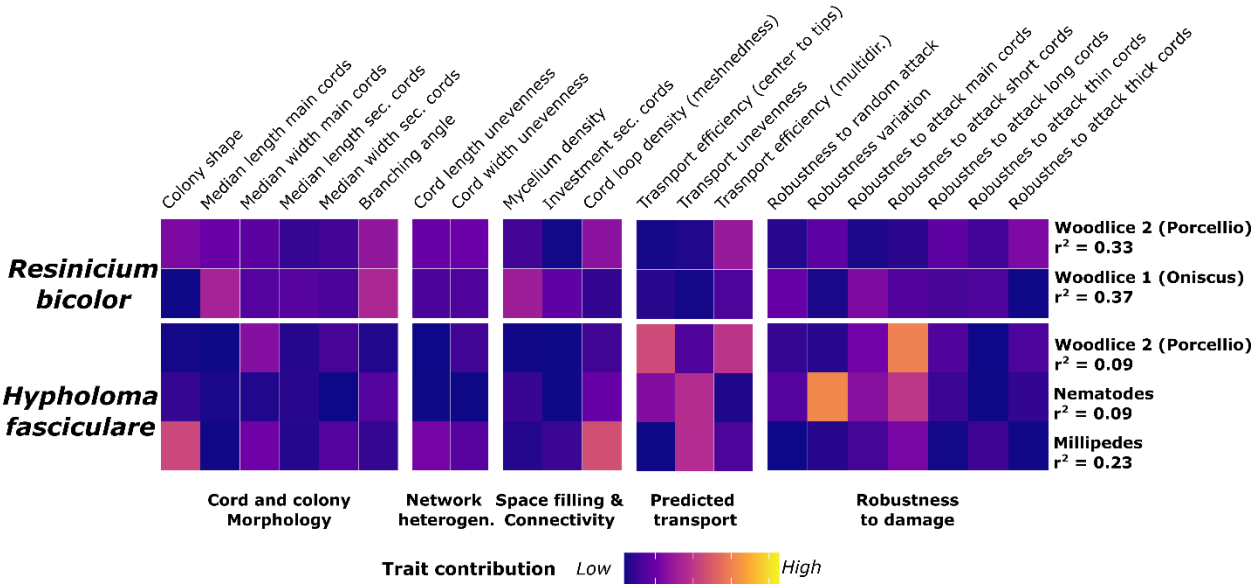


Figure 6. Heatmap of morphological trait shifts between grazed and non-grazed genotypes at the end of the experiment for *R. bicolor* and *H. fasciculare*. These two species showed the strongest grazer-induced deviations in morphology (see Figure 5). For each species, the grazer treatment with the largest deviation is shown, along with the percentage of explained variation. Corresponding RDA biplots can be found in Figure S2.

414 **Discussion**

415 We tested whether grazing pressure induced convergence towards a common mycelial
416 phenotype or, alternatively, whether plasticity remained constrained within the trajectories of
417 non-grazed genotypes. We found support for the latter, where despite grazer induced effects
418 altering the morphology of each species, the effect was small and genotypes retained
419 characteristic patterns across all treatments. In fact, we found that changes induced led to
420 increased dissimilarities among grazed genotypes rather than convergence to a particular
421 grazing-resistant phenotype, as observed for grazing in other kingdoms. These results are
422 surprising based on the assumption that fungi have the capacity to vary their morphology
423 significantly (extreme plasticity) in comparison to that of animals and plants (Slepecky &
424 Starmer 2009). These results suggest that the mycelium morphology has a more limited
425 flexibility to reconfigure itself than previously thought. It also suggests that morphological
426 plasticity may not be the most effective means to deal with grazing pressure.

427 *Strong species differences suggest limits in morphological plasticity*

428 Although some of our species exhibited considerable plasticity in response to grazers, these
429 changes were not strong enough to override genotype-specific differences. In other words, the
430 morphological trajectories of each fungal genotype remained constrained within distinct
431 boundaries defined by genotype identity. This pattern suggests that the plasticity of cord-
432 forming fungi is constrained within certain species-specific limits, at least in response to
433 grazing. In addition, these limits varied among species, with *R. bicolor* showing up to 30%

434 variation in morphology due to grazing pressure, while *P. impudicus* exhibited only around 10%
435 change in response to grazers.

436 The low levels of plasiticity in *P. impudicus* might be explained by several factors. It could be
437 that the architecture of *P. impudicus* constitutively embodies grazing-resistant features, such as
438 a high level of cross-linking, however this explanation is unlikely as there was no conversion
439 into a common grazed phenotype for the other species. In theory, when genotypes show low
440 levels of grazer-induced plasticity it can explained by the lack of sensory systems to detect and
441 respond to grazers (Murren *et al.* 2015) or show variation in the metabolic cost of changing the
442 phenotype (Auld *et al.* 2010). While we do not have direct evidence of sensory mechanisms for
443 our species, we expect they are present as they are in closely related taxa (e.g. *Coprionopsis*
444 *cinerea* and *Pleurotus ostreatus*) are known to respond to at least nematodes and collembola
445 (Plaza *et al.* 2016; Schmieder *et al.* 2019). Thus, more likely, lower levels of plasticity may be
446 associated with variation in the metabolic cost of altering the morphology of mycelial
447 phenotype sufficiently to achieve resistance to grazing in comparison to allocation to other
448 forms of defence. We speculate these costs may reflect differences in the construction of cords.
449 There is evidence that the level complexity in the histology of the cords varies across species
450 (Thompson & Rayner 1983; Townsend 1954). For example, *P. impudicus,* the least plastic
451 species in our study, is at the more complex end of the spectrum with clear differentiation of
452 anatomical regions within the cord compared to that of other cord-forming fungi (Eamus *et al.*
453 1985; Townsend 1954). Likewise, *P. velutina*, which also shows relatively low plasticity in this
454 study, has also been reported as showing zone differentiation in its cord anatomy (Thompson &
455 Rayner 1983). In contrast *H. fasciculare*, with relative higher levels of plasticity in our study, is

456 reported to on the lower end of cord complexity (Thompson & Rayner 1983). We have not
457 found anatomical studies of the cords of *R. bicolor* (the species with the most plasticity in our
458 study), but it is a fast-growing-species (Zakaria & Boddy 2002) a life-history trait that is
459 commonly associated with lower investment in resources in the construction of an organism´s
460 body (Wright *et al.* 2004). Thus, we hypothesized with increasing complexity of the cord-
461 forming mycelia is part of a trade-off with the ability to respond to grazer pressure. For species
462 with less plastic and more complex phenotypes, modifying the network architecture may
463 jeopardize efficient transport which might outweigh any benefit to morphological responses to
464 grazing.

465 We also emphasize that these observed limits to grazer-induced plasticity may not reflect the
466 potential for morphological plasticity in response to other environmental factors. For example,
467 we observed relatively high variation of mycelium morphology at the start of the experiment
468 within replicates of the same genotype reflecting initial differences in mycelial growth before
469 the addition of grazers (Fig. S3). Such differences may arise from inherent heterogeneity in
470 resource quality, or the extent colonization of wood blocks used as inoculum in these
471 experiments. Such differences may have long lasting effect determining the trajectory of the
472 developing mycelia. Such effects have been reported for cord-forming fungi, where the
473 intensity of colonization of wood blocks can strongly the development of the mycelium even
474 across generations (Fukasawa *et al.* 2020). Overall, while our results indicate limits to grazer-
475 induced plasticity in cord-forming fungi, they also suggest that other factors such as resource
476 heterogeneity may drive greater levels of morphological plasticity.

Lack of convergence may be due to weak evolutionary pressure of fungal grazing for induced morphological plasticity

Although we cannot determine whether the observed grazer-induced plasticity is adaptive—such a study would require long-term monitoring of fitness consequences of plastic phenotypes—our findings do not support that fungal grazing exerts strong evolutionary pressure for the development of adaptive morphology. We found no evidence of a convergent 'grazing-resistant phenotype' across species and instead observed substantial variation in plastic responses. The emergence of a common adaptive phenotype typically requires strong and consistent environmental pressures. Given the diverse community of fungal grazers present in soils, optimizing morphology for one grazer may offer limited fitness benefits if it compromises adaptability to other grazers or environmental factors (Einum *et al.* 2019). Even if such optimal phenotype exist, the fact that we did not observe a response in our time window indicates that individuals would spend a significant part of their life cycle in a sub-optimal transitioning phenotype, a feature of weak selection pressure (Einum & Burton 2023).

We also note, however, that grazing can still impose strong evolutionary pressure for the evolution of plasticity in other traits that are not morphological. Phenotypic convergence could be exhibited in traits not related to morphology that we did not measure in this study as “hidden plasticity” (Forsman 2015), such as production of chemical compounds that reduce palatability and intoxicate the grazers. This type of responses is well documented in in plants (Fernández De Bobadilla *et al.* 2022) and more recently it has been found that some fungi produce compounds with similar functionality (Lee *et al.* 2020; Plaza *et al.* 2016; Schmieder *et al.* 2019). So, while the apparent target morphological phenotype between grazed and

499 ungrazed environment are different, “hidden” grazer induced plasticity in the form of altered
500 chemistry may act as compensatory mechanism to maintain the apparent dissimilar phenotype
501 between the two environments (Metcalfe 2024).

502 *Implications of plasticity limits in trait-based fungal ecology*

503 The significant differences between species but limited morphological plasticity with species
504 suggest that mycelium morphology embodies useful functional traits to understand community
505 assembly and ecosystem processes (Mcgill *et al.* 2006). In community ecology, traits that
506 exhibit lower within-species than among-species variation can reflect consistent ecological
507 strategies and predict patterns of species composition along environmental gradients (Franklin
508 *et al.* 2020; HilleRisLambers *et al.* 2012). For example, lab-derived traits in marine plankton and
509 zooplankton have successfully predicted seasonal shifts and habitat filtering in natural settings
510 (Edwards *et al.* 2013; Vogt *et al.* 2013). If fungal mycelial traits reflect species differences, they
511 could also predict how shifts in fungal community composition along environmental gradients
512 impact ecosystem processes, such as carbon cycling. Cord-forming fungi, for instance, play a
513 key role in lignin decomposition, and morphological traits may influence decomposition
514 efficiency, thereby affecting carbon budgets. Although speculative, these findings suggest that
515 predictable relationships between mycelial morphology, environmental conditions, and
516 ecosystem functions warrant further exploration.

517 *Implications of using fungi as model systems for studying the ecology and evolution of plasticity*

518 Moving forward, it remains to be tested whether non-cord-forming fungi exhibit the expected
519 extreme plasticity attributed to fungi (Klein & Paschke 2004; Slepecky & Starmer 2009). Given

that phenotypic plasticity tends to evolve in highly variable environments (Lande 2009), fungi inhabiting soils—among the most heterogeneous habitats—would be expected to show strong selective pressures for high morphological plasticity. Evolutionary models suggest that filamentous growth forms, which are particularly advantageous in spatially heterogeneous environments like soils, would not have evolved in more homogeneous aquatic habitats (Heaton *et al.* 2020). This parallels findings in ectotherms, where greater plasticity is observed in terrestrial species compared to aquatic ones, likely due to higher environmental variability on land (Einum & Burton 2023). However, our results suggest limited plasticity in cord-forming fungi, a phenotype that has evolved repeatedly in the Basidiomycota (Monk 2014). One possible explanation for this reduced plasticity is that cord-forming fungi, with their complex and energetically costly growth structures, may have evolved to optimize efficiency in specific ecological roles. This specialization could limit the need for extensive morphological plasticity. Understanding the evolutionary pressures that led to the emergence and maintenance of such behavior in less plastic fungi would be crucial, particularly in relation to their role in ecosystem processes like carbon cycling.

Overall, our study highlights how fungi can expand our understanding of plasticity. Existing theories, largely developed for organisms with deterministic body plans, need refinement to better explain how phenotypic plasticity emerges and is maintained in organisms with complex, adaptable structures that evolved in variable environments. Our findings suggest that investigating these questions in cord-forming fungi may provide new insights into the constraints of morphological plasticity under diverse and dynamic ecological conditions.

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