Testing the extreme plastic mycelium hypothesis: Does grazing induce developmental plasticity in saprotrophic fungi?

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Conflict of interest

All authors declare no conflict of interest.

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Abstract

Phenotypic plasticity—the ability of a genotype to alter its phenotype in response to environmental changes—can play a crucial role in maximizing fitness in fluctuating environments. However, plasticity comes at a cost, as the energy required to transform a phenotype imposes limits on its extent. While limits to plasticity are well-documented in many organisms, they are not well understood in modular organisms like filamentous fungi. Fungi have highly flexible morphologies, allowing individual organisms 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 interesting system for studying plasticity mechanisms. To test this idea, we analyzed the grazer-induced morphological plasticity of four cord-forming fungal species exposed to different soil microfauna with varying feeding behaviors, using a phenotypic trajectory analysis framework. We hypothesized that grazing would drive convergence towards a common "grazing-resistant phenotype" across species, due to the extreme flexibility of fungal mycelia. Alternatively, we proposed that species would follow unique developmental trajectories in response to grazers, reflecting species-specific plasticity limits. Our results showed that fungal species accounted for most of the morphological variation, with grazers having only a small but significant effect. Moreover, we did not observe convergence towards a common grazing-resistant phenotype; instead, each species followed distinct developmental pathways within species-specific limits, which varied widely. Some species altered up to 30% of their phenotype in response to grazers, while others showed as little as 1% variation. These findings suggest a more nuanced view of fungal plasticity, where morphological plasticity might not be the most effective response to grazing pressure. We speculate that the observed differences in plasticity limits may correlate with the complexity of cord formation in different species. These constraints on fungal morphological plasticity offer new insights into how fungi adapt to dynamic ecological conditions and underscore the need to explore alternative phenotypic responses to environmental pressures. Our study highlights the need to refine plasticity theories, particularly for organisms with complex, modular structures like fungi.

Introduction

Phenotypic plasticity—the ability of a genotype to alter its phenotype in response to environmental changes—is widespread across the tree of life and has been a focal point of research in ecology and evolution (West-Eberhard, 1989; Pigliucci, 2001). In fluctuating environments, plasticity can be crucial for maximizing fitness in the new conditions (Forsman, 2015; Pfennig, 2021). However, the extent of phenotypic plasticity varies widely. Studies using "reaction norms"— quantitative tracking of phenotypic changes of a given genotype along environmental gradients (Gomulkiewicz & Stinchcombe, 2022)—have shown that the extent of plasticity varies across major clades and life forms (Sultan, 2021) as well as within species in a clade (Sultan, 2003). This variation highlights that while phenotypic plasticity can provide adaptive advantages, it is also constrained by costs. These costs arise from the need to maintain and activate the physiological machinery to detect environmental changes and the energetic demands required to reshape the phenotype, ultimately limiting the extent of plastic responses in many organisms (Auld *et al.*, 2010; Schneider, 2022).

The phenotypic plasticity of filamentous fungi, a dominant group of organisms in terrestrial ecosystems (Anthony *et al.*, 2023), has only recently gained formal attention in ecological and evolutionary studies (Behm & Kiers, 2014; Alster *et al.*, 2021). Yet, filamentous fungi possess exceptional attributes to help understand plasticity. Most fungi are modular organisms, and as such, have no definitive adult phenotype. Instead, their body consists of a network of interconnected hyphal filaments forming a mycelium (Fricker et al 2017). This structural flexibility allows them to alter their overall morphology in response to local variation in their environment. At the most extreme, fungi can switch between unicellular (yeast-like) and

filamentous (mycelium-like) growth depending on conditions such as temperature, pH or nutrient availability (Boyce & Andrianopoulos, 2015; Gauthier, 2015). Within filamentous phenotypes, morphological changes—including shifts in space occupancy, colony shape, and color—have been documented across various resource gradients and in response to biotic and abiotic stress (Boddy, Yu Fukusawa papers (Slepecky & Starmer, 2009; Veresoglou *et al.*, 2018). This high degree of morphological flexibility has led some researchers to characterize fungi as exhibiting "extreme phenotypic plasticity"(Klein & Paschke, 2004; Slepecky & Starmer, 2009; Ugalde & Rodriguez-Urra, 2014). As such, fungi provide an opportunity to understand how filamentous modular growth can potentially accommodate the cost of plasticity that constrain other taxonomic groups.

While plasticity can be evaluated along any environmental cue, the universe of potential environmental variation to test it can be daunting. Particularly for saprotrophic fungi, environmental variation may stem from, among others, differences in resource type, quantity, and spatial distribution, in addition to abiotic factors, such as temperature, humidity or soil structure. However, predator-induced plasticity—where a genotype alters its phenotype in response to the presence of a predator— may provide a more tractable experimental set up where the extent of plasticity can be probed using a simpler presence-absence experimental design. This approach has been widely used in other kingdoms as a model system to understand the ecology and evolution of plasticity (Tollrian & Harvell, 1999; Hoverman & Relyea, 2007). These studies typically involve quantitative comparison between the phenotype of clonal genotypes in the presence and absence of a predator. Classic examples come from animal systems, such as *Daphnia*, where exposure to predators induces significant changes in

91 their morphological traits (Spitze & Sadler, 1996), as well as other animals including rotifers
92 (Gilbert, 2013; Yin *et al.*, 2017), dragonflies (Flenner *et al.*, 2009), snails (Auld & Relyea, 2010)
93 and aphids (Sentis *et al.*, 2019). Likewise in plants there is a growing number of studies
94 investigating how the presence of herbivores triggers morphological plasticity (see for example,
95 Dorey & Schiestl, 2022). Furthermore, the changes induced depend on the feeding habits of the
96 herbivore (Lebbink *et al.*, 2024). This plasticity is believed to have arisen in evolution because,
97 when predation exerts a strong pressure, the new phenotype becomes more robust and thus
98 confers higher fitness.

99 Fungal grazing by soil fauna is analogous to animal predator-prey or plant-herbivore
100 interactions, thus making it a candidate to study plasticity of fungal phenotypes. In a similar
101 manner to experiments conducted in animals, fungal plasticity can be assessed by growing
102 clonal replicates of the same fungal genotype in the presence or absence of naturally occurring
103 grazers, using densities that reflect natural conditions. Moreover, other lines of evidence have
104 shown the existence of basic elements for plastic response of the fungal morphology to grazing.
105 First, grazing reduces fungal fitness (Tordoff *et al.*, 2006; Tordoff *et al.*, 2008) thus, likely
106 imposing strong evolutionary pressure for the emergence and maintenance grazer-induced
107 plasticity. With a diverse array of grazing soil fauna, including nematodes, collembola, and
108 millipedes, fungi encounter varying degrees of grazing pressure due to differences in the
109 biology and behavior of these organisms (Crowther *et al.*, 2012a).

110 Second, there is evidence that fungi have sensory systems to detect the presence of grazers.
111 For example, Schmieder *et al.* (2018) identified sensory pathways activated in a species of
112 *Coprinopsis* that was exposed to a fungivorous nematode. Moreover, there is evidence of

sophisticated evolutionary dynamics between fungi and potentially fungivorous soil fauna, with elements emerging for warfare strategies by carnivorous fungus, including toxins and traps, to incapacitate and then consume prey items (a famous example is the edible mushroom *Pleurotus ostreatus* that produces chemicals that paralyze nematodes upon contact). This An adaptation that is related to poor nutrient conditions (Lee *et al.*, 2020).

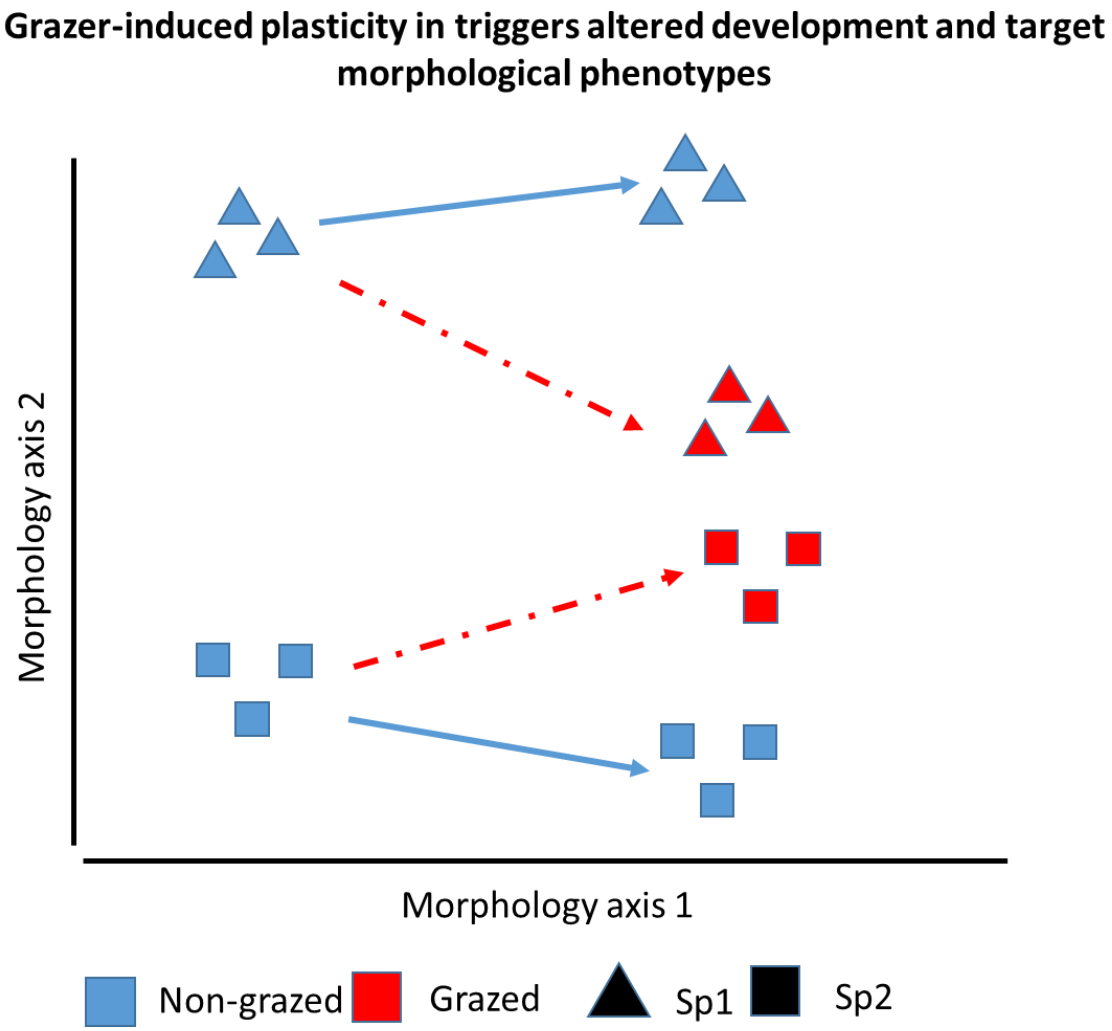
Until recently one of the challenges of studying morphological plasticity of fungi has been limited quantitative measurements of the network structure of the mycelium. However, semi-automated pipelines have now been developed that provide multiple metrics describing fungal network morphology and predicted function in mesocosms (Heaton, L *et al.*, 2012; Vidal-Diez de Ulzurrun *et al.*, 2015; Du *et al.*, 2016; Fricker, M. D. *et al.*, 2017; Dikec *et al.*, 2020; Sten *et al.*, 2024). These methods are particularly appropriate to quantify the morphology of the mycelia while they forage for resources from a nutrient base (Aguilar-Trigueros *et al.*, 2022b). Thus, whilst fungi cannot forage for resources by active movement like animals, they can explore their surrounding environment by growing out of a resources patch even when those surroundings have 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 millimetre thick hyphal aggregates termed cords that forage on a centimeter to meter scale (Fricker, M. D. *et al.*, 2017; Yafetto, 2018). Resources are transported within these cords enabling a single genotype to span several resource patches (Tlalka *et al.*, 2002). These group of fungi provide a tractable model system to investigate fungal morphological plasticity, particularly to grazing, given the numerous studies reporting the impact of grazing on their fitness (reviewed by (Crowther *et al.*, 2012b). Possible plastic responses of cord-forming mycelia to grazing include the formation of

new segments that increase cross-connectivity of the existing network to provide alternative routes to transport, developing a denser mycelial with multiple transport routes or thickening of cords to limit damage (Rotheray *et al.*, 2008).

To study the extent of grazer-induced plasticity in these fungi, we reanalyzed an existing image dataset of cord-forming mycelia from four fungal species exposed to grazing by a range soil micro-fauna with different feeding behaviour (Crowther *et al.*, 2011). The original study quantified changes in radial extension rate, hyphal coverage, fractal dimension, and wood decay rate and detected significant fungal-grazer interactions (Crowther *et al.*, 2011).The experimental design parallels reaction norm studies typically used to evaluate predator-induced plasticity and allowed us to quantify changes in the network architecture of each fungal genotype in the presence and absence of various grazers. Specifically, we measure a suite of mycelial morphological traits that capture the network architecture of the fungal mycelium from time series images (Fricker, M *et al.*, 2007; Fricker, MD *et al.*, 2007; Fricker, Mark D *et al.*, 2017; Aguilar-Trigueros *et al.*, 2022a). We used phenotypic trajectory analysis to quantify the extent of developmental plasticity in response to grazing. This method, widely used to study both evolutionary (Adams & Collyer, 2009) and developmental plasticity (Metcalfe, 2024) using multiple traits (Hoverman & Relyea, 2007), has been suggested as a powerful tool for studying fungal plasticity (Behm & Kiers, 2014).

Given the expected extreme flexibility of fungal mycelial phenotypes and the fitness costs imposed by grazing, we tested two hypotheses. First, we hypothesized that under the same grazing pressure, the developmental trajectories of different fungal species might converge towards a common morphological phenotype (Fig. 1), suggesting the presence of an optimal

157 “grazing resistant phenotype” shared across fungal species appropriate to each fungivore that
158 arises due to the extreme plasticity of the mycelia. Alternatively, if plasticity is more
159 constrained, we hypothesized that instead of convergence, each species would exhibit unique
160 developmental trajectories in response to grazers, reflecting fungal species-specific levels of
161 morphological plasticity.



162

163 Figure 1. Schematic representation of grazer-induced plasticity. Each fungal species occupies a
164 region of the network morphospace and follows a genotype specific developmental trajectory
165 in the absence of grazing (blue). Grazing-induced plasticity alters the developmental

trajectories (red). Strong selection may result in convergence of phenotypes towards similar grazing resistant phenotypes, such as an increasingly cross-linked network (red). Alternatively, each fungal species may adopt a species specific response (green).

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 wood or cords in Wales and are maintained in the Cardiff culture collection (Boddy et al., 1999). These species form network of cords (hyphal aggregates) growing out of wood blocks into the surrounding environment. Network growth occurs by extending the cords (in length) and by 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. Differences in morphology arise by changing the rates of cord extension, thickening, branching and fusion. Briefly, for each species, 5 clones from the same genotypes were cultivated from 20 × 20 × 10 mm beech blocks on compressed soil. The clones grew for 8-12 days until at least half of the replicates had reached a diameter of 160-200 mm. Five clones per species (i.e. replicates) were exposed to five types of grazing pressure, along with a set that was maintained grazer free that served as control. Grazing pressure (i.e. grazing treatment) consisted of five grazing levels: collembola (*Folsomia candida*), millipedes

187 (*Blaniulus guttulatus*), woodlice 1 (*Oniscus asellus*), woodlice 2 (*Porcellio scaber*), nematode
188 (*Panagrellus redivivus*) and a grazer-free control.

189 The developing mycelia were photographed for all four fungal species across grazer treatments.
190 Images were taken at the start of the experiment (day 1, before grazer addition), and
191 subsequently on days 2, 4, 8, 16, and 32, to monitor mycelial development over time. Here we
192 restrict analysis up to the 8-day time-point to ensure the network architecture was not
193 perturbed by physical constraints as some species reached the edge of the petri dish beyond
194 this day. During the experiment, all mycelia were grown at 18°C, and images were captured
195 with pixel sizes ranging from 80 to 155 µm.

196 *Quantitation of mycelial network architecture*

197 We use a set of image processing algorithms to extract the mycelial morphology from pictures
198 as described in (Aguilar-Trigueros *et al.*, 2022b). Briefly, each picture was loaded into a
199 graphical user interface and processed through a standard pipeline that included background
200 correction, network enhancement, segmentation, skeletonization, width estimation and
201 network graph representation using protocols optimized across a range of biological network
202 systems (Fricker, Mark D. *et al.*, 2017, Obara, 2012; Pain *et al.*, 2019; Xu *et al.*, 2021)}. The
203 software package and complete manual explaining in detail the steps involved is freely available
204 from Zenodo (DOI: 10.5281/zenodo.5187932). The output of this pipeline is a network graph
205 representation of the mycelia where the tips, cord branching and cord fusion events are as
206 nodes whilst the cords themselves form 'edges' connecting the nodes. The length and width is
207 automatically measured for each edge, whilst the (*x,y*) position and branching angle recorded

208 for each node. It was not possible to resolve edges within the inoculum, so these are
209 represented as strong connections between each incident edge on the resource and a central
210 node (termed the 'Root' of the network). This information was exported in two tables as .csv
211 and .xlsx files containing the cord (edge) information and the tips, branching and fusion points
212 (node) information. The node and edge tables from the image processing pipeline were
213 imported into R (using the *read_excel* function from the readxl package (Wickham & Bryan,
214 2019) and used to construct a spatially explicit transport network embedded in 2D space given
215 by the Euclidean coordinates of the nodes using the igraph package in R (Csardi & Nepusz,
216 2006) (Figure 2. See also details in the code available in the github repo
217 https://github.com/aguilart/Fungal_Networks).

218 *Traits used to define mycelial network architecture*

219 We measured a set of 22 network metrics that reflect the architecture of the mycelial network,
220 the investment in connectivity of the network, predicted transport dynamics within the
221 network and robustness to damage of the network (Table 1, Table SX). Connectivity of the
222 network was measured by, first, identifying the pathways representing the main routes of the
223 network (by detecting the minimum spanning tree (MST) that confers the least transport
224 resistance from the wood resource patch to the foraging tips at the front. The remaining cords
225 that represent secondary routes that enhance cross-linking, nutrient redistribution and
226 resilience to attack, but also incur an additional cost. Using this distinction, we then measured
227 four type of traits as follow: (1) Summary values (medians or means) per colony of
228 morphological traits, such as length, width, or branching angle, for the main and secondary
229 cords; (2) Skewness in the distribution of these traits as a proxy of the heterogeneity in the

230 construction of the network; (3) Mycelium wide metrics of the investment of main and
231 secondary routes in terms of volume of mycelium allocated to secondary routes and the
232 predicted transport efficiency within the entire network or specifically from the inoculum
233 ('root') to the tips; (4) Metrics of the expected robustness of the network to simulations to
234 attack (i.e. removal of cords) of the main routes and at random (see Table 1).

235 Predicted transport in the network was simplified as unidirectional fluid flow from the wood
236 block to the growing front of the network by assuming cords can be represented as a
237 contiguous network of interconnected bundles of cylindrical shape. The predicted resistance to
238 flow scales with cord length and inversely to the square of the radius of the cord (i.e. *resistance*
239 $\mu\ l/r^2$) (Bebber *et al.*, 2007; Fricker, M *et al.*, 2007). Thus, long and thin hyphae/cords provide
240 higher resistance. This is a simplification of transport dynamics of mycelia but matches well
241 with empirical distributions of radiolabeled nutrient movement in these networks (Heaton *et*
242 *al.*, 2010; Heaton, LLM *et al.*, 2012).

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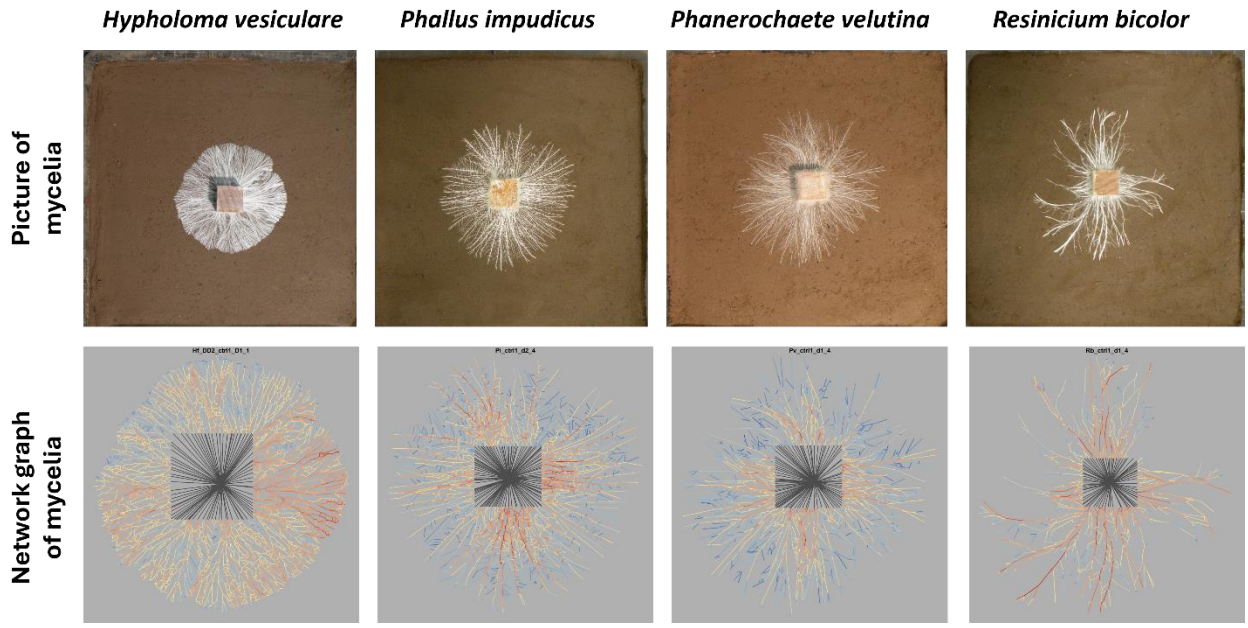


Figure 2. Translation of cord-forming fungi into a weighted transport network.

248 Table 1. Trait description.

Type	Biological meaning	Metric
1. Morphological traits	Average size limits in the construction of the cords	Medians of cord length and widths of main and secondary routes of the network, median branching angle
2. Heterogeneity of morphological traits	Heterogeneity (evenness) of the construction of the cords and the shape of the whole network	Skewness of cord width and cord length of the main routes; skewness of transport routes, colony circularity.
3. Investment in main and secondary routes	Relative cost and benefit of the connectivity of the network	Meshnedness (alpha coefficient), Root-tip transport efficiency, global transport efficiency, ratio total mycelial volume to volume of the main routes of the network.
4. Robustness to damage	Expected robustness to removal of the main routes of the network and removal of cords with distinct properties	Percentage of the number of cords than can be removed until the volume of the mycelial network falls below 50%. Removal of cords of the main routes, at random and targeted to cords depending on their width and length.

249

250 *Phenotypic trajectory analysis to measure morphological developmental plasticity*

251 Phenotypic trajectory analysis relies on the use of multivariate ordination methods to
252 summarize the change through time in the in phenotypes across multiple traits combined using
253 ANOVA-like frameworks for statistical testing (Adams & Collyer, 2009; Behm & Kiers, 2014). In
254 this study, we used three types of permutational multivariate analyses of variance models
255 (PERMANOVA) to determine level of grazer-induced plasticity across the fungal species using
256 the 22 metrics (network traits). In all the models, we controlled for initial differences in
257 inoculum growth at the start of the experiment by using the first two axes from a principal

258 component analysis (PCA) of traits at time 1 (i.e. immediately before the addition of the
259 grazers) as covariate in the PERMANOVA.

260 In the first and more general model, we tested whether the development of the morphology of
261 the network is altered (i.e. plastic trajectory) across all fungal species and all grazing pressures.
262 In this model, time, fungal species identity, and grazer type were use as fixed factors and PC1
263 and PC2 of the traits used at the start of the experiment to control for initial conditions (see
264 table 2 for the full formula used in this model). If fungal species converge to a common
265 phenotype, we expect to detect a strong (and significant) effect of the interaction between
266 grazer and time. That is, the more deviation of the development of the morphology of grazer-
267 exposed clones compared to the grazer-free clones the stronger is this interaction term effect
268 (i.e. effect size).

269 In the second model, we tested whether specific grazers induced to a common phenotype at
270 the end of the experiment. To do so, we run five PERMANOA models (i.e. one for each grazer)
271 where the network traits of all four fungal species were used as response variables while the
272 only fixed factors was the grazer treatment with two levels (presence or absence). This model
273 tested which grazer altered the developmental trajectory towards convergence to a common
274 phenotype. This convergence would be shown by a significant, large effect size of grazer
275 identity. To further visualize and validate the output of these models, we complemented it by
276 testing whether the grazers increase the similarities among fungal species compared to the
277 control. This test was done by measuring the distances between each fungal species across the
278 respective grazer treatments to their multivariate mean (centroids) followed by a permutation
279 based-test for multivariate homogeneity of group dispersions (implemented with the function

280 *betadisper* from the package *vegan* in R). If grazers increase the similarity (or decrease it) it
281 would be shown as a strong and significant reduction in the distances between each species
282 and their respective centroids.

283 The third model tested which fungal species had the most plastic response to grazers. To do so,
284 we run PERMANOVA models for each fungal species and grazer combination. Thus, this test
285 measured the extent of development plasticity of fungal and grazer combinations irrespective
286 convergence to a common phenotype.

287 In all PERMANOVA models, we used a forward model selection approach to determine which
288 fixed factors should be retained. We used Principal Component Analysis (PCA) to visualize the
289 changes in the phenotype through time. Analysis of the distances to each centroid were done
290 using Euclidean distances. Effect sizes were measured as the proportion of explained variance
291 of the fixed factors. PERMANOVA, PCA, analysis of distances to centroids, model selection and
292 effect sizes were calculated using functions from the vegan package (Oksanen *et al.*, 2012). All
293 statistical analysis were conducted in R (R Development Core Team, 2017).

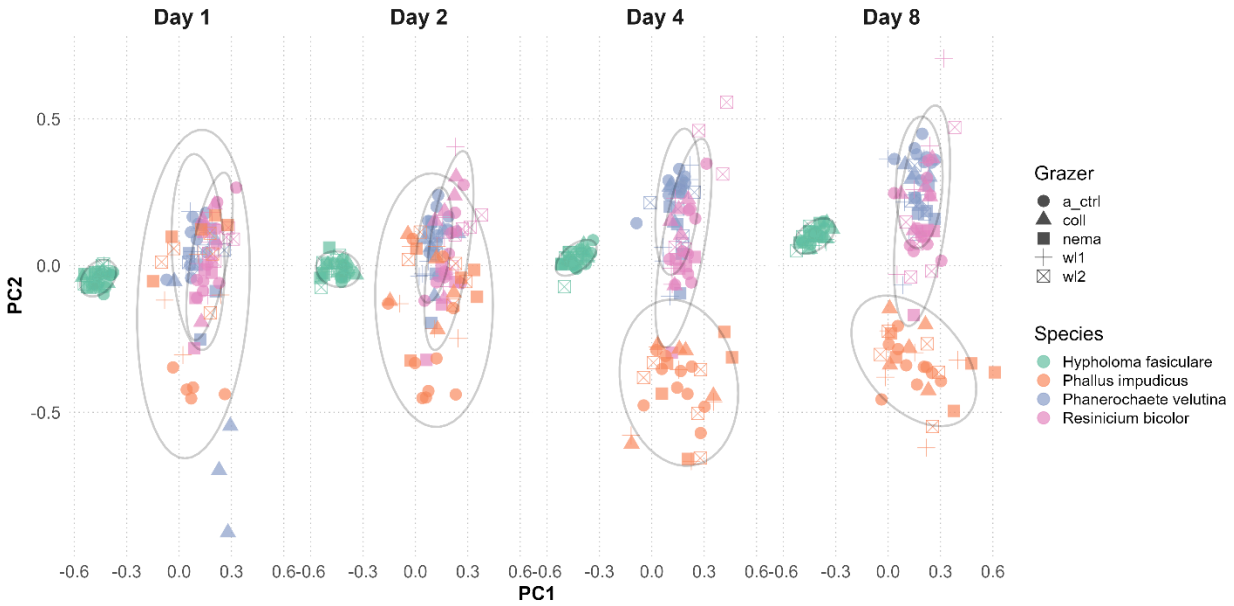
294 **Results**

295 *There is only weak convergence to common grazer-induced phenotype but strong species-*
296 *specific effects*

297 There was a strong effect of species identity that explained most (~90%) of the variation in
298 morphology across all treatments and time points using model 1 (Fig. 3). Within this overall
299 pattern, there was a small effect of grazer identity and an interaction with time, but neither

300 effect was larger than 5% of the total variation (Table 2). Likewise, there was little evidence for

301 convergence to a common phenotype across all fungal species and grazers.



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303 Figure 3. Clustering of fungal species in a PCA morphospace. The PCA analysis includes the

304 complete data set, but results for different days are shown as separate panels for clarity. Each

305 fungal species (indicated by colour) occupies a different region in the morphospace and follows

306 a different trajectory over time. However, the effect of different grazers (indicated by symbol)

307 accounts for a much lower variability in the overall dispersion.

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

Source	Df	Variance	F	R2	p-value
Species	3	3.7293	83.0941	0.2795	1e-04
Grazer	4	0.3861	6.4523	0.0289	1e-04
day	1	0.5632	37.6466	0.0422	1e-04
Species:day	3	0.9448	21.0512	0.0708	1e-04
Species:Grazer	12	0.7766	4.3258	0.0582	1e-04
Grazer:day	4	0.1696	2.8344	0.0127	2e-04
Species:Grazer:day	12	0.4138	2.3051	0.031	1e-04
Residual	425	6.3581		0.4766	

311 In the second models that focus on the effect of each grazer separately, we found weak support
312 for convergence to a common phenotype only for millipedes, while for woodlice and
313 nematodes caused a mild increase in the dissimilarity among species (Fig. 4). More specifically,
314 for these grazers, their main effect was retained (i.e. significant at a p-value lower than 0.05).
315 Nevertheless, the effect, was weak, accounting for only 2 to 3 percent of the total variation (Fig.
316 4). A similar trend was observed when comparing the distances of fungal species to centroids in
317 the control compared to that of the grazers (however this trend was not statistically
318 significant). Taken together, these results show that grazer-induced plasticity was detectable
319 across fungal species, but there was only weak evidence for convergence into a common grazer
320 resistant phenotype for a subset of the grazers.

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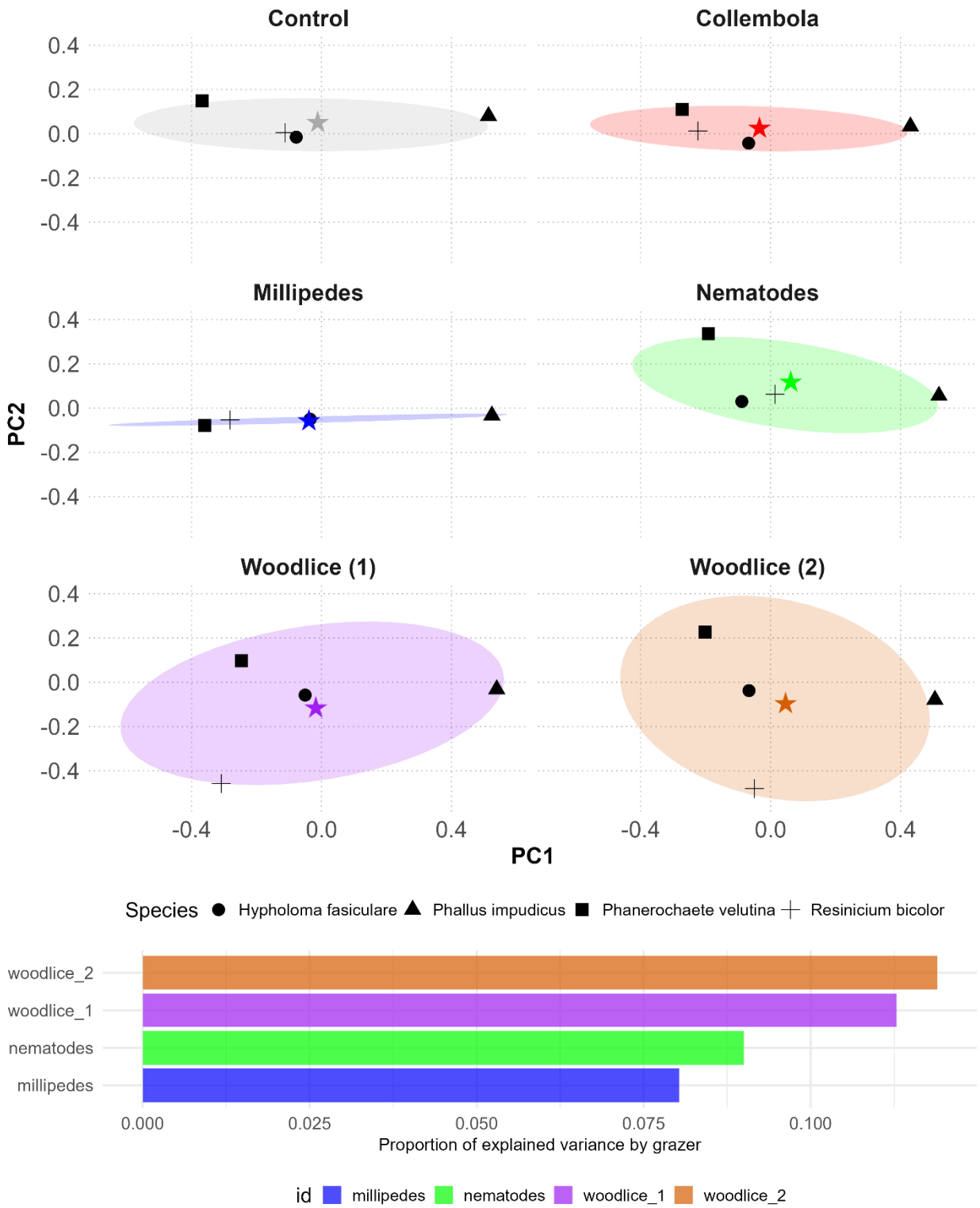
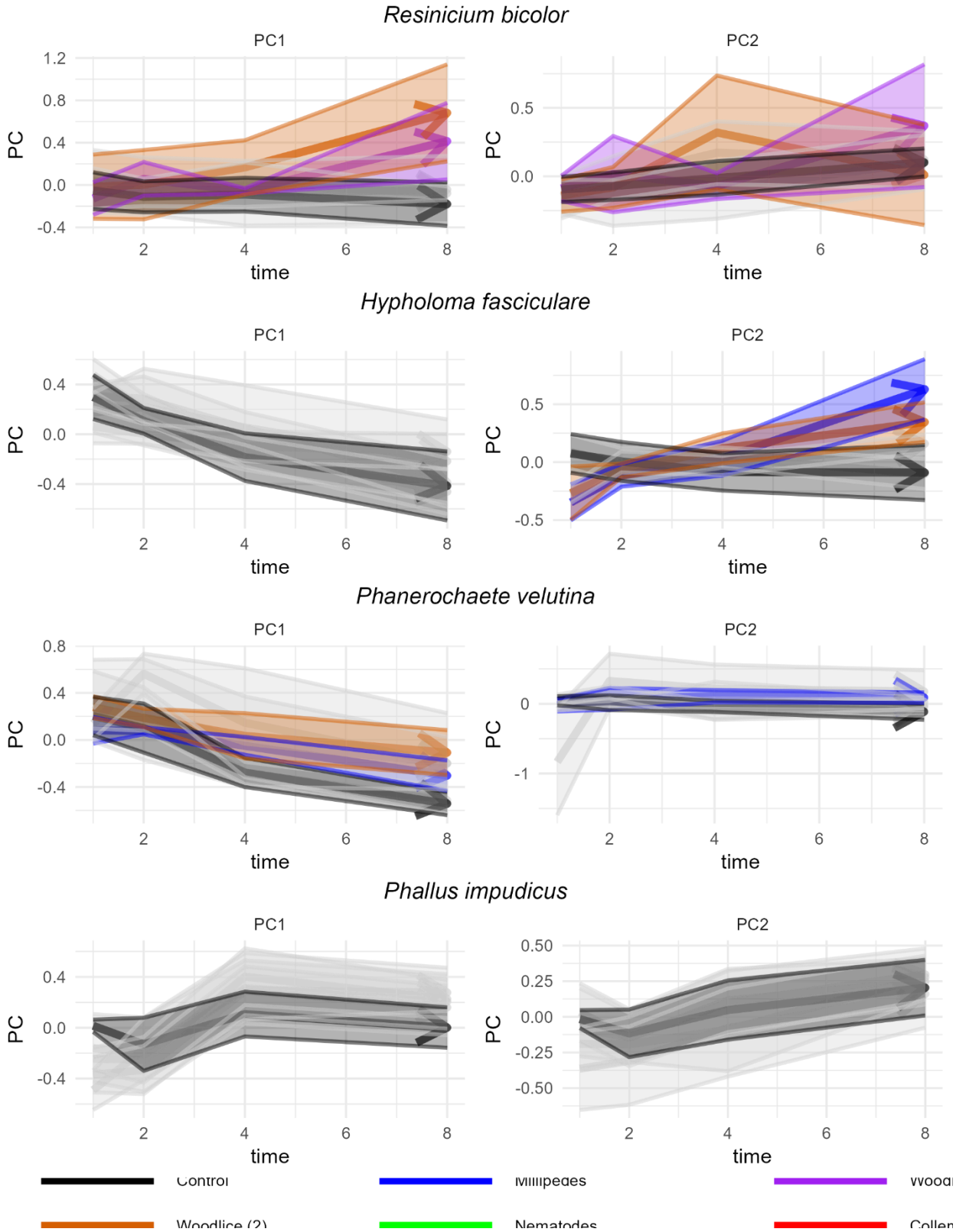


Figure 4. Testing convergence to a common phenotype due to grazer pressure.

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

Given there was some evidence for grazer-induced plasticity, but not coherent behaviour across all fungal species, we next sought to explore the time-dependent interaction pairwise for each fungal species and grazer type. At this level of resolution, we found large variation in the level of plasticity in response to grazer among the fungal species tested (Fig. 5). *Resinicium bicolor* displayed the largest response to 4 out of the 5 grazer types, and also exhibited the largest change in morphology. For example, the proportion of variation explained was greater than 305 for woodlice and millipedes). *Phanerochaete velutina* and *Hypholoma fasciulare* showed lower overall responses with 10% to 20% variation in morphology attributed to the presence of grazers. In contrast, development in *Phallus impudicus* showed the least sensitivity to grazers with less than 10% in the variation in morphology attributable to the presence of any grazer (Fig. 5).


Resinicium bicolor
PC1
PC2
Hypholoma fasciculare
PC1
PC2
Phanerochaete velutina
PC1
PC2
Phallus impudicus
PC1
PC2
PC
time
Control
Millipedes
Woodlice (2)
Nematodes

339

Figure 5. Species specific morphologically induced changes to exposure to different types of grazers. We show the fungal species x grazer interaction where grazer induced variation is significant and they are color coded when the deviation explains more than 10% of the total variation.

The traits that underpin the grazer-induced plasticity can be inferred from the loadings on the RDA axes and differed among fungal species and type of grazers (Fig. 6). For *Resinicium bicolor* the traits that were more responsive to grazers included branching angle, median cord length of both the main and secondary routes, and transport efficiency from inoculum to the tips. For *Hypholoma fasciculare*, the most plastic traits were median length of the cords in the main route, robustness to attack towards to the thin or thick cords, loopiness, investment in secondary routes, colony circularity and transport efficiency from inoculum to the tip. For *Phanerochaete velutina*, the most plastic traits were robustness to most types of attack (random or directed), branching angle, colony circularity and cord density.

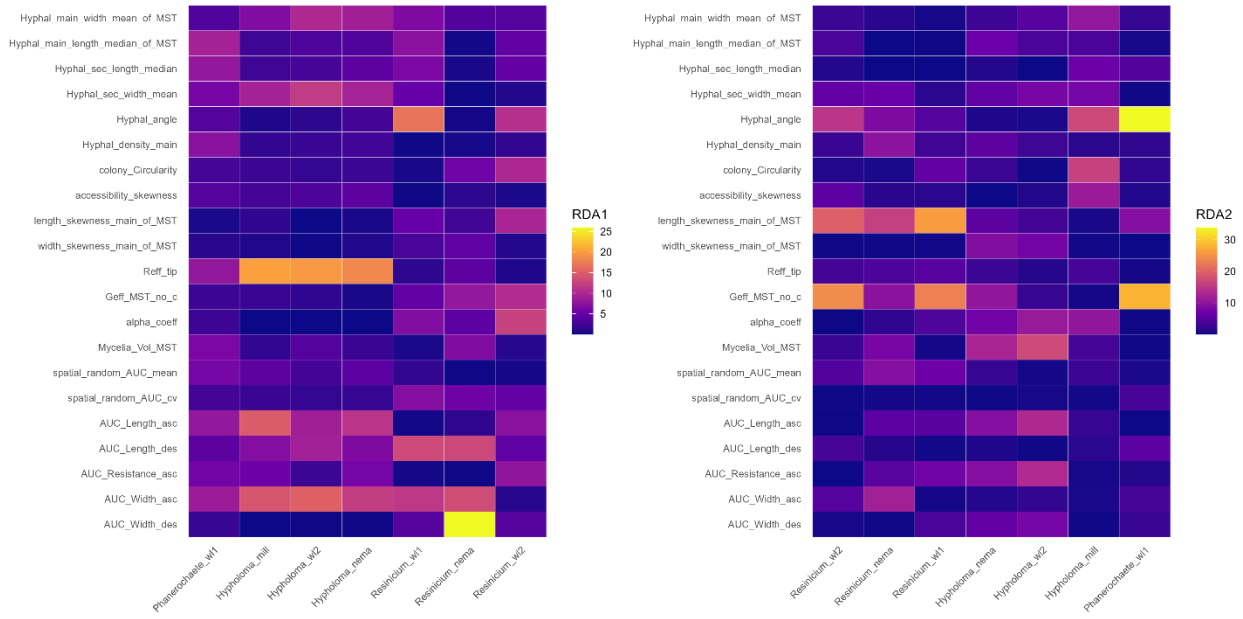


Figure 6. Heatmap of shifts in morphological traits between grazer and non grazer exposed genotypes during development. Color coding of a given trait indicates the magnitude by which the trait changes due to development (time), the presence of the grazer (comparison of grazed phenotype to ungrazed control), and the interaction between these two. Only fungal species and grazer combinations where there is a significant effect are shown (see table X below).

Discussion

We tested whether grazing pressure induces convergence towards a common mycelial phenotype or, alternatively, that genotypes of different species exhibit distinct responses to particular grazers. Perhaps surprisingly, the level of overall variation was dominated by fungal species, with only a small, although significant effect of grazers. Within this, we did not find much evidence for convergence to a particular grazing resistant architecture, instead the genotypes of each fungal species followed distinct developmental trajectories when exposed to different grazing pressures within species-specific developmental limits. These results are surprising based on the assumption of extreme morphological plasticity in fungi (Slepecky & Starmer, 2009). We infer that these findings can be explained by either limited flexibility to reconfigure fungal network architecture (through varying branching angles or the rate of cord thickening for example), and/or that morphological plasticity is not the most effective means to combat grazing. Thus, grazing may only impose a weak pressure for the emergence of morphological plasticity in fungi. This does not rule out alternative phenotypic responses, such as reducing palatability or producing toxins, which might represent more cost-effective measures than morphological change.

Strong species differences suggest limits in morphological plasticity

Although some of our species exhibited considerable plasticity in response to grazers, these changes were not strong enough to override species-specific differences. In other words, the morphological trajectories of each fungal genotype remained within distinct boundaries defined by species identity. This pattern suggests that the plasticity of cord-forming fungi is

381 constrained within certain species-specific limits. These constraints varied among species, with
382 *Resinicium bicolor* showing up to 30% variation in morphology due to grazing pressure, while
383 *Phallus impudicus* exhibited less than 10% change in response to grazers. In theory, this
384 heterogeneity could correlate with either the lack of sensory systems in certain taxa (Murren *et*
385 *al.*, 2015) or variation in the actual metabolic cost of changing the phenotype (Auld & Relyea,
386 2010). While we do not have direct evidence of sensory mechanisms for our species, we expect
387 they are present as they are in closely related taxa (e.g. *Coprionopsis cinerea* and *Pleurotus*
388 *ostreatus*) are known to respond to at least nematodes and collembola (Plaza *et al.*, 2015;
389 Schmieder *et al.*, 2018). Thus, more likely, lower levels of plasticity may be associated with
390 variation in the metabolic cost of altering the morphology of mycelial phenotype sufficiently to
391 achieve resistance to grazing. We speculate those costs are associated to differences in the
392 construction of cords. While all cord-forming fungi have more complex chemistry and structure
393 compared to non-cord forming fungi (Yafetto, 2018; Porter *et al.*, 2022), there is a gradient in
394 the level complexity in the histology of the cords across species (Townsend, 1954). For example,
395 *Phallus impudicus,* the least plastic species in our study, is at the more complex end of the
396 spectrum with clear differentiation of distinct zones in the anatomy of the cord compared to
397 that of other cord-forming fungi (Townsend, 1954; Eamus *et al.*, 1985). Likewise,
398 *Phanerochaete velutina*, which also show relatively low plasticity in this study, has also been
399 reported as showing zone differentiation in its cord anatomy (Thompson & Rayner, 1983). In
400 contrast *Hypholoma fasciculare*, with relative higher levels of plasticity in our study, is reported
401 to on the lower end of cord complexity (Thompson & Rayner, 1983). We have not found
402 anatomical studies of the cords of *Resinicium bicolor* (the species with themost plasticity in our

403 study), but it is reported as a fast-growing-species (Zakaria & Boddy, 2002) a trait that is
404 commonly associated with lower investment in resources in the construction of an organism´s
405 body (Wright *et al.*, 2004). Thus, we hypothesized with increasing complexity of the cord-
406 forming mycelia is part of a trade-off with the ability to respond to grazer pressure. For species
407 with less plastic and complex phenotypes, modifying the network architecture may jeopardize
408 efficient transport which might outweigh any benefit to morphological responses to grazing.

409 We also emphasize that these observed limits to grazer-induced plasticity may not reflect the
410 potential for morphological plasticity in response to other environmental factors. For example,
411 we observed relatively high variation in the starting point in the PCA morphospace within
412 replicates of the same genotype reflecting initial differences in mycelial growth before the
413 addition of grazers. Such differences may arise from heterogeneity in resource quality, or the
414 extent colonization of wood blocks used as inoculum in these experiments. As addition of
415 grazers was initiated once half of the replicates had reached 8 cm diameter, variation in the
416 initial growth and resource availability are maintained over time. Overall, while our results
417 indicate limits to grazer-induced plasticity in cord-forming fungi, they also suggest that other
418 factors such as resource heterogeneity may drive greater levels of morphological plasticity.

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

421 Although we cannot determine whether the observed grazer-induced plasticity is adaptive—
422 such a study would require long-term monitoring of fitness consequences—our findings do not
423 support that fungal grazing exerts strong evolutionary pressure for the development of

424 adaptive morphology. We found no evidence of a convergent 'grazed phenotype' across species
425 and instead observed substantial variation in plastic responses. The emergence of a common
426 adaptive phenotype typically requires strong and consistent environmental pressures. Given
427 the diverse community of fungal grazers present in soils, optimizing morphology for one grazer
428 may offer limited fitness benefits if it compromises adaptability to other grazers or
429 environmental factors (Einum *et al.*, 2019). Even if such optimal phenotype exist, the fact that
430 we did not observe a response in our time window indicates that individuals would spend a
431 long time of their life cycle in a sub-optimal transitioning phenotype, a feature of weak
432 selection pressure (Einum & Burton, 2023).

433 We want to note, however, that grazing can still impose strong evolutionary pressure for the
434 evolution of plasticity on other traits that are not morphological. Phenotypic convergence could
435 be exhibited in traits not related to morphology that we did not measure in this study (as
436 “hidden plasticity” (Forsman, 2015)). Phenotypic convergence in response to grazers could be in
437 the form of production of chemical compounds that reduce palatability and intoxicate the
438 grazers. This type of responses is well documented in in plants (ref) and more recently it has
439 been found that some fungi produce compounds with similar functionality (Plaza *et al.*, 2015;
440 Schmieder *et al.*, 2018; Lee *et al.*, 2020). So, while the apparent target morphological
441 phenotype between grazed and ungrazed environment are different, “hidden” grazer induced
442 plasticity in the form of altered chemistry may act as compensatory mechanism to maintain the
443 apparent dissimilar phenotype between the two environments (Metcalfe, 2024).

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

445 The limits to morphological plasticity observed in our study suggest that fungal traits may serve
446 as reliable indicators of community assembly and ecosystem processes (McGill *et al.*, 2006). In
447 community ecology, traits that exhibit lower within-species than among-species variation can
448 reflect consistent ecological strategies and predict patterns of species composition along
449 environmental gradients (HilleRisLambers *et al.*, 2012; Franklin *et al.*, 2020). For example, lab-
450 derived traits in marine plankton and zooplankton have successfully predicted seasonal shifts
451 and habitat filtering in natural settings (Edwards *et al.*, 2013; Vogt *et al.*, 2013). If fungal
452 mycelial traits reflect species differences, they could also predict how shifts in fungal
453 community composition along environmental gradients impact ecosystem processes, such as
454 carbon cycling. Cord-forming fungi, for instance, play a key role in lignin decomposition, and
455 morphological traits may influence decomposition efficiency, thereby affecting carbon budgets.
456 Although speculative, these findings suggest that predictable relationships between mycelial
457 morphology, environmental conditions, and ecosystem functions warrant further exploration.

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

459 Moving forward, it remains to be tested whether non-cord-forming fungi exhibit higher levels
460 of plasticity. Given that phenotypic plasticity tends to evolve in highly variable environments
461 (Lande, 2009), fungi inhabiting soils—among the most heterogeneous habitats—would be
462 expected to show strong selective pressures for high morphological plasticity. Evolutionary
463 models suggest that filamentous growth forms, which are particularly advantageous in spatially
464 heterogeneous environments like soils, would not have evolved in more homogeneous aquatic
465 habitats (Heaton et al., 2020). This parallels findings in ectotherms, where greater plasticity is
466 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 thesis). 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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