Trade-offs in soil filamentous fungi

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"It is not the strongest of the species that survives,

Nor the most intelligent,

It is one that is most responsive to change".

***On the Origin of Species*, Charles Darwin, 1859.**

Foreword

This Dissertation is a cumulative work of manuscripts, selected from my publication list, either published or prepared to submit. This thesis is based on the following work:

I. Veresoglou SD, **Wang DW**, Andrade-Linares DR, Hempel S and Rillig MC. 2018. Fungal Decision to exploit or explore depends on growth rate. Microbial ecology 75 (2): 289-292.

II. **Wang DW**, Veresoglou SD, Forstreuter M and Rillig MC. Fast-growing fungi have a higher sensitivity to a mechanical disturbance but tolerate better its reoccurrence. Prepared to submit (based on the new phylogenetic system).

III. **Wang DW**, Veresoglou SD, Andrade-Linares DR, Mardhiah U and Rillig MC. Fungal fast growers exert lower sensitivity to temperature. In preparation.

IV. **Wang DW**, Veresoglou SD, Lehmann A and Rillig MC. A trait-based framework to understand the life history of filamentous fungi. Prepared to submit (based on the new phylogenetic system).

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Chapter One:

General Introduction

1.1 Background and problem definition

1.1.1 Functional Traits in fungi

Fungi are ubiquitous in nature and contribute immensely to ecosystem functioning (Egbuta et al., 2016; Amend et al., 2019; Perotto et al., 2013; Bindschedler et al., 2016; Hart and Trevors, 2005). The Red Queen hypothesis (Dieckmann et al., 1995; Clay und Kover, 1996; Brockhurst, 2011; Liow et al., 2011; Benton, 2010) justifies evolution across organisms in terms of a never-ending arms race to survive. Resulting character evolution should be apparent in most phenotypic characteristic influencing fitness, the functional traits. The functional traits that are most widely used in filamentous fungi relate to growth, reproduction and survival (Chagnon et al., 2013; Treseder and Lennon, 2015), but trait-based approaches in fungi remain under-explored compared to bacteria and macroscopic organisms (Aguilar-Trigueros et al., 2015).

Functional trait approaches present an arsenal of tools to test mechanisms underlying the community assembly of species and associated ecosystem processes. Bauman et al. (2016) found that functional traits of host tree species and chemical soil parameter shape the spatial distribution of the ectomycorrhizal fungal community. However, standardized measurements of functional traits are lacking for many organisms and ecosystems, including fungi. Fungi are known to be important for plant nutrition and resistance to stresses. A number of focus-points are given based on empirical and theoretical evidence that could be utilized to slow down negative selection pressures on fungal functioning, therein increasing crop benefit (Verbruggen and Kiers, 2010). As the research field of

fungal functional ecology is growing, there is a need for standardized ways to measure fungal traits within and across taxa and spatial scales (Halbwachs and Bässler, 2015). Most soil fungi are endemic to particular bioregions, underlying that factors operating at large spatial scales, like climate and spatial location, are the dominate reason of fungal community structure (Talbot et al., 2014). However, description and understanding of the rules measuring fungal functional traits remains scarce.

The application of the life history strategy framework has been expanded from plants to the microbes. There have already been a handful of studies addressing how functional traits relate to life strategies in filamentous fungi (Ho et al., 2017). Frameworks that group species into functional groups along a few trait axes have helped to summarize biological variation (Winemiller, 2011). Chagnon et al. (2013) identified three major axes of specialization in arbuscular mycorrhizal fungi by matching Glomeromycotina to Grime's competitive, stress tolerant, ruderal framework.

1.1.2 Environmental factors and fungal response

Functional traits determine the occurrence of species along environmental gradients and their coexistence with other species. Understanding how traits evolved among coexisting species could hint on community assembly processes (Treseder et al., 2018a). There are various environmental factors (i.e. nutrient limitation, disturbance, stress) which induce profound physiological changes in living organisms and as a result regulates their fitness. These results suggest that environmental filtering favors the co-existence of related and similar operational taxonomic unit within the Basidiomycota community assembly, whereas the Ascomycota and Glomeromycotina communities seem to be impacted by competitive interactions (Le Van et al., 2017).

Proliferation of hyphae, resource allocation (carbon and nutrients) within a mycelium and spatial distribution of resource capturing structures (internal mycelium for carbon and external mycelium for nutrients) can be considered as foraging strategies (Olsson et al., 2002). Growth allocation strategies in sessile organisms have significant fitness consequences and are thus subject to evolutionary selection. Yet, fungal foraging strategies remain largely under-explored. Theoretical work has proposed that optimal resource allocation strategies in fungi change with ages (Gilchrist et al., 2006) and empirical

evidence suggests that fungi exhibit adaptive foraging behavior in response to heterogenous resource availability (Tlalka et al., 2008). So, *the research question of whether allometric partition of fungal biomass is regulated by site fertility or not was assumed (Postulate 1)*.

Disturbances are characterized by three properties, intensity, duration and frequency, promote increases in resource availability (Ryckier, 1985) and the temporary unfettering of niche space, which may favor organisms with specific life history traits, such as fast growth rates, high investment in propagules and fast development (Overpeck et al., 1990). Microbial responses to disturbance can be complex. Williamson and Wardle (2007), for example, found that a moderately severe fungivory-associated disturbance is more detrimental to soil filamentous fungi than a more severe fungivory treatment. Under specific circumstances, the frequency of disturbance can be at least as important as its intensity (Miller et al., 2011). Climate warming is predicted to increase the frequency and severity of extreme weather events which from the perspective of soil organisms can be viewed as disturbances (Ummenhofer and Meehl, 2017; Thom and Seidl, 2016). Moreover, anthropogenic activity throughout the globe renders associated disturbances to aboveground but also soil biota more frequent (Swaddle et al., 2015). Hence, *it is emergent to explore the manifestations of fungal sensitivity under frequent disturbance (Postulate 2)*.

Temperature (i.e. stress factor) regulates the fitness of living organisms by triggering profound physiological changes (Newsham et al., 2016; Zogg et al., 1997; Zucconi et al., 1996; Pietikäinen et a., 2005). This should be particularly the case in fungi (McCurchie et al. 1973), which maintain close to ambient body temperatures. Proliferation of fungi with the genetic capacity for C and N acquisition may contribute to the maintenance of biogeochemical cycling under stress (Treseder et al., 2018b). The metabolism of an organism is regulated by numerous enzymes, which, subject to environmental conditions, catalyze key chemical reactions (Mackie et al., 1990). A widely used metric for temperature sensitivity is Q_{10}, which quantifies how much the rate of a physiological process changes when we alter the temperature by 10^{o}C (Lloyd et al., 1994). Another key factor influencing the sensitivity of filamentous fungi to changes in temperature relates to their origin and distribution. The niche breadth hypothesis (Brown, 1984) proposes that the organisms that inhabit the poles occupy wider ecological niches than those in the tropics and can tolerate a wider range of environmental conditions (Morin et al., 2006). Latitudinal changes overlap greatly with those of temperature that peaks in the tropics. It is thus likely that fungi adapted to lower temperatures, have a wider ecological niche and are less sensitive to temperature (Curie, 1991). Temperature was the dominant driver of these diversity gradients, with weak

influences of edaphic properties, including soil pH (Nottingham et al., 2018). Consequently, *it is interesting and necessary to reveal the growth of the isolates and aspects of the functioning under temperature stress (Postulate 3).*

1.1.3 Life-history trade-offs of fungi

A trait-based framework was applied to address whether and how environmental filtering and niche differentiation influence the fitness of soil filamentous fungi. Two widely used frameworks of life history strategy were showed in this thesis (Fig. S1.1). The r-K strategy trade-off depicts reasonably well life strategies in fungi, useful for example for predicting successional dynamics across species (Richardson, 2002). However, there have been cases where it performed less well. For example, Maynard et al. (2017) found that differences in competitive ability remained despite correcting for growth rate showed that the r-K trade-off fell short of describing life-history strategies in fungi. An alternative informative model is the CSR model which was proposed for plants by Grime (1974). The model decomposes the ability of organisms to grow into three niche axes describing their abilities to outcompete other species, tolerate unfavorable conditions and deal with disturbance (Grime, 1974, 1977). This framework been shown to perform well for plants (Grime et al., 1997). There has also been an effort to apply this model to Glomeromycotina (Chagnon et al., 2013). Unlike the case of the r-K continuum model where it is sufficient to show a trade-off between an aspect of growth rate or generation time and a measure of competitive ability, usually the CSR model is tested after a variance decomposition with multivariate techniques (Grime et al., 1997; Pierce et al., 2013; Pierce et al., 2017). In this study, CSR model was manipulated to filamentous fungi in order to better understand the life-history strategies of them. Taken together, *it is essential to modify fungal functional traits to be able to establish soil filamentous fungal life history strategy framework (Postulate 4).*

1.2 Objectives of this thesis

The principal aim of this thesis is to conduct an integrated study of fungal functional traits and its impact on the investigation of their life history strategies, which includes 1) a case study on fungal optimal foraging strategy; 2) measuring fungal disturbance sensitivity; 3) calculating fungal stress-tolerance; 4) modeling fungal functional traits into CSR framework.

The specific objectives of each research problem are respectively present as follows:

Postulate 1

To develop a novel method to test fungal foraging strategies according to calculating the biomass ratio on various cultures.

To modify the experimental setup to get an ideal heterogenous resource environment.

Postulate 2

To obtain fungal disturbance sensitivity based on a disturbance-sensitivity score which was calculated by averaging mean growth data per isolate across the two disturbance treatments.

To calculate the response ratio of sensitivity to disturbance in the single disturbance treatment over the sensitivity to disturbance in the iterative disturbance treatment.

Postulate 3

To assess the impacts of stress tolerance and estimated temperature sensitivity by using the Q_{10} index.

To explore fungal optimal growth variability across isolates with a Gaussian curve of square root transformed temperatures.

Postulate 4

To measure competitive ability by exploring their colony growth in pairwise interactions.

To assess temperature sensitivity as a proxy of stress tolerance.

To set disturbance tolerance as a proxy of persistence in ruderal environments.

To map fungal isolates into CSR strategies.

1.3 Outline of this thesis

This thesis is composed of six chapters.

Chapter One introduces the fundamental traits in fungi, review existing literature on the topic of this thesis.

The following four core chapters (chapters 2 to 5) comprise the main research work conducted within this thesis. They have been written as manuscripts for publication in international peer-reviewed scientific journals and accordingly, each of them can be independently read as self-contained pieces of work. For a consistent layout, these four manuscripts have been re-edited as follows:

Chapter Two tests the hypothesis if fast-growing fungi have a tendency towards localized growth whereas slow-growing fungi tend to explore their environment in attempt to locate further resources.

A set of 30 isolates of filamentous fungi was used to quantify the growth preference of isolates for nutrient-rich PDA agar. To account for phylogenetic dependencies, phylogenetically independent contrasts (PICs) was employed with an existing phylogenetic reconstruction of the isolates. *Fast-growing fungi would allocate a larger fraction of biomass to the nutrient rich habitat compared to slow growers were hypothesized.*

Chapter Three addresses the question if the sensitivity of various fungi towards singular and iterative mechanical disturbances is determined in relation to the growth rate and carbon use efficiency of the fungi.

A controlled experiment was established to evaluate comparative responses to disturbance and disturbance frequency in our 30 fungal core set. Mechanical disturbances were simulated via vortexing liquid cultures containing glass beads to establish disturbance treatments. Fast growing fungi are less sensitive to disturbances because they can compensate with their faster growth rates (*Hypothesis One*) and that frequent disturbance events suppress growth and reduce physiological activity in all filamentous fungi (i.e. augment the detrimental effects of disturbance) proportionally to the influence of a single disturbance event (*Hypothesis Two*).

Chapter Four applies various calculation methods to assess the temperature sensitivity of fungi.

An experiment was carried out to assess fungal temperature sensitivity (Q_{10}) and optimal growth temperatures. The experimented with growth at four different temperature settings: 15 ^{o}C, 20^{o}C, 25^{o}C & 30^{o}C and assessed growth as radial expansion of the fungal colony. Faster-growing fungi will have lower Q_{10} values (*Hypothesis One*) and that fungi with lower optimal growth temperatures will have lower Q_{10} values (*Hypothesis Two*).

Chapter Five proposes a novel method to map the life-history traits of fungi into the competitive-stress tolerant-ruderal model.

The suitability of the competitive-stress tolerant-ruderal classification system for describing growth traits in filamentous fungi was addressed. A novel way to map trait data into a CSR triangle was proposed and *two hypotheses, (i) that the two dimensional classification system of the CSR model adds meaningful life-history information compared to the existing r-K one; and (ii) that all three CSR traits are phylogenetically conserved*.

Chapter Six combines the results of the previous chapters as a general discussion elaborating the relevance of fungal functional traits for fungal life history strategies. This chapter also presents the conclusions of this thesis, by summarizing the major outcomes of the aforementioned chapters and the highlights of this thesis, as well as providing an outlook for the future research.

Appendix contains additional figures that have been included in the original manuscripts as supplementary materials.

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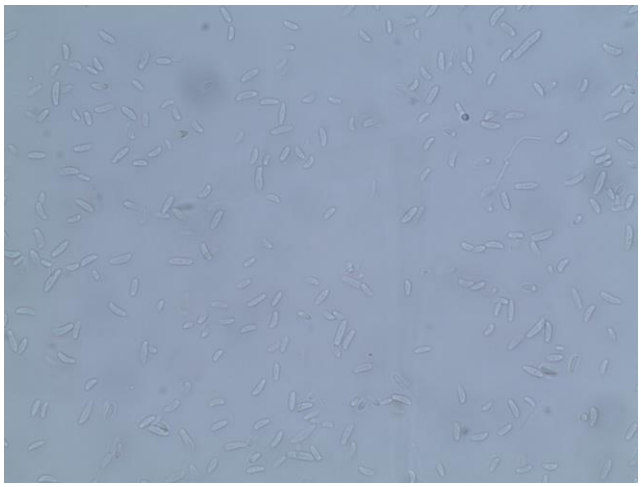
Chapter Two

Fungal decision to exploit or explore depends on growth rate



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Abstract

Theoretical work has proposed that optimal resource allocation strategies in fungi change with age and empirical evidence suggests that fungi exhibit adaptive foraging behavior in response to heterogenous resource availability. Yet, fungal foraging strategies remain largely under-explored. We used a set of 30 isolates of filamentous fungi originating from one grassland to address the hypothesis that fast-growing fungi would allocate a larger fraction of biomass to the nutrient rich habitat compared to slow growers. We present evidence that fungal growth rates relate to their biomass-allocation strategies. This relationship holds for the entire range of diverse fungi considered. It is of interest to further test whether the findings are generalizable to other microorganisms.

Introduction

Biomass allocation decisions in sessile organisms have significant fitness consequences and are thus subject to evolutionary selection. Most studies focus on allocation decisions in plants. For example, allometric partition of plant biomass to roots and shoot is regulated by site fertility (Mamolos et al., 1995) and symbiotic associations (Veresoglou et al., 2012). Relative investment to primary and secondary metabolism is another allocation trade-off (Neilson et al., 2013). Plant exploitation strategies of local soil-nutrient patches have seen extensive study; roots may either proliferate in patches at the risk of not finding other patches or explore the soil interface at a risk of under-exploiting existing known patches (Hodge, 2004). Finally, authors have tried to explain patch-exploitation strategies based on optimal foraging theory (Neilson et al., 2013).

Filamentous fungi are an under-explored group of eukaryotic, modular organisms. They are sessile, and optimize their growth-strategies chemotactically (Arkowitz, 1999). A group of ubiquitous mutualistic fungi, Glomeromycota, likely optimizes growth-strategies based on economical markets (Werner et al., 2014). Theoretical work has proposed that optimal resource allocation strategies in fungi change with age (Gilchrist et al., 2006) and empirical evidence suggests that fungi exhibit adaptive foraging behavior in response to heterogenous resource availability (Tlalka et al., 2008). Yet, fungal foraging strategies remain largely underexplored.

Several life history and phenotypic parameters can describe a fungus and many of these are of high functional importance (Aguilar-Trigueros et al., 2015). However, the single phenotypic parameter that summarizes much of this information is growth rate. Growth rate is a key determining feature of the r-(high growth rate)–K-(high competitive ability) tradeoff and has been used to summarize life history traits of fungi ranging from plant pathogens to wood decomposers (Boddy and Heilmann-Clausen, 2008). It has also been used to explain patterns of successional trajectories, such as in dung and decomposing leaves (Richardson, 2002). High growth rates in short-lived, fast-growing fungi imply high metabolic costs (Arendt, 1997) making these fungi less likely to explore nutrient patches outside those currently exploited. By contrast, slow-growers with high competitive ability might use resources more dynamically to secure longevity through exploitation of multiple patches. Here we report on two experiments that aimed at quantifying relative risk-taking affinity of saprotrophic fungal isolates to

nutrient rich (nutrient acquisition) over co-occurring nutrient poor (exploration) habitats and their relation to isolate growth-rates.

Materials and Methods

We used a set of 30 isolates of filamentous fungi (Andrade-Linares et al., 2016) originating from one grassland to address the hypothesis that fast-growing fungi would allocate a larger fraction of biomass to the nutrient rich habitat compared to slow growers.

The second experiment was carried out to assess growth rates of the 30 isolates. Fungi were incubated in 9cm Petri dishes with PDA agar at 20°C. Radii (four replicates per isolate) of the fungal colonies were measured every second day and growth rates were averaged across the course of the experiment. The experiment lasted until majority of isolates reached the plate perimeter.

To quantify the growth preference of isolates for nutrient-rich PDA agar (lrPDA-growth) we used the log-response-ratio of mean (per isolate) biomasses in the two different sectors of the plate. The log-response ratio of mycelium area densities (lrPDA-density) in the two media was also calculated, with density being defined as $2m_{media}/\pi R_{media}^2$ (m: biomass, $\pi \approx 3.14$; R: radius; see supplement). Total biomass was the sum of the two biomass estimates in the plate. Because in our Petri dishes the compartmentalized nature of the plate could result in confounding effects of colony size and growth-preferences across isolates, we validated relationships between growth preferences and biomass accumulation with independently measured growth rates (Experiment Two). Relationships between the measured and calculated variables were analyzed using linear mixed effects models with harvest as random effect. Additionally, we also analyzed the same relationships for both harvests independently using correlations tests. Upon violation of the assumptions of normality-homoscedasticity we used non-parametric Kendall tests instead of Pearson tests to assess relationships. To account for phylogenetic dependencies, we employed phylogenetically independent contrasts (PICs) with an existing phylogenetic reconstruction of the isolates. Please see the supplementary material for more details on the statistical analyses employed.

Results

The first experiment consisted of 9cm Petri dishes with half of the plate covered with Potato-Dextrose Agar (PDA) and half with water-agar (Fig S2.1). To facilitate later solution the medium agar concentration was 0.8%. Plugs (0.7cm diameter) of the 32 fungi (maintained in PDA) were positioned in the center of the plate and were incubated at room temperature. Each isolate was replicated ten times. The isolates were harvested six (four plates) and nine days (remaining six plates – time point when the fastest fungi reached the plate perimeter) after inoculation. At harvest we averaged three measurements of the colony radius in the PDA and water-agar sectors of each plate. Subsequently, sectors of the plate were separated, submerged in 200 ml of deionized water each, liquefied under heat and vacuum-filtered to a pre-weighted filter. Filters were subsequently dried for 48h at 60^{o}C. The difference in filter weight after and before filtration was used to calculate fungal biomass.

Phylogenetic signal (Blomberg K-statistic) was detected for growth-preference and biomass (Fig. S2.2). Positive relationships were found between lrPDA-growth and either growth rate (after correcting for phylogeny) or total biomass, and no relationships between total biomass and lrPDA-density (Fig. 2.1; Fig S2.4). There was no relationship between lrPDA-density and growth-rate (Fig. 2.1). Fungal colonies grown in nutrient-poor media tend to adopt a low mycelium density per surface which is termed herringbone-pattern-topology (Ott et al., 2013) but we could not detect this in our experiment (Fig. 2.1). The ability of fungi to adjust their mycelium density depending on nutrient availability was not phylogenetically conserved (Fig. S2.2). We conducted a paired *t*-test of the relative-preferences in the two harvests to address the possibility that relative preferences were affected by colony size, and we found no colony size dependencies (t=-0.044, *P*=0.97). We also confirmed that the biomass of the plugs was negligible compared to the size of harvested mycelium.

Discussion

How can our results be interpreted from a functional perspective? That we experimented with artificial media could make generalization debatable (but see supplement). We think that fast-growing fungi can proliferate faster in patches and eventually acquire more resources. Slow-growing fungi pursue an

alternative strategy to remain competitive and this should involve a more dynamic risk management including more effectively exploring their environment. This involves allocating less biomass in existing patches relative to the biomass used for exploring their environment. It is unclear if it is inherent attributes of their mycelium that prevent fast-growing fungi to use their mycelium more plastically or if they have adopted a life-strategy that prevents them from exploring soil (or in our case media). Many of the fast-growers in our experiment, such as all Mucoromycotina, belonged to orders/phyla that are found early in succession and often exhibit a live-fast-die-young strategy (Richardson, 2002). For such fungi exploration of the environment could simply represent a waste of resources. By contrast, for fungi that persist for long periods with active mycelia in soil, such as many Basidiomycota, exploring surroundings may represent a crucial facet of their persistence.

In conclusion, we present evidence that fungal growth rates relate to their biomass-allocation strategies. This relationship holds for the entire range of diverse fungi considered. It is of interest to further test whether the findings are generalizable to other microorganisms.

Legends to figures

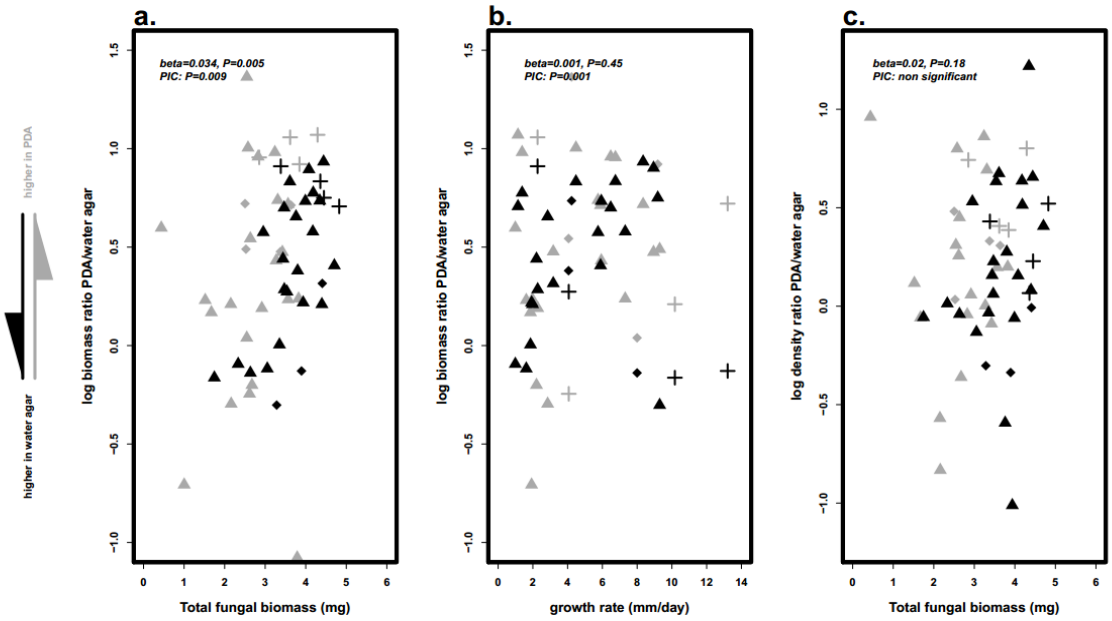

Fig. 2.1 Relationships between the log biomass ratio PDA/water agar (lrPDA growth) and total fungal biomass (a) or the growth rate of the isolates independently assayed in the second experiment (b) and between mycelial area density and total fungal biomass (c). Statistics from a mixed-effects linear model are integrated in the panels and total biomass - lrPDA growth and growth rate - lrPDA growth relationships were significant after correcting for phylogenetic signal. By contrast we found no relationship between total biomass and lrPDA density in any of our tests. Grey points originate from the first harvest whereas black ones from the second. Triangles stand for Ascomycota isolates, rhombuses for Basidiomycota and crosses for Mucoromycotina isolates, respectively. We present raw data (uncorrected for phylogeny) in this figure. We also present the *P* values after correction with phylogenetic independent contrasts (PIC see supplement).

Acknowledgments

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Supplementary data to this chapter can be found in Appendix 2.

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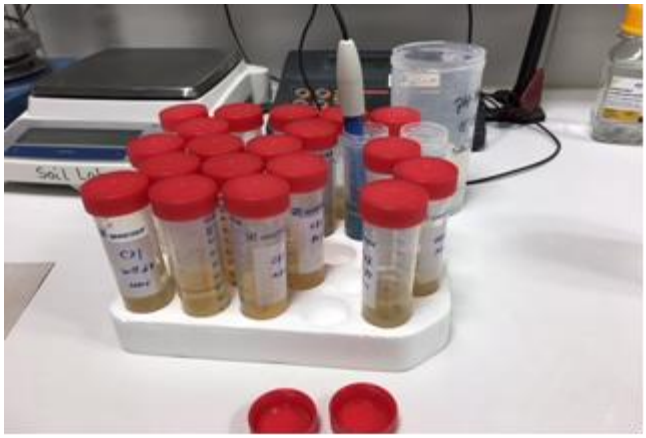
Chapter Three

Fast-growing fungi have a higher sensitivity to a mechanical disturbance but tolerate better its reoccurrence



Dongwei Wang, Stavros D. Veresoglou, Manfred Forstreuter, Matthias C. Rillig

Abstract

In the future, we expect further increases in the intensity and frequency of anthropogenic disturbances to natural and managed ecosystems. By studying disturbance responses of key groups of organisms, such as fungi, we could better assess whether these projected changes will impair delivery of ecosystem services. Here we established a controlled experiment to evaluate comparative responses to disturbance and disturbance frequency in a well described set of 30 soil filamentous fungi. We simulated mechanical disturbances via vortexing liquid cultures containing glass beads to establish three disturbance treatments: No disturbance, low frequency disturbance (i.e. a single vortexing event) and high frequency disturbance (i.e. six vortexing events). We observed a higher sensitivity to disturbances in fast growing fungi, which was associated with a higher carbon use efficiency proxy. We also show that a single disturbance, compared to iterative disturbances, was more deleterious to fungi with fast growth rates. Linking small-scale mechanistic experiments such as the one we present here with high-resolution molecular studies could help interpret community-level responses.

Introduction

We describe as disturbance a disruption in a system, usually involving the death of individuals (Rykier, 1985). Disturbances promote increases in resource availability (Ryckier, 1985) and the temporary unfettering of niche space, which may favor organisms with specific life history traits, such as fast growth rates, high investment in propagules and fast development (Overpeck et al., 1990). A disturbance is characterized by three features, its intensity, duration and, if it is intermittent, its frequency. Microbial responses to disturbance can be complex. Williamson and Wardle (2007), for example, found that a moderately severe fungivory-associated disturbance is more detrimental to soil filamentous fungi than a more severe fungivory treatment. Under specific circumstances, the frequency of disturbance can be at least as important as its intensity (Miller et al., 2011). Kim et al. (2013) observed a lower Shannon diversity index in bacteria at higher frequencies of a physical disturbance, which in this study was the sterilization of part of the soil substrate. Disturbances may induce rapid growth of opportunistic saprotrophic fungi that presumably use the dead microbial biomass as a substrate (e.g. Kristensen et al., 2000); thus predictable changes in the structure of the microbial communit are likely to occur (e.g. Schnoor et al., 2011b; Crowther et al., 2012).

Much of our existing understanding of how fungi tolerate disturbances originates from meta-barcoding studies. A group of fungi that have been particularly well-studied in regards to their disturbance tolerance are arbuscular mycorrhizal fungi (AMF). Community changes in AMF following mechanical disturbances to plants might exceed those of host selectivity (Schnoor et al., 2011a) but they remain weak in magnitude (Lekberg et al., 2012). In general, small-spored mycorrhizal families such as Glomeraceae, Archaeosporaceae and Paraglomaceae share a higher tolerance to disturbances than large-spored families such as Gigasporaceae (van der Heyde et al., 2017), which could explain the predominance of small-spored AMF in agricultural landscapes (e.g. Sýkorová et al., 2006). The colonization rate of the roots by AMF might change, however, more dramatically following disturbances than their community structure (Jansa et al., 2003). Simulated disturbance via canopy gap formation and additions of coarse woody debris could increase the abundance and richness of wood-inhabiting phylotypes (Brazee et al., 2014). In forests, clearcutting was shown to have minimal impacts on the community structure of decomposer fungal communities (Meurisse et al., 1998). By contrast, soil compaction in forest ecosystems has been found to reduce fungal abundance, increase fungal diversity,

and persistently alter the structure of the microbiota (Hartmann et al., 2014). Saprobic taxa, such as ascomycetes and ectomycorrhizal fungi are sensitive to timber clear-cut harvesting disturbance (Hartmann et al., 2012).

Climate warming is predicted to increase the frequency and severity of extreme weather events which from the perspective of soil organisms can be viewed as disturbances (Ummenhofer and Meehl, 2017; Thom and Seidl, 2016). Moreover, anthropogenic activity throughout the globe is projected to further increase, rendering associated disturbances to aboveground but also soil biota more frequent (Swaddle et al., 2015). Because filamentous fungi regulate manifold ecosystem processes in soil (Treseder and Lennon, 2015) and support ecosystem services such as clean water provision and sustainable crop yield (Mace et al., 2012), it is important to investigate how changes in frequency of disturbances may affect filamentous fungi and associated ecosystem functioning. For example, a meta-analysis by Holden and Treseder (2013) quantified the contributions of three common soil disturbances, fire, harvesting of plant biomass and storms induce to declines of microbial biomass, finding average effects sizes of 48.7%, 19.1% and 41.7%, respectively. Microorganisms can influence ecosystem functioning through their physiological activity (Schimel et al., 2007), here defined as the aggregate of intracellular and extracellular changes in the metabolism of an organism. It remains unclear how such declines in microbial biomass influence physiological activity. To fill this gap in the literature we designed a study where we manipulated the disturbance regime and disturbance frequency for a set of 30 isolates of filamentous fungi and studied the implications of these manipulations on the growth rate of the isolates and aspects of the functioning of the cultures (i.e. CO_2 respiration). We hypothesized that fast growing fungi are less sensitive to disturbances because they can compensate with their faster growth rates (*Hypothesis One*) and that frequent disturbance events suppress growth and reduce physiological activity in all filamentous fungi (i.e. augment the detrimental effects of disturbance) proportionally to the influence of a single disturbance event (*Hypothesis Two*).

Materials and Methods

Organisms

We used a well-described set of fungi (Andrade-Linares et al. 2016; Solivaires et al. 2018; Veresoglou et al. 2018) which were isolated from a single grassland (Mallnow Lebus, Brandenburg, Germany, 52°27.778' N, 14°29.349' E) in Germany. We selected for this study a subset of 30 fungal strains with representatives of the three phyla Ascomycota, Basidiomycota and Mucoromycota and which additionally, varied considerably in growth rates (TABLE S3.1).

Experimental Design

For this experiment, we used liquid cultures established in 50mL conical bottom centrifuge tubes (Sarstedt, Germany) filled with 20mL potato dextrose broth (PDB). Tubes were inoculated with 0.7cm diameter plugs (two plugs per tube) excised from the growing edge of a stock culture in Potato Dextrose Agar (PDA). Liquid cultures were incubated at room temperature (25°C) on a horizontal shaker (New Brunswick Scientific, Excella E24 incubator shaker series, USA) at 120 rpm over a period of eight days. To prevent anoxic conditions, the tubes were uncapped daily. In each tube we had added three sterilized glass beads (diameter of 4±0.3mm, Carl Roth, HH55.1, Germany) so that we could induce disturbances (i.e. disruption of fungal mycelium) through vortexing (Scientific Industries, Vortex Genie 2, gear: 10, Germany). For each isolate, we applied in triplicate the following three disturbance treatments: (i) no disturbance (120 rpm); (ii) low frequency disturbance (a single disturbance event on the second incubation day); (iii) high frequency disturbance (daily disturbance starting from the second incubation day, totaling six disturbance events; Fig. 3.1). Each disturbance event (vortexing) lasted 10 seconds. Disturbance events was intended to resemble the activity of burrowing animals in the soil; however, here we aimed at reproducing disruptions in the integrity of the fungal mycelium; such disruptions are likely common in soil following mycelial grazing (Tordoff et al., 2008). Even though bead beating is well known to disrupt filamentous fungi and is thus often used in DNA extraction protocols (e.g. Müller et al., 1998), to the best of our knowledge no studies have used this approach to study disturbance in fungi. We applied a fully randomized factorial design with the two factors being the fungal isolate and the disturbance treatment each with three replicates, yielding a total of 31 x 3 x 3 =279 Tubes. On the eighth

day, we harvested the fungal cultures and assayed fungal biomass by thawing the agar in a microwave oven and filtering it through a pre-weighted, pre-dried 11-µm pore size filter (Whatman, 90mm diameter, 1001090). Biomass was determined as the weight difference of the filters after drying them for 48h at 60 °C.

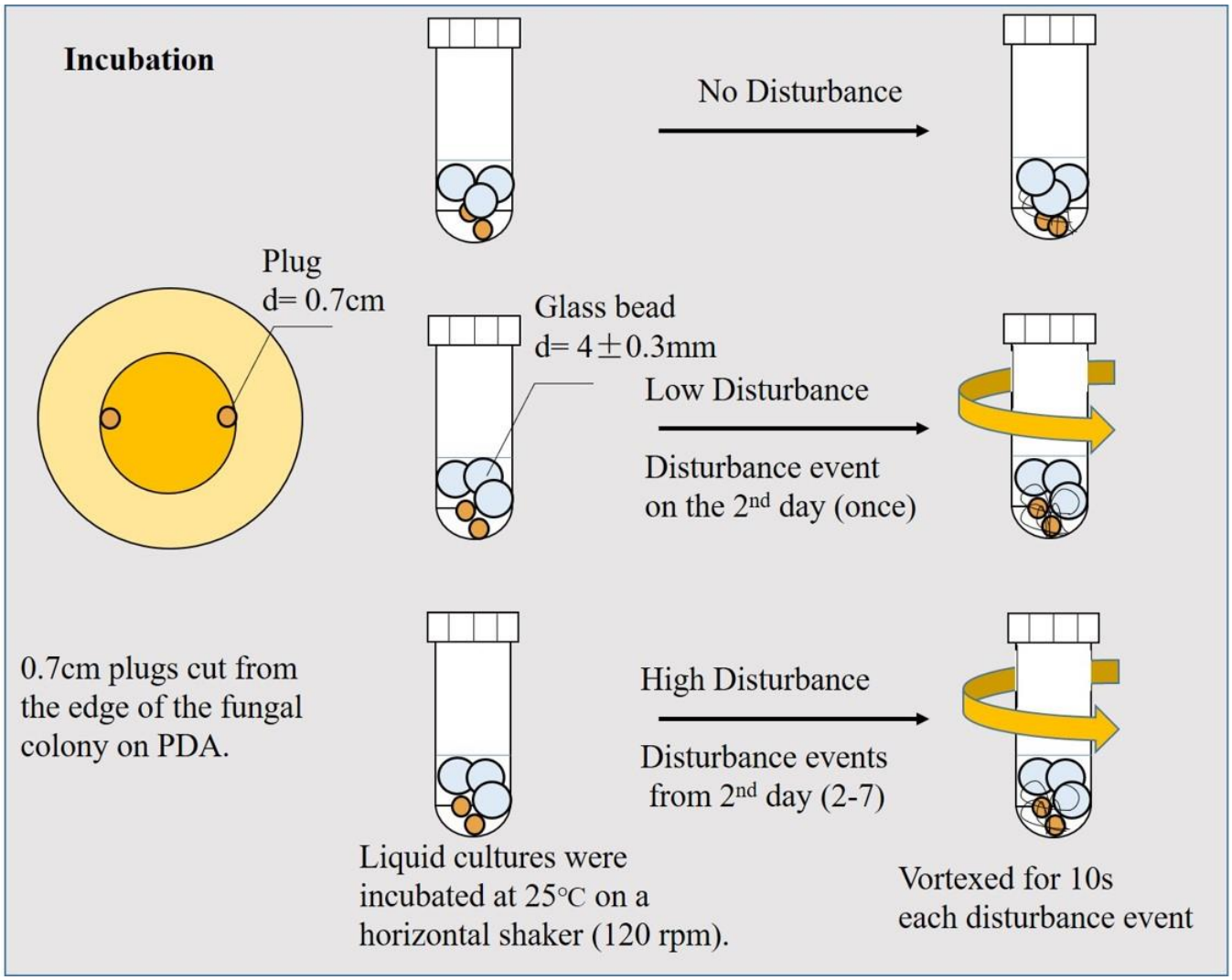


Fig. 3.1 Illustration of the experimental design. Cultures contained two plugs taken from a culture maintained in PDA and three beads. There were three treatments: no disturbance, low disturbance, and high disturbance. Measurements included assaying qCO_2 at harvest and fungal biomass.

CO_2 measurements

Respiration rates were measured following Bradford et al. (2010). Centrifuge tubes were fitted with caps modified for gas analysis, flushed with air and headspace CO_2 exchange rate were determined using a portable gas-exchange system (HCM-1000, Walz, Effeltrich, Germany) with an infrared gas analyzer (BINOS-100/4PS, Rosemount). We assayed the exchange rate of CO_2 in $\mu molCO_2 m^{-2} s^{-1}$. We used the CO_2 measurements to calculate a proxy of carbon use efficiency (Maynard et al., 2017), the microbial metabolic quotient (qCO_2 describing the respiration rate per unit of microbial biomass). This quotient has been used extensively in disturbance studies (Anderson and Domsch, 1990; Pabst et al., 2016 but see Wardle and Ghani, 1995) and addresses the mechanisms underpinning our observations of disturbance tolerance.

Statistical analyses

We assessed growth rate of the fungi by dividing biomass of the isolates in the undisturbed treatment by the number of days of growth (eight days). To assess overall sensitivity to disturbance (i.e. address *Hypothesis One*), we used a disturbance-sensitivity score which we calculated by averaging mean growth data per isolate across the two disturbance treatments. By averaging the two types of disturbance (i.e. occasional and iterative) we made the sensitivity score descriptive of both types of disturbances. We subsequently adapted an expression from the competition literature quantifying competition intensity as the difference in biomass with and without competition divided by the larger of these two values (Callaway et al. 2002; Brooker et al. 2005). Our adaptation of the metric to our disturbance experiment had the following form (Formula 3.1): $S = \frac{M_{ND} - M_D}{x}$. M_{ND} is the mean biomass without disturbance, M_D the mean biomass with disturbance and x is the maximum of M_{ND} and M_D. The specific formulation of sensitivity allows for negative sensitivity values if a species proliferates better following disturbance and scales between -1 and 1. Our expectation was that overall sensitivity to disturbance correlated positively with growth rate (i.e. fast growing fungi were more susceptible to disturbance – *Hypothesis One*). We assessed this relationship with all fungi, irrespective of their responses to disturbance and with the subset of isolates that both at low and high frequencies of disturbance showed a biomass reduction (i.e. negative sensitivity values).

To address how the frequency of disturbance alters the fitness of a fungus (i.e. *Hypothesis Two*) we calculated the response ratio of sensitivity to disturbance in the single disturbance treatment over the

sensitivity to disturbance in the iterative disturbance treatment which we term here the disturbance ratio. Interpreting ratios becomes challenging when the variable ranges include both negative and positive values. A positive response (i.e. ratio value), for example can arise from either the division of two positive or two negative effects. For this analysis we thus only used the 15 isolates which met the following two conditions: (i) the mean biomass at harvest in the low frequency disturbance treatment was below that of the no-disturbance treatment (14 isolates did not meet this criterion); (ii) the mean biomass at harvest in the high frequency disturbance treatment was also below that in the no disturbance treatment (10 isolates did not meet this criterion). This resulted in a semi/standardized variable that had values close to one when there were no differences between the two disturbance treatments and large positive values when the fitness losses for the fungus were higher in the iterative disturbance treatment. Our expectation was that growth rate did not correlate with the ratio of mean per isolate biomass loss (*Hypothesis Two-a*) or with sensitivity to disturbance (*Hypothesis Two*-b).

To address above-mentioned relationships, we carried out Pearson correlation tests. When assumptions of normality and homogeneity of variances were not met, we used Kendall Tau tests instead. To address whether the disturbance frequency effect is phylogenetically conserved, we assessed phylogenetic signal for each of them with the Blomberg's K method (Blomberg et al., 2003; Drummond and Rambaut, 2007) and corrected for phylogenetic dependencies with Phylogenetically Independent Contrasts (PICs; Freckleton, 2009). We used a phylogenetic reconstruction of the fungal isolates, which has been published in Andrade-Linares et al. (2016). Each of the P values for Blomberg's K method was produced following a series of 9999 permutations where the tips of the tree were shuffled. The phylogenetic reconstruction was based on sequence information on a single locus, ITS. There is a consensus use of the ITS locus to classify taxa to species (Schoch et al. 2012) which comes at the cost of ITS having a lower resolution at taxonomic ranks above the family level. Because PICs require that the topology of the phylogeny be accurate (Cooper et al. 2016), ITS represents an obvious choice of a locus to recreate single locus phylogenies for PIC analyses. The species names for the 33 fungal isolates represented best hits of BLAST searches in NCBI and species names may not necessarily be accurate; we preferentially reconstructed the phylogeny for our isolates rather than using published phylogenies to maximize accuracy at low taxonomic ranks.

Results

Disturbances induced a median 7.4% decline in biomass production in the treated fungi. Fungi that received iterative disturbances were on average 7.3% smaller than those that received a single disturbance treatment. The carbon use efficiency proxy varied between 8.2% (first quartile) and 61% (third quartile; the median was 34%). We did not observe phylogenetic conservatism (i.e. significant Blomberg'S K values) in any of the traits in this experiment.

There was a positive relationship between sensitivity to disturbance and mean growth rate (Fig. 3.2a) which was robust to a correction for phylogeny (Fig. 3.2b). Moreover, there was a positive relationship between growth rate and the carbon use efficiency proxy, irrespective of phylogenetic corrections (Fig. 3.3). Sensitivity to disturbances ranged from -68% (i.e. a 68% increase in biomass following disturbance) to 44%.

Slow growers were more sensitive to iterative disturbances irrespective of phylogenetic corrections (Fig. 3.4). There was, however, no relationship between our metric of frequency of disturbances and sensitivity to disturbances (Fig. 3.5).

Discussion

Fungi represent indispensable constituents of terrestrial ecosystems and drive many key ecosystem processes. Yet, our understanding of their ecology lags behind those of macroscopic organisms and bacteria (e.g. Veresoglou et al., 2015). We address here how the disturbance ecology of fungi relates to their growth rate. For our analyses, we used representatives of the three main phyla in the Eumycota and our findings should thus reflect general trends across filamentous fungi, at least for this particular site. We show that the sensitivity to disturbance increases for fast-growing fungi but it is slow-growing fungi that suffer most from iterative disturbance events.

It has been argued that it is possible to scale up fundamental trade-offs in filamentous fungi to large spatial scales (Maynard et al., 2019). Here we worked at a laboratory microcosm scale and the degree to which these trade-offs can be found at regional scales remains unclear. A challenge, however, remains to

rationalize such trade-offs. A possibility why slow- growing fungi can tolerate better disturbance is that they possess traits which are conserved at crude taxonomic scales which facilitate tolerance to disturbance. As an example, hyphae in Basidiomycota and Ascomycota contain regular septa (Stajich JE, et al., 2009) which could minimize damage following injury. Nevertheless, we were unable in this study to detect phylogenetic signal for any of the traits we examined (Fig. 3.2, 3.3, 3.4, 3.5). Traits which are only conserved at crude taxonomic scales are suggestive of low evolution rates, and may generate non-detectable phylogenetic signal (e.g. Revell et al., 2008). Another possibility is that recovery responses of fungi to disturbance are linked to their C nutrition (Holden and Treseder, 2013). Depending on the severity of the disturbance a considerable fraction of the fungal community might experience death. Mucoromycotina pioneer microbial succession (Schneider et al., 2012) but rely on readily available carbon which following disturbances, at the recovery stage, should be in high demand. By contrast, isolates in Basidiomycota possess the ability to degrade less available C such as lignin (e.g. white-rot fungi - Steffen et al., 2000) the availability of which should be influenced less by disturbances. It may thus be the case that isolates in Basidiomycota and other slow-growing fungi feature physiological adaptations that allow them to better tolerate disturbances without slowing down their metabolism. To this end, we detected a higher (proxy of) carbon use efficiency for slow-growers (Fig. 3.3).

A high frequency of disturbances could potentially negate the effectiveness of survival mechanisms in filamentous fungi such as compensatory growth (Bentgtsson et. al., 1993) and anastomosis (Roca et al., 2005), rendering more important tolerating disturbance than avoiding it. In agreement to our hypothesis (i.e. *Hypothesis Two*) which predicted that tolerance would be proportional to the tolerance to single disturbances, it was mainly fast growers that tolerated well intermittent disturbances (i.e. showed a low biomass loss response ratio). This suggests that disturbances may induce predictable shifts in the community structure of filamentous fungi in soil. Existing attempts to monitor traits in filamentous fungi (e.g. Aguilar-Trigueros et al., 2015), could thus have the added value of becoming integrated into observational meta-barcoding studies on fungi from disturbance experiments.

Despite attempts to unify definitions of disturbance (e.g. Rykier, 1985), these vary across experimental systems. Also “death-inducing perturbations” which we commonly describe as disturbances, can speed up growth rates in the longer term, for example through the release of nutrients, compensatory growth or increased sporulation and dispersal (e.g. McNaughton, 1983). Fungal isolates in the single disturbance treatment of our experiment (as well as in the first mechanical disturbance in the iterative disturbance

treatment) were allowed seven days to recover from the mechanical disturbance to which they exposed them which should have represented sufficient time to recapture mineralized nutrients (Tao et al., 2008; Boberg et al., 2010). For example, disturbance through mowing can induce overcompensation responses in plants (Agrawal, 2000). Increases in growth rate thus remain likely following disturbances. The experimental settings in our experiment here might have been subject, however, to the shortcoming of vortexing increasing the efficiency of propagule dispersal and possibly improving the oxygenation state in the cultures (these two changes should also be associated with the activity of burrowing animals), which we could not control for in our undisturbed treatment. However, the cultures were incubated on a horizontal shaker, meaning that some dispersal structures such as conidia, had a high dispersal rate in the culture and might have not benefitted from vortexing. Moreover, actively growing mycelial fragments were likely located in the outer surface of the growing culture irrespective of votexing. It thus came to us as no surprise that we observed little influence of our disturbance treatments on the overall growth rate. Because, however, these changes in growth rate were predictable (Fig. 3.2; Fig. 3.4), we believe that our experiment effectively measured disturbance tolerance of filamentous fungi.

We highlighted the significance of fungi in regulating key ecosystem processes which brings into question the implications of our findings for overall ecosystem functioning in the introduction. We showed that fast-growing fungi are more sensitive to disturbance (Fig. 3.2), but cope best with intermittent disturbances (Fig. 3.4). Based on these findings, it is the slow-growing fungi that should benefit from small intensity, infrequent disturbances. Nevertheless, the nature and intensity of disturbances varies considerably with some types of disturbances such as fire being considerably more destructive than others (Holden and Treseder, 2013). We believe that high-intensity disturbances which disrupt all species of filamentous fungi should eventually benefit fast-growing taxa because (a) fast-growing fungi are more likely to invest heavily in propagule/conidial production and show a better dispersal (Metcalfe and Monaghan, 2003); (b) fast-growing fungi should be more effective in recolonizing habitats (Cadotte, 2007). Slow-growing fungi should thus excel in resistance to disturbance (i.e. the property of an ecosystem and form of ecosystem stability that allows it to remain unchanged following environmental perturbations; Shade et al., 2012) whereas fast-growing fungi in resilience (i.e. the property of an ecosystem and form of ecosystem stability that allows it to reach again a stable equilibrium quickly after an environmental perturbation has made it unstable; Shade et al., 2012). It

could be the case that ecosystem stability peaks under conditions that allow slow- and fast-growing fungi to coexist, such as intermittent localized high-intensity disturbances.

We presented data on how a diverse set of 30 fungi responds to low and high-frequency disturbances. We showed that in both cases it is fungi with slow growth rates that tolerate disturbance better and argued for possible implications of our results at an ecosystem level. Linking small-scale mechanistic experiments such as the one we present here with high-resolution molecular studies could help interpret community-level responses (Hall et al., 2018).

Legends to figures

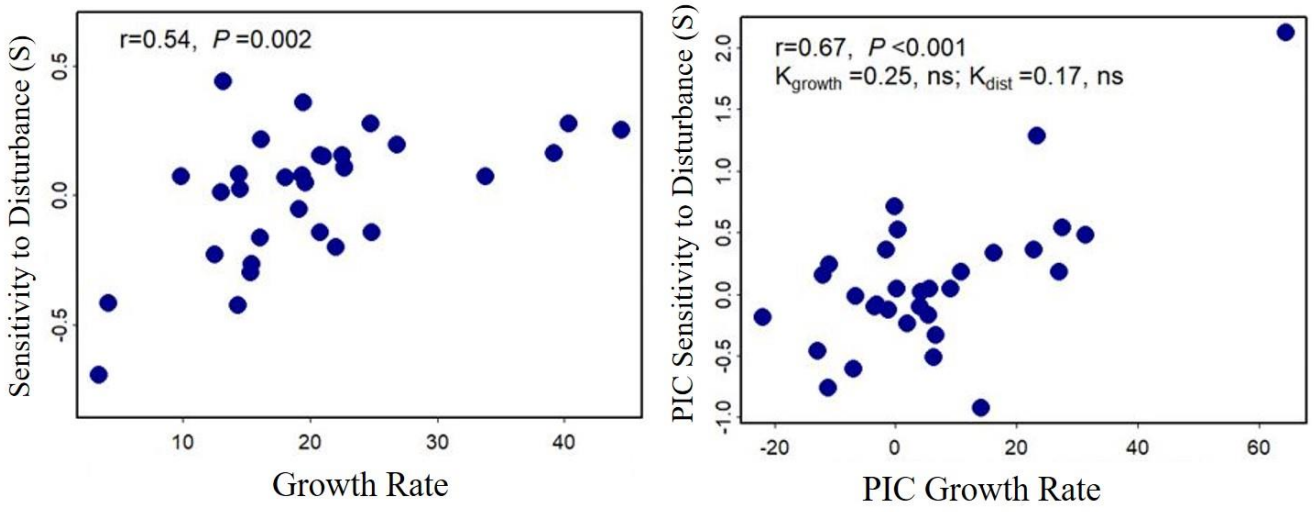


Fig. 3.2. Relationships between sensitivity to disturbance and mean growth rate with (a) and without (b) phylogenetic corrections. In the absence of phylogenetic corrections, each point represents a fungal isolate; in the presence of phylogenetic corrections each point represents a node in the phylogenetic tree.

We defined sensitivity to disturbance as $S = \frac{M_{ND} - M_D}{x}$, where M_{ND} is the mean biomass without disturbance, M_D the mean biomass with disturbance and x is the maximum of M_{ND} and M_D. We assayed fungal biomass at harvest to determine growth rate. The relationships were significant in both cases, even though none of the two traits was phylogenetically conserved.

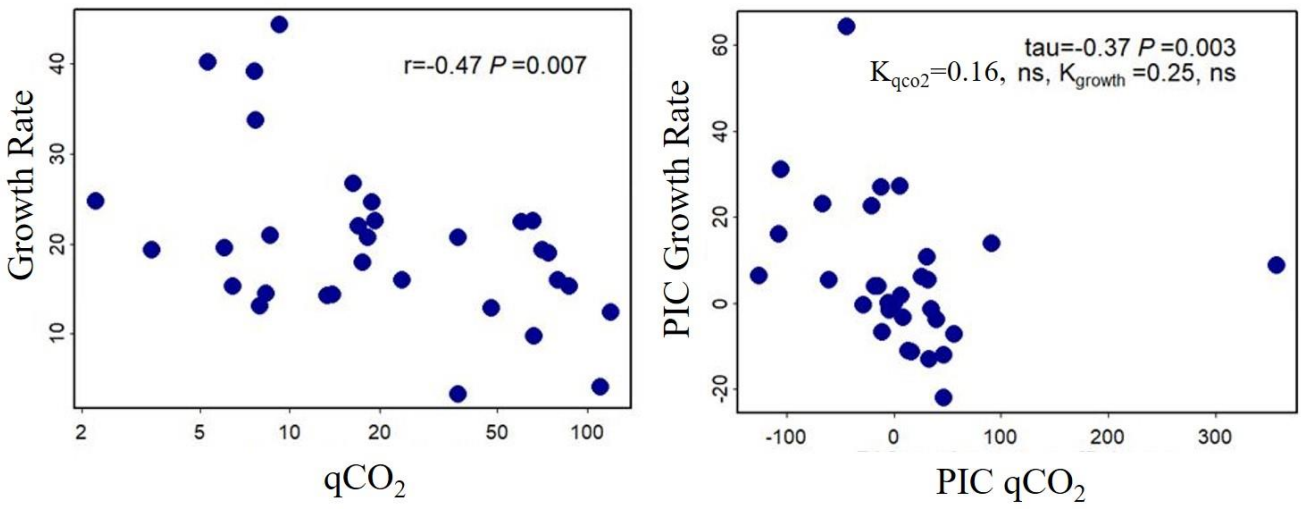


Fig. 3.3 Relationships between growth rate and the qCO_2 we used in this study with (a) and without (b) phylogenetic corrections. In the absence of phylogenetic corrections each point represents a fungal isolate; in the presence of phylogenetic corrections each point represents a node in the phylogenetic tree. For this analysis, we preserved only the isolates which showed a lower biomass following disturbance in both the disturbance treatments. The relationships were significant in both cases.

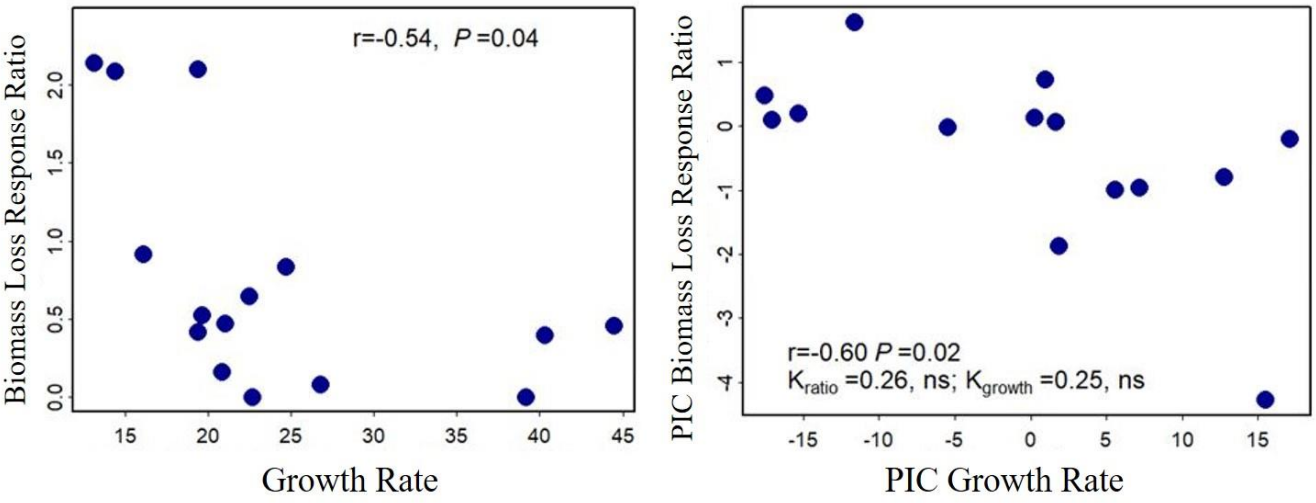


Fig. 3.4 Relationships between the ratios of biomass loss in single versus iterative disturbances (i.e. high values suggest higher losses in the single disturbance treatment) vs mean growth rate with (a) and without (b) phylogenetic corrections. In the absence of phylogenetic corrections each point represents a

fungal isolate; in the presence of phylogenetic corrections each point represents a node in the phylogenetic tree. For this analysis we preserved only the isolates which showed a lower biomass following disturbance in both the disturbance treatments. The relationships were significant in both cases, even though none of the two traits was phylogenetically conserved.

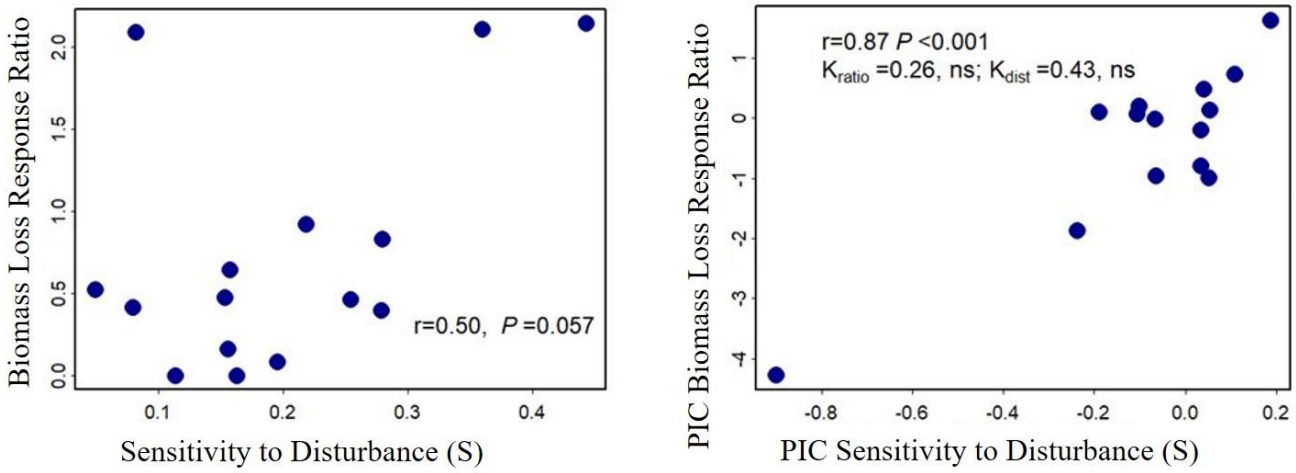


Fig. 3.5 Relationships between the ratio of biomass loss in single versus iterative disturbances (i.e. high values suggest higher losses in the single disturbance treatment) vs sensitivity to disturbance with (a) and without (b) phylogenetic corrections. In the absence of phylogenetic corrections each point represents a fungal isolate; in the presence of phylogenetic corrections each point represents a node in the phylogenetic tree. For this analysis we preserved only the isolates which showed a lower biomass following disturbance in both the disturbance treatments. The relationships were significant only following a phylogenetically correction.

Acknowledgments

DW, MCR conceived the idea, DW prepared material and carried out the experiment, analyzed the data with the help of SDV and wrote the manuscript with contributions of SDV. MF contributed to methods and instruments. All authors commented on the manuscript and approved the final version.

Supplementary data to this chapter can be found in Appendix 3.

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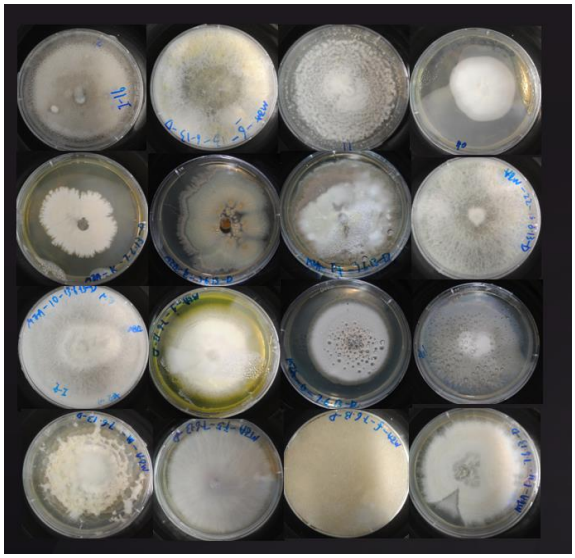
Chapter Four

Fungal fast growers have lower sensitivity to temperature



Dongwei Wang, Stavros D. Veresoglou, Diana R. Andrade-Linares, Ulfah Mardhiah, Matthias C. Rillig

Abstract

Plasticity of phenotypic traits has profound implications for survival. Here we carried out an experiment to assess temperature sensitivity (Q_{10}) and optimal growth temperatures in a set of 30 filamentous soil fungi. We experimented with growth at four different temperature settings: 15^{o}C, 20^{o}C, 25^{o}C & 30^{o}C and assessed growth as radial expansion of the fungal colony. We hypothesized that faster-growing fungi will have lower Q_{10} values (*Hypothesis One*) and that fungi with higher optimal growth temperatures will have lower Q_{10} values (*Hypothesis Two*). Optimal growth temperatures ranged from 19.0 to 35.5^{o}C. We observed no significant relationships between Q_{10} and growth rate at 20°C, which was disagreement to *Hypothesis One*. We found, however, a significant positive relationship ($P < 0.001$) between Q_{10} and optimal temperature in support to *Hypothesis Two*. The relationship was robust to consideration of phylogeny via Phylogenetically Independent Contrasts (PICs) ($P = 0.006$). Following phylogenetic corrections there was also a negative relationship ($p = 0.046$) between Q_{10} and growth after PICs. Our results suggest that fast growing fungi maintain narrower temperature optima. Our results support (i.e. *Hypothesis Two*), the niche breadth hypothesis suggesting that cold-acclimated organisms which originate at high latitudes have a broader ecological niche, which was here manifested with a lower sensitivity to temperature.

Introduction

Temperature induces profound physiological changes in living organisms and as a result regulates their fitness (Newsham et al., 2016; Miyamoto et al., 2015; Zogg et al., 1997; Li et al., 2009; Zucconi et al., 1996; Pietikäinen et a., 2005). This should be particularly the case in poikilotherm organisms (McCurchie et al., 1973), such as fungi, which maintain close to ambient body temperatures. Filamentous fungi play a key role in the functioning of most terrestrial ecosystems, in particular in regards to the processes of litter decomposition, nutrient cycling, nutrient uptake and soil aggregation (Paszkowski, 2006 Martinez-Garcia et al., 2017; Morris et al., 2019; Rillig and Mummey, 2006; Lehmann et al., 2017; Gryndler et al., 2009; Shah et al., 2016). Despite the range of life-history and phenotypic parameters that can be used to classify fungi (Aguilar-Trigueros et al., 2015), it is increasingly appreciated that we can summarize much of this information on a single axis, describing on one end fast-growing fungi, which invest little in producing secondary compounds that facilitate competition (r-strategies), and on the other end slow-growing fungi that rely on effective competition for survival (K-strategies) (Pianka, 1970; Andrews and Harris, 1986; Andrews and Rouse, 1986; Boddy and Heilmann-Clausen, 2008; Veresoglou et al., 2018).

The metabolism of an organism is regulated by numerous enzymes, which, subject to environmental conditions, catalyze key chemical reactions (Mackie et al., 1990). This makes it difficult for organisms, for example, to function under temperatures below zero or above 50 ^{o}C (Weinstein et al., 2000; Malcolm et al., 2008). Some organisms have evolved an arsenal of genes that allow them to cope with sub-optimal temperature settings (Barcenas-Moreno et al., 2009). This adaptation, however, should come at a fitness cost. Thus, we expect that the fungi that possess genes that allow them to grow under sub-optimal temperature will underperform under optimal growth conditions (*Hypothesis One*). In other words, we expect that fungi that grow fast under optimal conditions will have narrower temperature optima.

A widely used metric for temperature sensitivity is Q_{10}, which quantifies how much the rate of a physiological process changes when we alter the temperature by 10 ^{o}C (Lloyd et al., 1994). The Q_{10} metric has been often used to describe temperature responses in systems with filamentous fungi. Malcolm et al. (2008), for example, showed that the subset of ectomycorrhizal fungi that could not tolerate high temperature (i.e. had lower temperature sensitivity) required less carbon from their host

plants. Bai et al. (2017) calculated Q_{10} by comparing PLFA concentrations (i.e. PLFAs were used as proxies for abundance of major taxonomic groups of organisms and how these responded to temperature) in response to altering temperature by 10^{o}C to show that diurnal changes in temperature can modify PLFA stress indicators to warming. In a synthesis of the then available literature, Hamdi et al. (2013) showed that Q_{10} values in filamentous fungi range between 0.5 to over 300. Alster et al. (2018) used Q_{10} values as a response variable in a meta-analysis to show that fungi are more sensitive to temperature than bacteria. Moreover, Bradford (2013) used Q_{10} for better understanding multiple types of thermal adaptation. Hence, the use of chose Q_{10} as a response variable in our study makes our results comparable to a substantial body of literature on filamentous fungi.

Another key factor influencing the sensitivity of filamentous fungi to changes in temperature relates to their origin and distribution. The niche breadth hypothesis (Brown, 1984) proposes that the organisms that inhabit the poles occupy wider ecological niches than those in the tropics and can tolerate a wider range of environmental conditions (Morin et al., 2006). Latitudinal changes overlap greatly with those of temperature that peaks in the tropics. It is thus likely that fungi adapted to lower temperatures (i.e. which implies that the kernel of their distribution occurs at high latitudes), have a wider ecological niche and are less sensitive to temperature (Curie, 1991). We would, thus, expect that the fungi that have lower optimal temperatures would be more plastic in terms of their temperature requirements (*Hypothesis Two*). In other words, we expect a negative relationship between Q_{10} and optimal growth temperature.

We present here a comparative study with filamentous fungi spanning across the entire Kingdom of fungi. In summary, our two hypotheses where that (1) faster-growing fungi have lower Q_{10} values than slow-growing fungi (*Hypothesis One*); (2) fungi with lower optimal growth temperatures have Q_{10} values (*Hypothesis Two*).

Materials and Methods

Organisms

We used a diverse set of soil fungi which were all isolated from a grassland (Mallnow Lebus, Brandenburg, Germany, 52°27.778’ N, 14°29.349’ E) in Germany (Andrade-Linares et al., 2016). The

fungi had been isolated with a range of different media including Malt Extract agar, Benomyl agar, Rose Bengal agar, Yeast Extract-Peptone-Dextrose agar and Czapek Dox Agar (Thorn et al., 1996). From an original set consisting of hundreds of fungi, Andrade-Linares et al. (2016) selected a subset of 30 fungi with the criteria of (1) including representatives of all major clades of fungi (i.e., Ascomycota, Basidiomycota and Mucoromycotina) and (2) including isolates showing a high variation in terms of growth rates. Phylogenetic relationships across these fungi were reconstructed with a maximum-likelihood (general time reversible clock with gamma distributed sites) phylogenetic tree.

Experimental Design

Our experimental design was a fully randomized mono-factorial (i.e temperature with four levels) design with four replicates (Fig. 4.1). We inoculated 9 cm Petri dishes filled with PDA with a 0.7 cm diameter plug extracted from the edge of an actively growing colony. The four incubation temperatures were 15 ^{o} C, 20^{o} C, 25^{o} C and 30 ^{o}C. The experiment was carried out in two batches so that there were sufficient incubators to replicate each set of temperature settings (i.e. there were only six incubators); first, we collected growth data at temperatures 15 ^{o}C, 20 ^{o}C and 25 ^{o}C and then at temperatures 30 ^{o}C and 35 ^{o} C. Each incubation temperature was replicated to a minimum of two incubators (Binder, 12-22064, Germany) so that we could detect any incubation-settings-related variation in growth conditions. There was a total of 4 x 4 x 30= 480 petri dishes. We assessed growth rate via measuring radial extension rate (mean value of two colonial diameters perpendicular to each other). We then estimated temperature sensitivity by using the Q_{10} index (Formula 4.1):

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}, \qquad \ldots(4.1)$$

Where R_2 and R_1 are the growth rates of the fungus at the highest temperature (T_2) and lowest temperature (T_1) out of two temperatures. We calculated the Q_{10} value for all possible pairs of temperatures (i.e. $\binom{4}{2} = \frac{4!}{2!.2!}$ C (4, 2) = 6 pairs and averaged them. A higher Q_{10} value indicates a higher sensitivity to temperature. Because two isolates did not grow at 30^{o}C, we generated Q_{10} values for a subset of 28 out of the 30 fungal isolates.

Assessing optimal growth temperature for the isolates in our study

We approximated optimal growth variability across isolates with a Gaussian curve of square root transformed temperatures (Fig. S4.1). We carried out the square root transformation of temperature (i.e. Appendix 4.1) to address issues arising from skewness. The Gaussian distribution can be expressed as:

$$f(x) = \hat{f}(x)e^{-(x-\mu)^2/\sigma^2} \qquad \ldots(4.2)$$

Where *f(x)* is an ecosystem process rate (e.g. growth rate) which depends on the parameter *x* (e.g. temperature). We can further simplify the formula by approximating $\hat{f}(x)$ with the maximum observed process rate in the dataset (here growth rate at any replicate petri dish) as:

$$f'(x) = e^{-(x-\mu)^2/\sigma^2} \qquad \ldots(4.3)$$

Where *f'(x)* is the z-score standardized value for the process *f(x)*.

We used formula (4.3) to analyze our data after square root transforming temperature settings. We fitted a non-linear model that had as a response variable the z-score standardized growth rate estimates for our cultures at four different temperatures (15, 20, 25, 30 ^{o}C) and the respective square root transformed temperatures as predictors. Our non-linear models can occasionally fail to converge (i.e. model fitting in non-linear models is iteration based which depending on the distribution of data could fail to converge in which case the model gives an error) or produce uninformative estimates (i.e. when there is a low confidence on the estimates of the parameters; here we set a threshold of a standard error for the optimal temperature below 10 – Appendix 4.2).

Colony diameter over time was compared between strains and temperatures for each species by a Kendall's tau rank correlation test and a Spearman rank correlation test (Sokal and Rohlf, 1995). Growth rate (mm d^{-1}) was determined from the slope of the linear regression fitted to the colony diameter data over time. Data were log transformed when necessary to achieve homoscedasticity. Some additional details on the experimental design are presented in Appendix 4. All statistical analyses were carried out in R version 3.5.1 (R core team 2018).

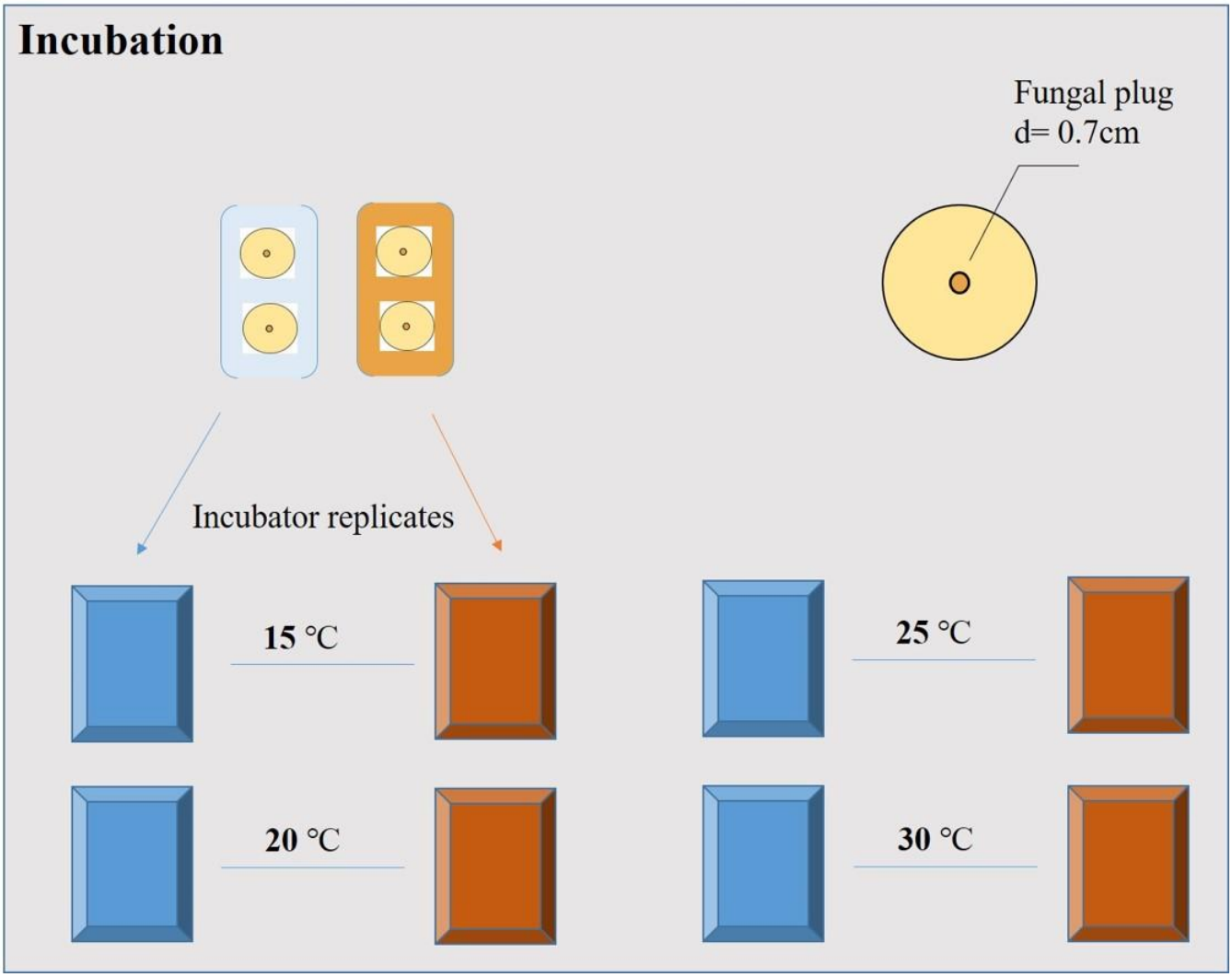


Fig. 4.1 Illustration of the experimental design. Cultures contained one plugs taken from a culture maintained in PDA. There were four temperature treatments: 15°C, 20°C, 25°C and 30°C. Measurements included radial extension rates.

Results

Optimal growth temperatures

We present growth information for the 30 fungal isolates and four ruderal traits at all four temperatures at Fig. S4.6 and Fig. S4.7. Over the course of the experiments all isolates were actively growing in

diameter. Optimal growth temperatures ranged from 19.0 to 35.5°C (Fig. 4.2). Most isolates had optimal temperatures between 20-25°C. We excluded three isolates (*Exophiala salmonis*-DF36 and *Phialophora sp*.-DF35) from further analysis, that were outside the range of temperatures we tested and thus the optimal temperature estimates were unreliable. We plotted the optimal fungal growth against the reconstructed phylogeny of the isolates and assessed phylogenetic signal (Fig. 4.3).

There was a significant negative relationship ($p = 0.046$) between Q_{10} and growth at 20°C after PICs were conducted (rho=0.39, *P*=0.046, Fig. 4.4b) but not in the absence of phylogenetic corrections (tau=-0.069, *P*=0.62). We arrived to similar conclusions when we used colony plasticity instead of Q_{10} (Fig. S4.4 and Fig. S4.5). Significant positive relationships ($p = 0.006$) between Q_{10} and optimal temperature were found irrespective of PIC corrections (Fig. 4.5). Taken together, fast growers showed lower temperature sensitivity of growth. The fungi that have lower optimal temperatures were less sensitive to temperature.

Discussion

We present here a study on temperature sensitivity of filamentous fungi. Even though we found little evidence (Fig. 4.4) that growth rate relates to the efficiency with which filamentous grow at an ambient temperature of 20°C, we observed that temperature sensitivity relates to optimal growth rate (Fig. 4.5). This relationship was robust to phylogenetic corrections. Our first hypothesis was that fast-growing fungi would be less sensitive to temperature change than slow-growing fungi. We standardized for temperature by comparing performance at 20°C which represents a temperature that most of the isolates should regularly experience at the grassland where they were isolated. We assessed temperature sensitivity through either the Q_{10} parameter or colony plasticity. We only found support for this hypothesis after correcting for phylogeny with PICs (i.e. rho=0.39, *P*=0.046, Fig. 4.4). It is unclear if there are inherent growth constraints of their mycelium that prevent fast-growing fungi from making more plastic use of resources or if they have adopted a specific life strategy that prevents them from adapting temperature change. High growth-rates in short-lived, fast-growing fungi imply high metabolic costs (Arendt, 1997), making these fungi less likely to explore nutrient patches. By contrast, slow growers with high competitive ability might engage in a more dynamic use of resources that could

secure longevity through an exploitation of multiple patches (Veresoglou et al., 2018). Climate change will lead to an increase in global temperature in the next century with estimates ranging between 1.4 and 5.8 ^{o}C (IPCC, 2014). There is evidence that temperature adaptations in fungi induce prominent changes in their carbon use efficiency, which is linked to survival (Crowther et al., 2013). It is thus expected that fungi maintaining a lower sensitivity to temperature will cope better with global change than those maintaining narrow temperature optima.

We found, by contrast, strong support that temperature sensitivity relates to the optimal temperature where a fungus grows (Fig. 4.5). Isolates with a low optimal growth temperature maintained lower Q_{10} values suggesting that they were less sensitive to temperature. This observation was congruent with the expectations of the Niche Breadth Hypothesis, provided that the kernel of the distribution of cold acclimated filamentous fungi is closer to the poles than that of warm acclimated fungi. Crowther et al. (2013) also found the warm-acclimated individuals having lower growth at intermediate temperatures than cold-acclimated isolates. Cold adapted fungi maintain a range of adaptations such as forming cysts and minimizing volume to surface area and thus exchange with environmental stressors (Selbmann et al., 2012; Wang et al., 2017). Even though we did not assay morphological adaptations in our fungi, a fruitful avenue to continue this project is to link temperature optima across fungi with metrics of mycelial morphology such as the fractal dimension, which has been measured for our set of fungi (Lehmann et al., 2018).

The optimal growth temperatures in our set fungi ranged between 19.6 to 35.5 ^{o}C. Most other studies have reported temperature optima for growth in most fungi around 30^{o}C (Barcenas-Moreno et al., 2009). To a certain degree, this difference could be due to the different techniques that authors use in the literature to assess optimal growth rate and temperature sensitivity. For our calculation of Q_{10}, for example, we aggregated many pairwise comparisons the way it was proposed by Alster et al. (2016). However, we used a crude temperature resolution of five degrees which implies that we might have missed the peak of fungal growth and underestimated temperature sensitivity. The same argument could be made for the calculation of optimal temperature, which was inferred from a statistical model. We experimented with a group of soil fungi that had been isolated from a single grassland which presents evidence that they share a potential of co-occur in some habitats and thus should not present as extreme differences in physiology as random sets of isolates from culture collections.. Another reason why we observe this mismatch may have been due to a different history of culturing the isolates. Common

practices, such as long-term incubation at 20^{o}C may have altered on the medium term the growth optima of our isolates. Finally, it is likely that the mismatch was due to the different origins of the cultures that here corresponded to the growth settings in temperate grassland.

We expect, however, that the general patterns between Q_{10} and growth rate would not change because these express key fungal investment axes that conserved genetically have a relatively low plasticity (e.g., Liu et al., 2006). The degree to which our findings might hold if we were to experiment with fungi from different ecosystems is unclear. The site where the isolates originated from experiences semiarid climatic conditions, which might have selected for isolates with specific adaptations and possibly the diversity of traits which we observe is lower that what we would observe if we had worked with isolates from a mesic system.

We worked with a key trait shaping the survival strategy of filamentous fungi, temperature, and addressed relationships with growth rate. Given the pace of global warming, we believe that our study aids to the establishment of response rules to global warming concerning filamentous fungi and thus ecosystem functioning.

Legends to figures

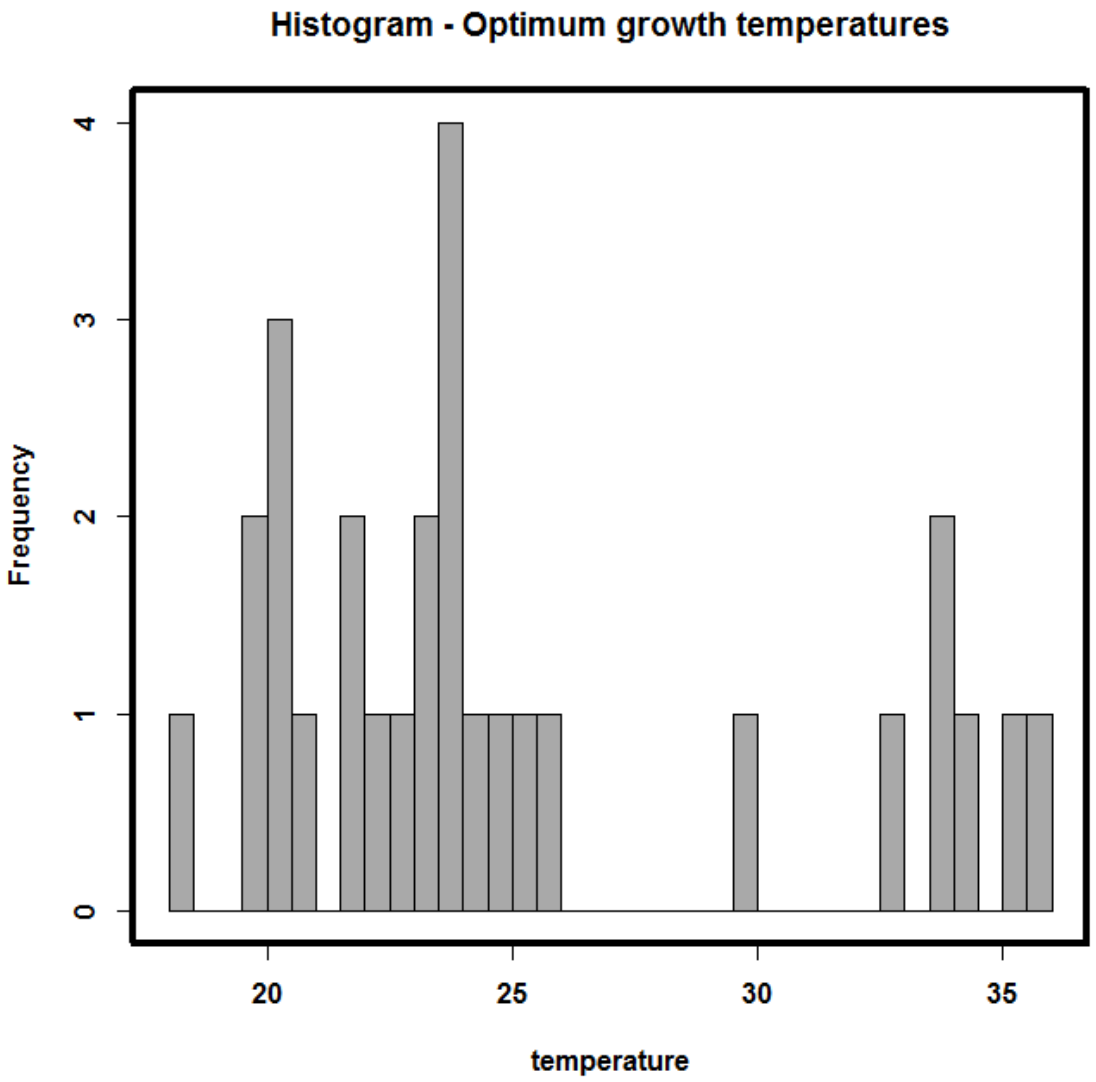


Fig. 4.2 A histogram of estimated values for the parameter optimal growth temperature in our set of 30 isolates. We fitted a Gaussian curve to our estimates of growth rates at four temperatures. We could assess optimal temperature for 28 out of the 30 fungi. Note that most of the fungi grow best between 20^{o}C and 25^{o}C.

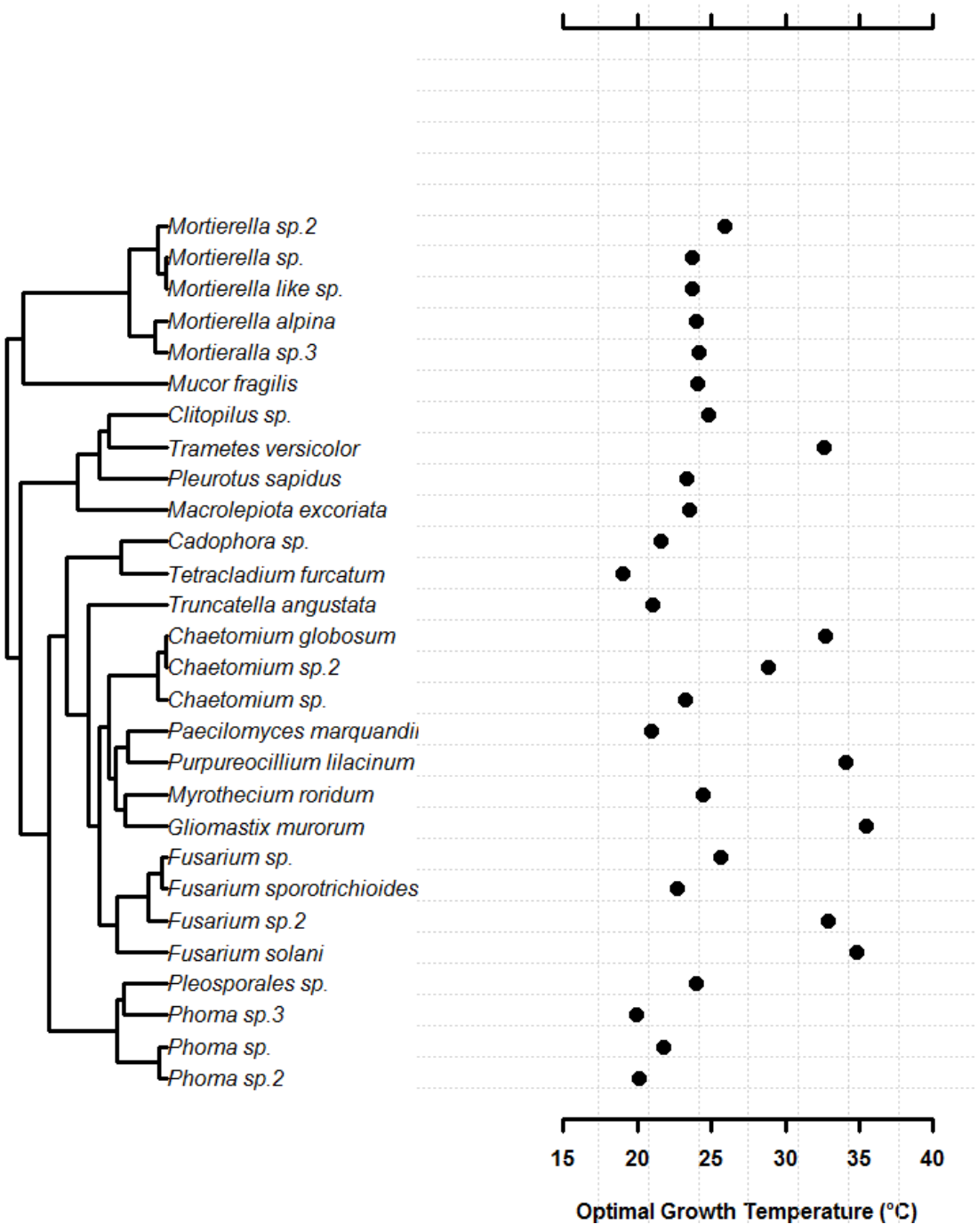


Fig. 4.3 Optimal specific growth information about 30 isolates. Phylogenetic relationships of the fungal isolates are illustrated via the overlaid phylogenetic tree that was presented in detail at Andrade et al. 2016. The dots correspond to the mean values for each species.

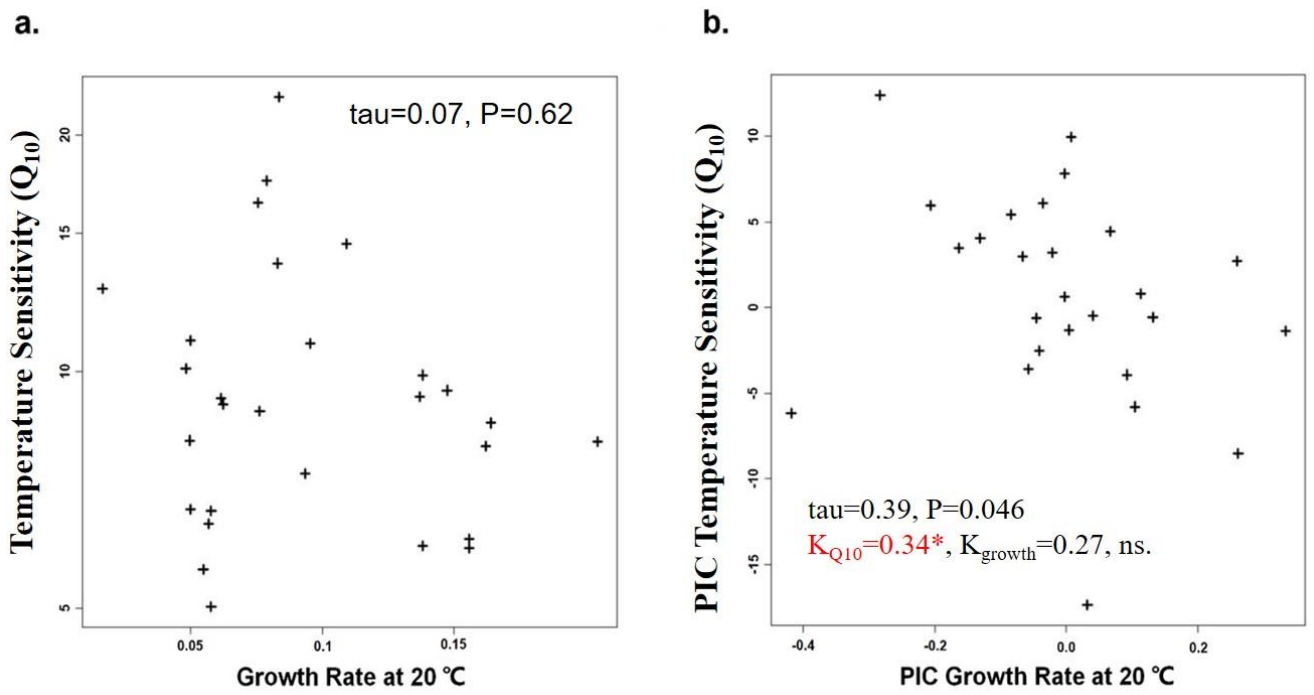


Fig. 4.4 Relationships between temperature sensitivity coefficients (Q_{10}) and growth rate at 20°C without (panel a) and with (panel b) a phylogenetic correction. Note that the relationship between PIC growth rate at 20°C and PIC Q_{10} becomes significant. Statistics are included in the panels. PIC stands for phylogenetic independent contrasts.

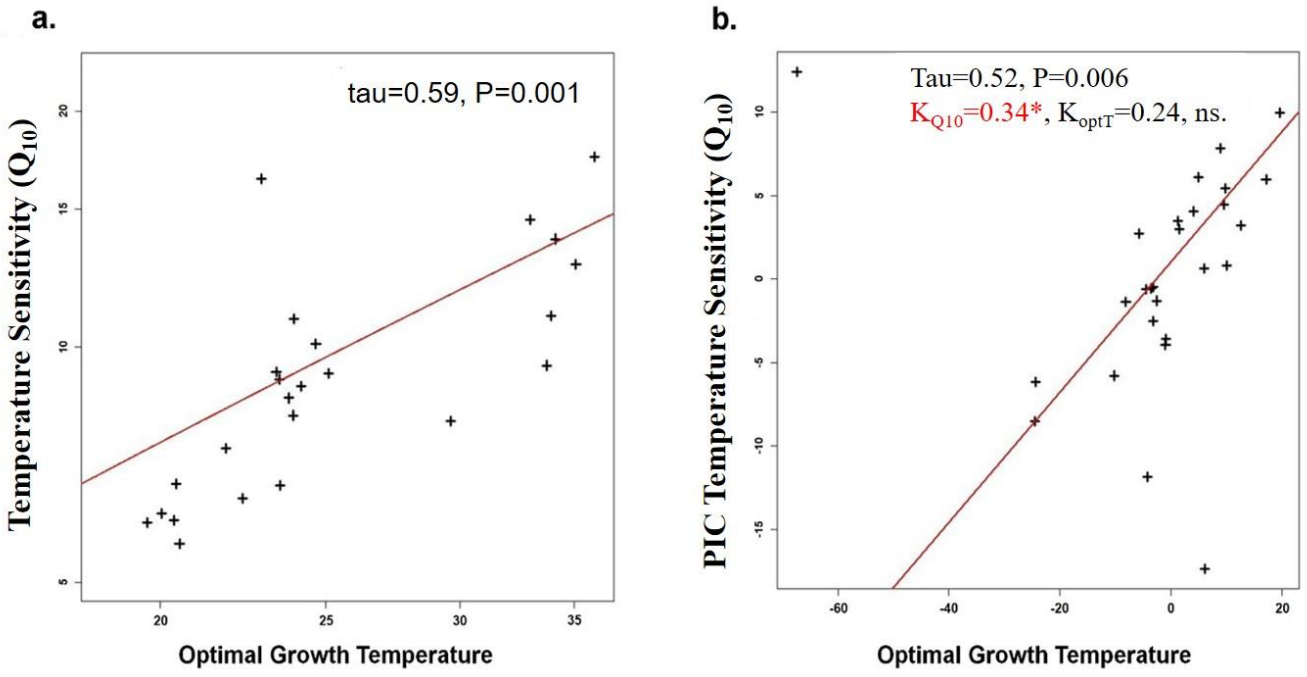


Fig. 4.5 Relationships between temperature sensitivity coefficients (Q_{10}) and optimal growth temperature without (panel a) and with (panel b) a phylogenetic correction. Note that the relationship between PIC optimal growth temperature and PIC Q_{10} becomes significant. Statistics are included in the panels. PIC stands for phylogenetic independent contrasts.

Acknowledgments

DW conceived the idea, prepared experimental material, and did the experiment. DW contributed to fungal isolations and the fungal identification based on ITS-sequencing. DW analyzed the data with the help of SDV and UM. DW wrote the manuscript. DA contributed to fungal isolations and deposited the fungal collections at DSMZ. MCR conceived the general idea to work with a large set of fungi and coordinated the project.

Supplementary data to this chapter can be found in Appendix 4.

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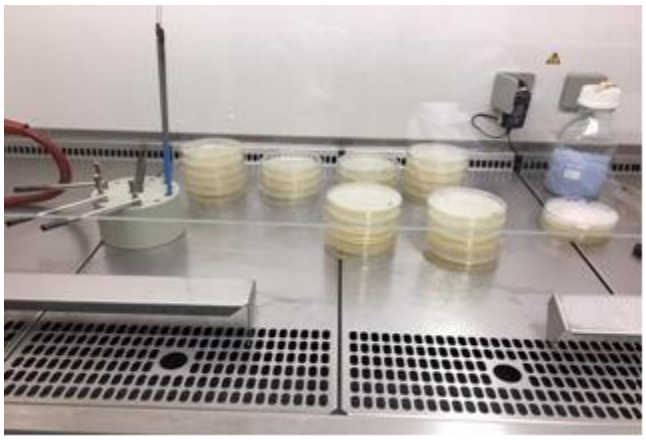
Chapter Five

A trait-based framework to understand the life history of filamentous fungi



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Abstract

Mapping life-history traits of fungi enables cross comparisons of communities under diverse settings and could speed up progress in the field of microbial ecology. With the exception of growth rate (i.e. r-K classification), however, no other life history traits have been sufficiently explored across Eumycota. Here we address the suitability of the competitive-stress tolerant-ruderal (CSR) classification system for describing growth traits in filamentous fungi. We propose a novel way to map trait data into a CSR triangle and pose two hypotheses, (i) that the two dimensional classification system of the CSR model adds meaningful life-history information compared to the existing r-K one; and (ii) that all three CSR traits are phylogenetically conserved. We show that the two-dimensional CSR model adds a new layer of information compared to the r-K system, but also that none of the three CSR traits are phylogenetically conserved. Our analysis hints at the potential of the CSR classification scheme to be integrated into programs aiming at a trait-based description of filamentous fungi, and that such a CSR-summary may facilitate interpretation of community data from microbiome studies. A sophisticated life-history classification scheme, such as the one we present here, could finally promote use of filamentous fungi by a much broader group of ecologists and experimental biologists.

Introduction

A Darwinian Demon describes a hypothetical organism that has evolved without biological constraints and maximizes simultaneously all aspects of its fitness (Law, 1979). Organisms need to manage finite sets of resources, that impose limitations on their ability to cope with environmental variability. Hence, depending on their lifestyle, organisms invest their resources in particular ways (Kraft et al., 2015; Thakur et al., 2017). This also applies to fungi. As an example, fast growing saprobic fungi invest more energy into reproduction, which comes at the cost of them not being able to recycle resources from their mycelium (Heaton et al., 2016). Moreover, slow growing filamentous fungi tend to devote more time to exploring their environment, which could balance their growth disadvantages when competing with faster growing fungal taxa (Veresoglou et al., 2018a). Unsurprisingly, there has been a lot of research aiming at unifying such observations across studies and at categorizing fungal investment strategies.

A large proportion of the existing literature uses the r – K continuum to characterize investment strategies in fungi (Pianka, 1970; Heaton et al., 2016; Veresoglou et al., 2018a): Fungi with fast growth, a trait typical of r strategists, should suffer penalties in terms of consumption competition (but most likely not overgrowth competition e.g. Magan and Lacey, 1984) compared to those with slow growth, the K strategists. The r-K strategy tradeoff depicts reasonably well life strategies in fungi, useful for example for predicting successional dynamics across coprophilous species (Richardson, 2002). However, there have been cases where it performed less well. For example, Maynard et al. (2017), after partially controlling for growth rate differences in a set of 37 wood-decay basidiomycetes, found that it was fungi that produced more enzymes that excelled in competition (i.e. the fact that differences in competitive ability remained despite correcting for growth rate showed that the r-K tradeoff fell short of describing life-history strategies in fungi). An altemative informative model is the competitive-stress tolerant-ruderal (CSR) model which was proposed for plants by Grime (1974). The model decomposes the ability of organisms to grow into three niche axes describing their abilities to outcompete other species, tolerate unfavorable conditions and deal with disturbance (Grime, 1974, 1977). This framework been shown to perform well for plants (Grime et al., 1997). There has also been an effort to apply this model to Glomeromycotina (Chagnon et al., 2013). Unlike the case of the r-K continuum model where it is sufficient to show a trade-off between an aspect of growth rate or generation time and a measure of competitive ability, usually the CSR model is tested after a variance decomposition with multivariate techniques (Grime et al., 1997; Pierce et al., 2013; Pierce et al., 2017).

We here aim to adapt and test the CSR classification system as a means of describing life histories of fungi. We first develop a procedure to map fungal traits to CSR strategies and compare our findings with those of the most widely used approach of ordination. We then ask how the CSR approach performs compared to the r-K classification. Second, we test the degree to which these CSR strategies are phylogenetically conserved, meaning that closely related groups of fungi are more likely to share a specific type of strategy. We then use a well-described set of 30 fungi (Andrade-Linares et al., 2016; Soliveres et al., 2018; Veresoglou et al., 2018) and describe them in terms of their CSR strategies (i.e. the degree to which they occupy the CSR space). We posed two hypotheses. 1): Expanding the classification of fungi from one dimension (r-K framework) to two dimensions (CSR framework) adds meaningful classification information on the life history of the organisms; or, in other words, no two of the CSR strategies will be positively or negatively collinear to each other (Fig. 5.1). 2): Because r-K traits are phylogenetically conserved as shown in Veresoglou et al. (2018), CSR traits also show phylogenetic conservatism.

Materials and Methods

Organisms

We used a diverse set of 30 well-described saprobic soil fungi which had been all isolated from a single grassland (Mallnow Lebus, Brandenburg, Germany, 52°27.778' N, 14°29.349' E) in Germany (Table S1; Andrade-Linares et al., 2016). Isolation had been carried out with diverse media including Malt Extract agar, Benomyl agar, Rose Bengal agar, Yeast Extract-Peptone-Dextrose agar, and Czapek Dox Agar (Thorn et al., 1996) at 25^{o}C. From an original set, which consisted of hundreds of fungi, 30 fungal strains had been selected covering the three phyla Ascomycota, Basidiomycota and Mucoromycotina, and which additionally, exhibit a wide range of growth rates. Phylogenetic relationships across these fungi were reconstructed in Andrade-Linares et al. (2016).

Measurements and data sources

For the purposes of the classification, we measured three fungal traits, which we mapped to the three strategies in the CSR model.

First, we used data on the competitive ability of our 30 fungal strains (Fig. S5.2), established by exploring their colony growth in pairwise interactions (these data are reported in Soliveres et al., 2018). In brief, the fungal pairs were grown on 9 cm Petri dished filled with potato dextrose agar (PDA). The fungal strains were introduced to the dishes by sterilized poppy seeds that get colonized by the corresponding fungal strain. For each fungal strain, one colonized poppy seed was placed in the Petri dishes with distance of 2cm between both strains (the target species and its competitor). We arranged our fungal strains in all possible combinations yielding a total of 435 experimental units. The combinations itself were not replicated since the unit of replication is the fungal species. Hence, each fungal strain had 29 replicates as there were 29 possible competitors available for each strain. The established cultures were incubated at room temperature in the dark for 28 days and scanned on seven occasions (day 0, 3, 5, 7, 14, 21 and 28). On harvest day, dishes were opened and photographed from above with a Canon EOS 70D and a standard objective. Using the scans and photographs, we reconstructed the growth behavior of the colonies over time and hence categorized fungal interaction types following Magan and Lacey (1984). There were three possible outcomes: (i) draw (the target strain intermingled or ended in a deadlock situation with its competitor), (ii) win (the target strain overgrew the competitor) and (iii) loss (the target strain was overgrown by the competitor). We calculated, here, the competitive ability as percentage of the number of wins the target species could accomplish against all possible 29 competitors.

Second, to assess temperature sensitivity of our 30 fungal strains (as a proxy of stress tolerance - S), we carried out here the following experiment investigating their colony extension under the influence of four different temperatures. We inoculated 9 cm Petri dishes filled with PDA with a 0.7 cm diameter plug extracted from the edge of an actively growing colony. The inoculated dishes were incubated at four different temperatures (15^{o} C, 20^{o} C, 25^{o} C, 30 ^{o}C). The experiment was carried out in two batches; first, we collected growth data at temperatures 15 ^{o}C, 20 ^{o}C and 25 ^{o}C and then at temperatures 30 ^{o}C and 35 ^{o}C. Due to a high rate of contaminations at 35 ^{o}C, however, we narrowed down the analysis to the four lowest temperatures. For the experiment we used six incubators (Binder, 12-22064, Germany), so that temperature settings were replicated to a minimum of two replicate incubators, which we included in our analysis as block factors (i.e. in the case of incubator-related differences, the categorical parameter incubator ID would be significant). Each fungal isolate was tested for its temperature sensitivity (four replicates of each fungal strain at each of the four temperature treatments). Overall, we had 4 x 4 x 30= 480 experimental units. Finally, we measured radial extension rate (mean

value of two colonial diameters perpendicular to each other) for all experimental units. From these data, we estimated temperature sensitivity by using the Q_{10} index (Formula 5.1):

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}, \quad \ldots(5.1)$$

Where R_2 and R_1 are the growth rates of the fungus at the highest temperature (T_2) and lowest temperature (T_1) out of two temperatures. We calculated the Q_{10} value for all possible pairs of temperatures (i.e. C (4, 2) = 4! /2! /2! = 6 pairs and averaged them. A higher Q_{10} value indicates a higher sensitivity to temperature. For ease of interpretation, we inversed the Q_{10} value $Q'_{10}=1/Q_{10}$ and larger values represent higher temperature tolerance. Because two isolates did not grow at 30°C, we generated Q_{10} values for a subset of 28 out of the 30 fungal isolates.

Third, we used data (Wang et al., *in preparation*) from a disturbance experiment. Disturbance tolerance (as a proxy of persistence in ruderal environments) was measured for the same 30 fungal isolates. For this experiment, we used liquid cultures established in 50mL conical bottom centrifuge tubes (Sarstedt, Germany) filled with 20mL potato dextrose broth (PDB). Tubes were inoculated again by 0.7cm diameter plugs (two plugs per tube) cut from the growing colony edge of the fungal strains growing on PDA. Liquid cultures were incubated at room temperature (25°C) on a horizontal shaker (New Brunswick Scientific, Excella E24 incubator shaker series, USA) at 120 rpm. For the application of the disturbance treatment, we also added three sterilized glass beads (diameter of 4±0.3mm, Carl Roth, HH55.1, Germany) per tube. Disturbance treatments were applied with a vortex mixer (Scientific Industries, Vortex Genie 2, gear: 10, Germany). There were three experimental treatments, each replicated three times, in a fully factorial experimental design consisting of a total of 30 x 3 x 3 = 270 experimental units: (i) no disturbance (120 rpm); (ii) low disturbance (one disturbance event on the second incubation day); (iii) high disturbance (daily disturbance starting at the second incubation day, totaling six disturbance events). Each disturbance event (vortexing) lasted 10 seconds. On the eighth day, we harvested the fungal cultures and assayed fungal biomass by filtering the culture through a pre-weighed and pre-dry 11-µm pore size filter (Whatman, 90mm diameter, 1001090). Biomass was determined as the weight difference of the filters after drying them for 48h at 60 °C. For the purposes of this study, we averaged biomass loss statistics of the two disturbance treatments to have an overall statistic describing how tolerant the isolates were to disturbances.

How does the CSR triangle compare with the r-K continuum classification?

To address the suitability of the *r-K* continuum in modelling fungal trait variability, we generated a correlogram for the three measured traits competitive ability, temperature sensitivity and disturbance tolerance. If we were to observe high collinearity (i.e. the variance inflation factors (VIFs) are below 0.2) between any two of these traits this would support that using two dimensions (i.e. CSR model) instead of one (*r-K* continuum) does not add meaningful trait information and that the *r-K* continuum is most likely a more suitable approach. We further produced expectations of how the CSR triangles would look if there is collinearity (Fig. 5.1b) or a trade-off (Fig. 5.1c) between any of the three life-history strategies we model here (the code for producing these triangles is reported in Appendix 5).

Modelling approach

Our modelling approach, which we developed here, aimed to rescale the three traits for competitive ability (C), stress tolerance (S) and disturbance tolerance (R) so that they possessed equal weights following averaging and summed together to (approximately) the value of one (i.e. 100%). Higher values implied a higher ability of a species to tolerate a type of environment. The purpose of the model was to generate a truncated Gaussian distribution of CSR values (Barr and Todd, 1999), with a mean of 0.333 (so that the aggregate for the three traits is 1 or 100%) and an upper bound of 1 at a 0.05% probability, which represents Fisher's significance threshold. Truncated parts of the distribution were assigned the two extreme values, 0 (i.e. poor performance in terms of the specific CSR strategy and 1 (i.e. the fungus invests exclusively in the specific CSR strategy). This led to a tri-modal distribution of life-history values with one peak for the value zero, one for the value one and one for the value 0.333 (Fig. S5.1), and the exact modelling procedure was as follows:

We first transformed the data to improve their distribution properties. For temperature sensitivity, we used a quadratic transformation of the values while maintaining their sign. For the same purpose, we log-transformed values on disturbance tolerance. We then *z*-score transformed each of the variables. For *z*-score transformation, we aimed at a standard deviation per trait after all corrections of 0.333, which would imply that the total variance for any species would approximate the value one. Based on a pre-analysis we selected a standard deviation for the *z*-score transformations of 0.42. We truncated values above one and below zero (i.e. which lowered our total variance). We then implemented a correction step where each of the three CSR parameters per species was divided by their aggregate to ensure that they summed up to the value one.

Traditional multivariate approaches

To represent the way CSR data are analyzed in the literature (Pierce et al., 2017), we used principal component analysis (PCA). We used three response variables (i.e. traits for C, S and R) and enabled the option of rescaling before ordination so that the three traits shared equal weights. We used the two first axes of the analysis, explaining over 85% of total variance and matched them with the three main specialization axes in the CSR triangle.

Mapping fungal isolates into CSR strategies

We assumed the threshold value 0.333 to project the continuous CSR scores into CSR categories. By definition, the aggregate of the scores for C, S and R was 1.0. We determined which of these scores were above the threshold.

Phylogenetic analyses

To address whether the CSR investment strategies are phylogenetically conserved, we assessed phylogenetic signal separately for each of them (Drummond and Rambaut, 2007). We used a phylogenetic reconstruction based on a phylogenetic tree which has been published in Andrade-Linares et al. (2016) and calculated the Blomberg *K* values (Blomberg et al., 2003) as these are implemented with the command *phylosignal* in the R package *picante (Kembel et al., 2010)*. Each of the *P* values was produced following a series of 9999 permutations where the tips of the tree were shuffled. Then we use phylogenetic corrections in our data analysis (Freckleton, 2009). All analyses were carried out in R version 3.5.1 (R Core Team 2018).

Results

Does the r-K continuum capture variability in CSR traits?

The variance inflation factors we observed between pairs of variables were all above 0.5 (CR: 0.68; CS: 0.51; SR: 0.96). There were no signs of extreme collinearity or tradeoffs in the resulting CSR triangle (Fig. 5.2a; compared to expectations from Fig. 5.1).

Mapping fungal isolates into CSR strategies

Tetracladium furcatum (C21) and *Phoma sp. 3* (A) failed to grow at 30°C and as a result we were unable to calculate Q_{10} values for these two isolates. Ten isolates followed a "C" strategy, six an "S" strategy and two an "R" strategy (Fig. 5.2a, Table S5.1). We further observed seven isolates with a combined "SR" strategy and three with a combined "CR" strategy. We did not find any isolate combining an "S" strategy with a "C" strategy.

Phylogenetic conservatism in CSR strategies

Our analysis with Blomberg *K* tests yielded no phylogenetic signal for any of the three traits. Blomberg *K* values for C, S and R were 0.14, 0.09 and 0.14, respectively (*P* values were 0.568, 0.793 and 0.6205, respectively; Fig. 5.3). We thus did not use phylogenetic corrections in our data analysis.

Comparing the two CSR mapping approaches

The ordination (PCA) on CSR scores resulted in comparable clustering patterns for the fungal isolates as in our CSR triangle approach. This was mostly the case with the first ordination axis (PCA1) which explained approximately 85% of the variability. An advantage of our triangle approach is that the three axes share equal weights and that it captures 100% of the total trait variability (Fig. 5.2). Moreover, it is based on *a priori* assumptions and not a *post hoc* test.

Discussion

We show here that it is possible to map life history strategies of a diverse set of filamentous fungi onto a framework that is popular in plant ecology, the CSR triangle, and that the classification adds functional information compared to the widely used *r-K* continuum. We further develop a novel approach that maps trait data onto CSR strategies, which weights better the three life history traits (i.e. competitive, stress tolerant and ruderal) and yet produces results comparable to currently used mapping techniques which are based on ordination. To a large degree, CSR strategies were evenly distributed over the triangle, meaning that the two dimensions in the analyses summarized a considerable proportion of the ecological information of the fungal strains. The CSR triangle thus represents a promising classification scheme

that could be adapted in mainstream Eumycota studies and integrated into fungal trait databases (Aguilar-Trigueros et al., 2015).

Unlike the *r-K* continuum (Veresoglou et al., 2018a), none of the CSR strategies showed phylogenetic signal, suggesting that the CSR triangle for fungi contains little information on evolutionary characters. This implies that it is impossible to infer the state of a fungus from phylogenetically related taxa and that each strain should be independently evaluated for a CSR life strategy. Most functional traits in fungi show phylogenetic signal (Martiny et al., 2013), including some key traits such as growth rate (Veresoglou et al., 2018a). Because CSR information does not overlap with phylogenetic information, it may be the case that CSR information complements that on evolution by describing a unique fraction of total fungal character variability and the CSR description has high added value from a trait-framework perspective. This was apparent even at a phylum level with the CSR mappings for the three phyla in the species set intermingling with each other (Fig. 5.2; Fig. 5.3). Moreover, the lack of phylogenetic conservatism represents an additional argument that the CSR triangle adds another layer of information beyond the widely used *r-K* continuum.

For our analysis we used an alternative classification procedure to the popular multivariate approach such as Grime et al. (1997) and Pierce et al. (2017). By using *a priori* classifications for the traits, the resulting triangle was balanced in terms of the weight of the different life strategies and was representative of 100% of the variance in CSR traits. A low representativeness (i.e. % variance explained) of the resulting ordination axes and an uneven weight across the three life-history strategies (i.e. competitive; ruderal and stress tolerant) represent two common shortcomings of variance decomposition techniques such as PCA. Moreover, our *a-priori* selection of traits made it possible to carry out the analysis with less data and required fewer assumptions (e.g. defining the axes in an ordination plot; determining extremes for CSR specialization in the ordination plot) for interpretation.

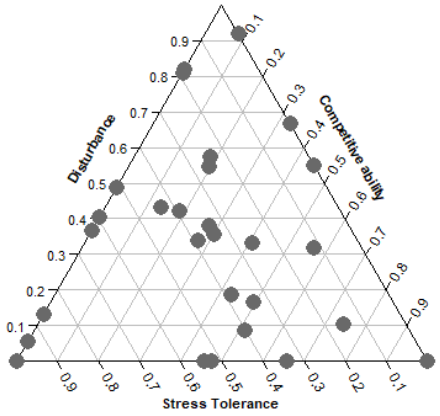
One of the main outputs of our analysis is a classification of the 30 fungi based on their CSR strategy (Fig. 5.2). Classifications like these could have multifold applications in ecology. For example, such information can be used in the planning of competition experiments. It is often desirable to experiment with groups of organisms that have a high likelihood of coexisting and this likelihood should increase by choosing species with a comparable CSR strategy (i.e. species experience comparable filters from the environment as for example SR strategists in tropical ecosystems; Rosardo and de Mattos, 2017). For example, Veresoglou et al. (2018b) used an existing CSR classification as a selection criterion for the plant species used in their experiment and this might be also possible for fungi. We found the trade-off

between a good competitive ability and possessing a symbiotic arsenal in the CSR triangle. An alternative way through which such classifications may be useful is in the form of indicators of the functioning of the system. This represents an idea originating from plant ecology and phytosociology where it is common to infer environmental conditions based on plant community information (Ellenberg, 1974; Clausman et al., 1987; Ellenberg et al., 2001). Fungal community structure is less conspicuous but could be informative of a range of ecosystem processes that are difficult to assess, such as long-term decomposition potential. Finally, such classification schemes can be later used to assess the consistency with which fungi contribute to ecosystem functioning. Existing studies on soil ecology have reached a consensus that at a short term and a specific environment a large proportion of microbes are functionally redundant e.g. (Torsvik and Øvreas, 2002; Cole et al., 2006; Talbot et al., 2014) and thus share equivalent CSR positions in the CSR triangle. We do not know if the functional role of fungi remains consistent across environments. By combining existing techniques measuring functional redundancy (e.g. Talbot et al., 2014) and a CSR framework it might be possible to address such questions.

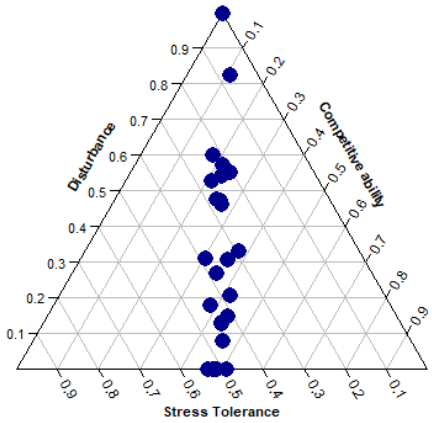
We developed here a new methodology to map traits onto CSR strategies and adapted it for a set of 30 fungal isolates. We formulated in the beginning of the paper two hypotheses, that expanding the classification of fungi from one dimension to two dimensions adds meaningful classification information on the life-history of the organisms and that CSR traits would be phylogenetically conserved. We show that the 30 fungal isolates were evenly spread over the CSR space (Fig. 5.2) which was in support of our first hypothesis. However, we observed no phylogenetic conservatism for any of the CSR classification traits. We adapt a popular idea from plant community ecology to filamentous fungi and present arguments on how it could benefit fungal ecology. By further refining existing classification schemes for filamentous fungi, we can advance fungal trait ecology (e.g. Hall et al., 2018) and promote a better characterization of existing microbiome data.

Legends to figures

(a)



(b)



(c)

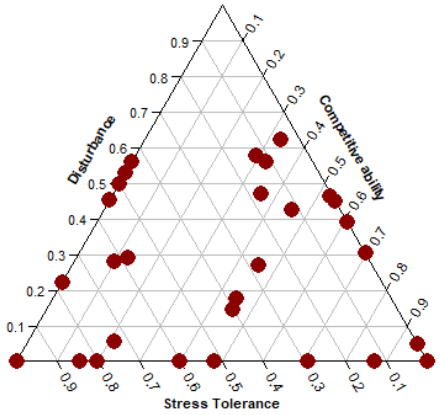


Fig. 5.1 Potential outcomes of CSR ordination based on different scenarios. (a) This CSR triangle depicts variance in a scenario where all three traits (C, S, R) vary independently of each other and share normal distributions. (b) This CSR triangle was constructed as in (a) but we allowed a 90% collinearity between disturbance and stress tolerance. Note that the "species" are aligned approximately in a vertical line (c) This triangle is as in (a) but we allowed for a tradeoff (90% of variance) between disturbance and stress tolerance. As a result, there are no "species" close to the center of the triangle or at the 100% disturbance tolerance tip. We provide the code we used to generate the panels in Appendix 5.

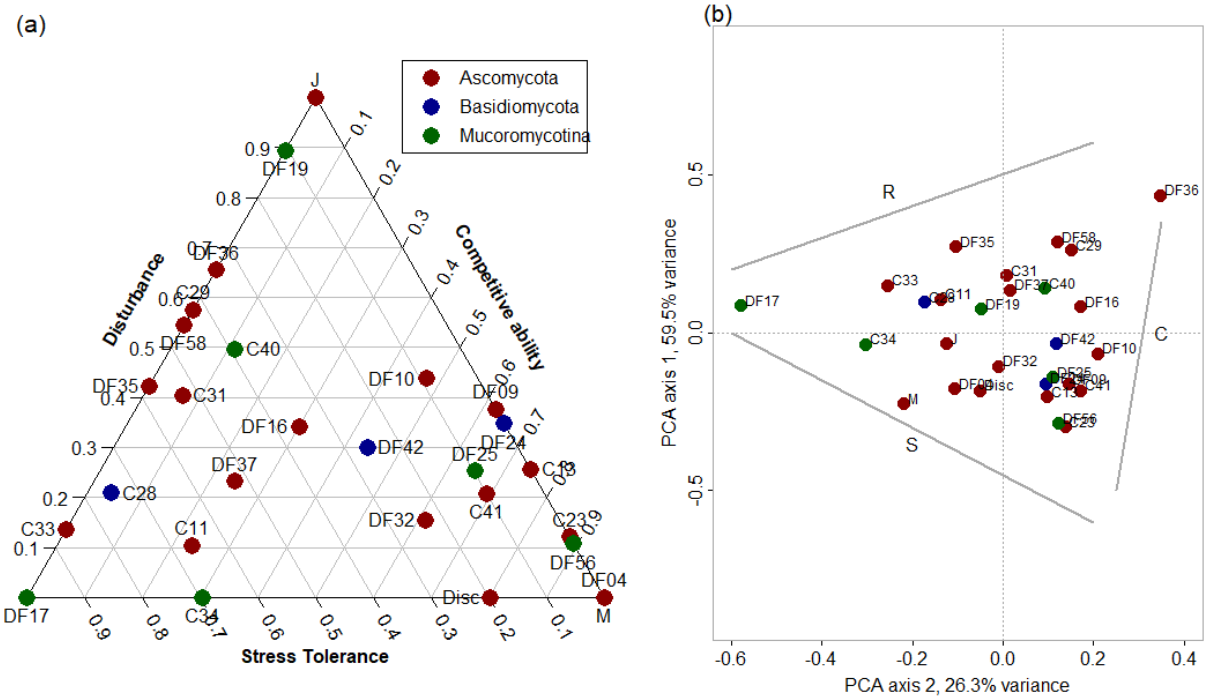


Fig. 5.2 (a) Alignment of the 28 fungal isolates in our culture collection in the CSR triangle based on the method we propose here. (b) PCA-based CSR triangle. For this panel we constructed the CSR triangle in a way compatible to the existing literature and visually identified the CSR axes, which matched well the CSR approach in panel (a). The technique we present here has the advantages of depicting the full variance in the distribution of the input traits; giving equal weight to the three life-history strategies (i.e.

C, S, R), relying on *a priori* assumptions and being easy to interpret (e.g. there is no need to determine the CSR axes in a separate analysis).

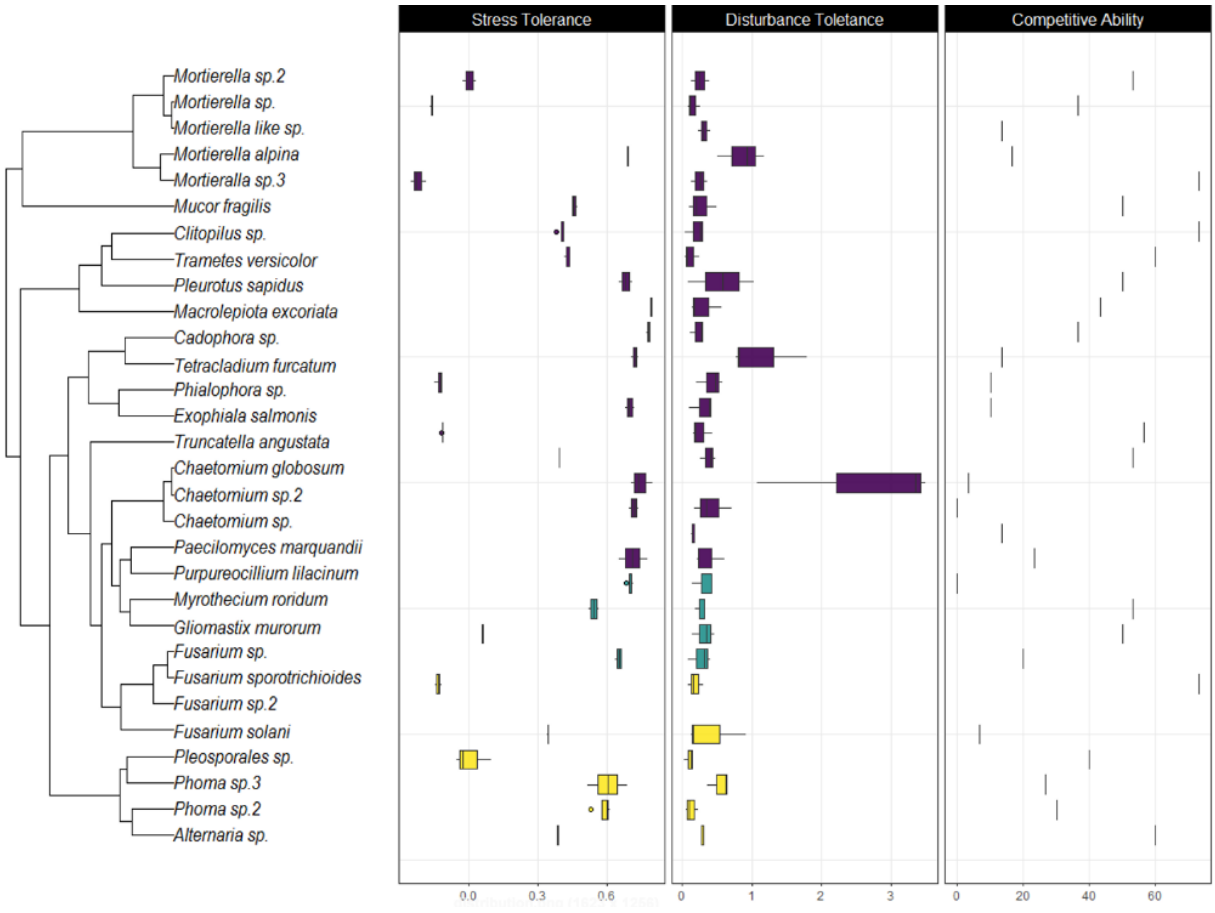


Fig. 5.3 Distribution of the traits of 30 fungi (Stress Tolerance, Disturbance Tolerance and Competitive Ability) across the phylogenetic reconstruction of the 30 fungi. Note that closely related fungi do not share more similar values which manifests an absence of phylogenetic conservatism.

Acknowledgments

DW, SDV, MCR conceived the study; DW contributed data on disturbance tolerance; DW contributed data on stress tolerance; AL contributed data on competitive ability; DW and SDV analyzed the data; DW with the help of SDV and AL wrote the manuscript.

Supplementary data to this chapter can be found in Appendix 5.

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Chapter Six

General Discussion

Trade-offs in fungal traits

In chapters 2, 3, 4, Temperature, disturbance and nutrient selection were observed to predictably modify the physiology and ecology of filamentous fungi (Table S6.1). This raises a question about establishing a life history strategies framework in filamentous fungi.

In the introduction, the importance of fungi for ecosystem functioning was highlighted. Many of the fast-growers in the experiments, such as Mucoromycotina, are found early in succession and often exhibit a live-fast-die-young strategy (Richardson, 2002). By contrast, filamentous fungi that persist for the long periods with active mycelia in soil, such as many Basidiomycota, exploring surroundings may represent a crucial facet of their persistence. Facilitation promotes the occurrence and persistence of communities in highly stressed and disturbed conditions (Le Bagousse-Pinguet et al., 2014). Fungal growth rates relate to their biomass-allocation strategies was approved in this thesis. This relationship holds for the entire range of diverse fungi considered.

A high frequency of disturbances could potentially negate the effectiveness of survival mechanisms in filamentous fungi such as compensatory growth (Bentgtsson et. al., 1993) and anastomosis (Roca et al. 2005), rendering more important tolerating disturbance than avoiding it. In agreement to our hypothesis which predicted that tolerance would be proportional to the tolerance to single disturbances, it was mainly fast growers that tolerated well intermittent disturbances. This suggests that disturbances may induce predictable shifts in the community structure of filamentous fungi in soil. Existing attempts to monitor traits in filamentous fungi (e.g. Aguilar-Trigueros et al., 2015), could thus have the added value of becoming integrated into observational meta-barcoding studies on fungi from disturbance experiments. The results showed that in both cases it is fungi with slow growth rates that tolerate

disturbance better and argued for possible implications of our results at an ecosystem level. Linking small-scale mechanistic experiments such as the one we present here with high-resolution molecular studies could help interpret community-level responses.

A key trait shaping the survival strategy of filamentous fungi, temperature, was addressed its relationships with fungal growth rate. Climate change will lead to an increase in global temperature in the next century with estimates ranging between 1.4 and 5.8 ^{o}C (IPCC, 2014). Given the pace of global warming, there is evidence that temperature adaptations in fungi induce prominent changes in their carbon use efficiency, which is linked to survival (Crowther et al., 2013 and 2014). It is thus expected that fungi maintaining a lower sensitivity to temperature will cope better with global change than those maintaining narrow temperature optima.

The evidence showed in this thesis differ from recent studies of fungal community structure that found that community composition at a site is more frequently phylogenetically clustered than even (Maherali and Klironomos, 2012). Functional traits have long been hypothesized to influence community assembly because organism function determines the ability to tolerate climatic conditions, acquire resources and interact with other individuals. When functional traits are shared by closely related species (conserved), phylogenies can be used to determine whether organisms function has played a role in the assembly of a given community. Yet, our understanding of fungal functional traits lags behind those of macroscopic organisms (e.g. Veresoglou et al., 2016).

Life history strategies of fungi

General assumption that there is a trade-off among competitive ability, stress-tolerance and disturbance sensitivity and disturbance increases the abundance of operational taxonomic units that are rare under undisturbed conditions (Lekberg et al., 2012). A conceptual model was proposed in this thesis to depict how trade-offs among stress-tolerance, disturbance sensitivity and combative dominance are likely to shape communities across environmental gradients. Filamentous fungi showed the activities of trade-

offs. The red queen hypothesis is a support of competition, that predicts good competitors should be favored at low levels of disturbance (K-selected), good colonizers (r-selected or ruderal strategies) at high levels of disturbance, and that coexistence should occur at intermediate levels of disturbance (Collins and Glenn, 1997).

Many fungi probably exhibit different life histories at different times during their life-cycle (Pugh and Boddy, 1988). Gilchrist et al. (2006) proposed theoretical work that optimal resource allocation strategies in fungi changing with age. Trade-offs can be plastic as well as genetic (Stearns, 1989). For our analysis, we used an alternative classification procedure to the popular multivariate approach such as that of Grime et al. (1997) and Pierce et al. (2017). By using a priori classifications for the traits, the resulting triangle was balanced in terms of the weight of the different life strategies and was representative of 100% of the variance in CSR traits. Moreover, our a-priori selection of traits made it possible to carry out the analysis with less data and required fewer assumptions for interpretation. The CSR triangle presents a promising classification scheme that could be adapted in mainstream Eumycota studies and get integrated into future fungal trait databases (e.g. Aguilar-Trigueros et al., 2015). Fungi tend to have many R-selected characteristics, is prevalent following disturbances. These are often combined with C-selected and S-selected characteristics depending upon the biotic and abiotic conditions to which their life strategies are best adapted. C, S and R species and habitats often occur in mixtures at varying scales though (Wilson and Lee, 2000).

Future perspectives

The approaches presented in this dissertation should be further refined and applied to a larger range of filamentous fungi. Specially about the set of the 30 fungi, the existing characterization would benefit from the inclusion of a stress factor other than temperature and drought. In relation to the disturbance experiment, assays of disturbance frequency should be coupled to those on disturbance intensity. A way to expand on the work in chapter 5 might include testing the dimension of the triangle data. Based on my previous work, it is possible to contribute to a fungal database for our lab.

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Summary

This thesis addresses how traits shape the ecology of filamentous fungi and determine life history strategies. I worked on a set of 30 filamentous fungi which were isolated in the AG Rillig from a grassland in northeast Germany. We analyzed the behavior of fungi in response to various environmental stimuli, namely temperature and mechanical disturbances. **Chapter 1** introduces the fundamental traits in fungal species, reviews existing literature on the topic of this thesis and establishes the framework and methodology used in my studies. **Chapter 2** explores the behavior of fungi in terms of resource allocation. We showed that fast-growing fungi have a tendency towards localized growth whereas slow-growing fungi tend to explore their environment in attempt to locate further resources. **Chapter 3 points out** the fitness changes in fast and slow-growing fungi in response to singular or iterative simulated mechanical disruption of the mycelium. Fast-growing fungi exhibited a lower carbon use efficiency which should explain their higher sensitivity towards mechanical disturbances. **Chapter 4** investigates the temperature sensitivity of fungi with the use of the Q_{10} indicator. We determined that the Q_{10} values (temperature sensitivity) exhibit a negative correlation with the growth rate of the fungi and a positive correlation with the optimal growth temperature. **Chapter 5** establishes a novel method to map the life-history traits of fungi into the competitive-stress tolerant-ruderal (CSR) model. My results showed that the two-dimensional CSR model has the potential to contain more information compared to the one-dimensional r-K model, but the CSR traits are not phylogenetically conserved. **Chapter 6** puts the outcomes of this thesis in the broader context of the literature, summarizing the conclusions of previous chapters.

Zusammenfassung

Diese Doktorarbeit befasst sich mit der Fragestellung, wie sich die Merkmalsbeschaffenheit von filamentösen Pilzen auf ihre Ökologie und spezifischen Lebensgeschichtenstrategien („life history strategy“) auswirkt. Zu diesem Zweck wurden 30 Stämme filamentöser Pilze untersuchte, die von der AG Rillig aus Grasslandboden (Mallnow, Deutschland) isoliert wurden. Diese Pilzstämme wurden auf ihre Reaktion hinsichtlich verschiedener Umweltstimuli untersucht. Bei diesen handelte es sich um Erwärmung und mechanische Störung bzw. Beschädigung des Pilzmyzels.

In **Kapitel 1** wird auf Basis einer Literatursynthese zum Thema Pilzmerkmale, das grundlegende Fundament dieses Forschungsvorhabens und der zugrundeliegenden Methodik herausgearbeitet. In **Kapitel 2** wird die Fähigkeit der Ressourcenzuweisung der 33 Pilzstämme erforscht. Dabei konnten wir zeigen, dass schnell wachsende Pilzstämme eine Tendenz zu lokalem Wachstum aufweisen, während langsam wachsende Stämme bevorzugt in ihre Umgebung auf der Suche nach weiteren Ressourcen expandieren. In **Kapitel 3**, wurden die Auswirkungen von wiederholter, mechanischer Myzelbeschädigung auf die Fitness von schnell und langsam wachsenden Pilzstämmen untersucht. Dabei zeigt sich, dass schnell wachsende Stämme eine geringe Effizienz in der Kohlenstoffnutzung und eine höhere Sensitivität gegenüber der zugefügten mechanischen Beschädigung aufweisen als ihre langsamer wachsenden Gegenstücke. In **Kapitel 4** wird die Temperatursensitivität der 30 Pilzstämme mittels Q_{10}-Indikator erhoben. Es zeigt sich, dass die Temperatursensitivität negativ mit der radialen Koloniewachstumsrate und positiv mit der optimalen Wachstumtemperatur korreliert. In **Kapitel 5** wird eine neue Methodik zur Darstellung der Lebensgeschichtenmerkmale von Pilzen mittels sogenanntem CSR-Model vorgestellt; das CRS-Model umfasst dabei

Merkmal, die die kompetetive, Stresstolerenz- und Ruderalkapazität abbilden. Es zeigt sich, dass das zweidimensionale CSR-Model das Potenzial hat, mehr Informationen zu enthalten, als das eindimensionale Gegenmodel der r-K-Strategien. Jedoch sind die CSR-Merkmale nicht phylogenetisch konserviert. In **Kapitel 6** werden die Ergebnisse dieser Arbeit in den breiten Literaturkontext gestellt und die Schlussfolgerungen der vorangegangenen Kapitel zusammengefasst.

List of publications or manuscripts and contributions

I. Veresoglou SD, **Wang DW**, Andrade-Linares DR, Hempel S and Rillig MC. 2018. Fungal Decision to exploit or explore depends on growth rate. Microbial ecology 75 (2): 289-292.

Own contributions: I contributed to fungal isolations, conducted the growth-rates experiment and provided assistances in the class of the experiments. All authors reviewed the manuscript.

II. **Wang DW**, Veresoglou SD, Forstreuter M and Rillig MC. Fast-growing fungi have a higher sensitivity to a mechanical disturbance but tolerate better its reoccurrence. Prepared to submit (based on the new phylogenetic system).

Own contributions: I conceived the idea, prepared material, and carried out the experiments, analyzed the data and wrote the manuscript. All authors reviewed the manuscript.

III. **Wang DW**, Veresoglou SD, Andrade-Linares DR, Mardhiah U and Rillig MC. Fungal fast growers exert lower sensitivity to temperature. In preparation.

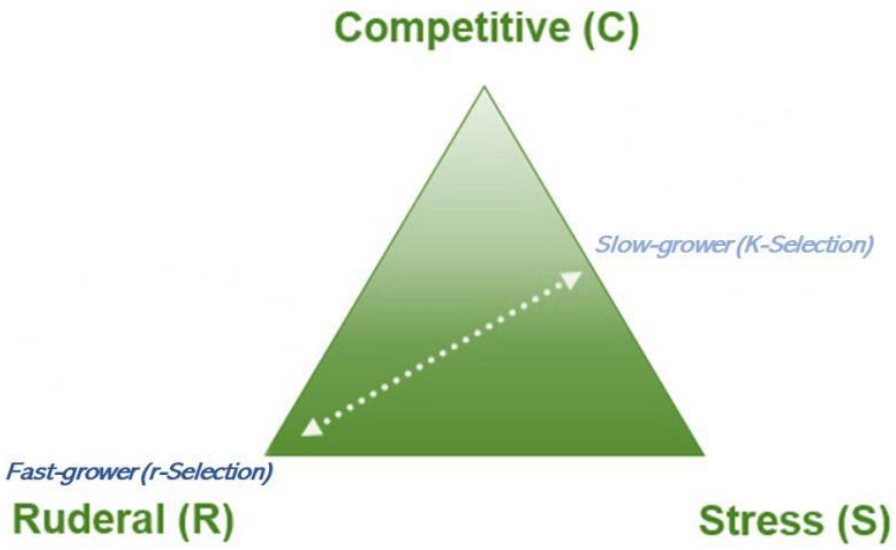
Own contributions: I conceived the idea, prepared the material, and did the experiments. I analyzed the data and wrote the manuscript. All authors reviewed the manuscript.

IV. **Wang DW**, Veresoglou SD, Lehmann A and Rillig MC. A trait-based framework to understand the life history of filamentous fungi. Prepared to submit (based on the new phylogenetic system).

Own contributions: I contributed and analyzed the data on disturbance tolerance and stress tolerance. I wrote the manuscript. All authors reviewed the manuscript.

Appendix 1

Table S1.1 Fungal life history framework (r/K and CSR).



Appendix 2

The rationale of our experiment was to establish two habitats that differed considerably with regards to their ability to promote fungal growth. We were aware that (i) there is no single medium where all fungi could grow at their maximum growth speed; (ii) even if we could find the ideal medium for a fungal species we could not assert that we lifted nutrient limitation entirely. We decided to use the standard media PDA and water agar because our preliminary results showed that all our fungi grew considerably better on PDA. We do not claim that PDA was not limiting for the growth of the fungi or that it might not have been more suitable for a subset of the fungi. However, we assert that the difference in the quality of the two habitats was substantial for all fungi and that we expected that our consideration of a large number of fungi should have evened out any differences in suitability of the PDA medium.

In our experiment we used biomass as a proxy of body size. It is true that it is challenging to describe body size for fungi. This is because the diameter of the hyphae and their branching differ across species and depend a lot on the habitat in which they grow. Moreover, fungal biomass does not differentiate between growing vegetative hyphae and biomass invested in sexual or asexual spores which are functionally very different. Nevertheless, we believe that for our purpose biomass represented a suitable proxy of body size.

The fungi (described in detail in Andrade-Linares et al., 2016) were all isolated from grassland soil (Mallnow - Lebus, Brandenburg, Germany, 52°27.778′ N, 14°29.349′ E). For isolation purposes a range of media were used including Malt Extract agar, Benomyl agar, Rose Bengal agar, Yeast Extract-Peptone-Dextrose agar and Czapek Dox Agar supplemented with antibiotics to suppress bacterial growth. To identify the fungi, Andrade et al. (2016) used the ITS region using the specific fungal primers ITS1F and ITS4.

To estimate fungal biomass, we first used a scalpel to separate PDA and WA sectors and then transferred the fungal culture to a 250 ml conical flask and added 200 ml deionized water. The agar was subsequent liquefied in a microwave oven at 400 W for approximately 3 minutes. An 11-µm pore size filter, dried and weighed, was used to vacuum-filter the suspension in order to obtain the hyphal biomass. Filters were subsequently dried for 48h at 60°C and reweighed. We used the difference in the weight of the filter (before and after filtration) to calculate fungal biomass.

The phylogenetic tree that we used for our study was from Andrade-Linares et al. (2016) and was constructed based on a Bayesian likelihood approach as implemented in BEAST v 1.7.2 (Drummond

and Rambaut, 2007). The authors ran a General Time Reversible substitution model with gamma-distributed substitution sites for 20 million generations and sampled over the last 18 million generations a tree every 2000 generations (9000 trees). They summarized the tree parameters through estimating the tree with the maximum clade credibility (median clade heights). The specific Bayessian approach does not produce support values for individual nodes. To correct for phylogenetic dependencies, we applied the technique for phylogenetically independent contrasts (PIC) that was proposed by Felsenstein (1985). The specific technique estimates values for each node in a phylogenetic tree that describe the difference of the two branches on a trait divided by their phylogenetic distance. PIC often results in values which are non-normally distributed. In many of our analyses we use non-parametric tests to address leverage issues with extreme values.

To calculate relative preferences of fungi we used as response variables log-response ratios. Log-response ratios have been used extensively in ecology and are believed to represent a very good way to summarize comparative experimental data (e.g. Laujeunesse, 2011) as they are easy to interpret and have excellent statistical properties. In our analyses we mostly relied on Pearson and Kendall correlations. Exceptionally, to combine data from the two harvests we used a linear mixed effects model approach. In this model we first transformed our values with a square root transformation (in the absolute value preserving the sign) and used the log-response ratio of biomass as dependent variable, total biomass as independent variable and harvest as a mixed effects factor (because all samples were independent to each other we did not use a repeated measure approach). To calculate densities, we used the formula $2m_{media}/\pi R_{media}^2$. We explain what each letter stands for in the main document. Here we would like to add that it is because the colonies were half circles (each media) we calculated the surface area as $\pi R^2/2$ which gave rise to the specific formula. All statistical analyses were carried out in R version 3.0.2 (R core team 2018)

We observed that many of the relationships when analyzing separately data from the two harvests were non-significant, only revealing trends. We believe that there are two main reasons behind this: (i) limited statistical power - we worked with 32 isolates which is not a particularly large number for a correlation study and some effect sizes might have not been strong; (ii) assessing some parameters such as colony radius involves a high measurement error, particularly when the size of the colony is small. This is why, for example, the relationships we found in the second study were so much stronger than at the first harvest. However, both studies showed the same trends, and this suggests that we are assaying real relationships.

An interesting point we wanted to further consider was how our growth rate results might have differed if we had measured growth rate in water agar instead of PDA agar. We think that colonies grown on water agar may have contained, particularly at the perimeter of the colonies, a higher proportion of hyphae that served the sole purpose of exploring the environment and the measurements may are less representative of actual growth rates and more representative of relative exploration strategies. Also, the fact that the colonies would contain a lower biomass density would make it more difficult to accurately determine the extent of the colony and thus our measurements would involve a higher measurement error. All in all, we believe that our choice of a PDA medium to measure growth rates resulted in more representative results.

Summarizing our results, we found that fast growing fungi (either assessed by colony extension radius or colony biomass) explored less the water agar sector of the plate and that there was no relationship between the log-response ratio of mycelium area densities in the two sectors of the plate and the growth rate of the fungi. The relationship in the case of colony biomass was present independent of phylogenetic correction whereas in the case of growth rates was present only after we corrected for phylogenetic relatedness (because we detected phylogenetic signal in both parameters, we valued more the results with a phylogenetically correction). The lack of a relationship between the log-response ratio of mycelium area densities in the two sectors of the plate and the growth rate of the fungi addresses the concern that larger colonies may have more effectively showed allocation preferences because they were less dependent on the allocation arrangement when we initiated the experiment.

Did the choice of growth media influence generalizability of results?

It is possible that the growth in artificial media has masked mechanisms that may facilitate exploration of the soil environment such as a growth affinity towards local moisture pockets or even induced unrealistically strong growth responses because the two media we used differed quite drastically in sugar availability.

However, the use of artificial media allowed us to filter out non-nutritional mechanisms which influence fungal growth/exploration and achieve a higher mechanistic understanding. In a way our experiment resembled behavioral experiments with macroscopic animals in which, even though the choice of rewards can sometimes be questioned, the payoffs are so remarkably different that it is straightforward

to interpret the results. In our case, to further account for the shortcoming that the "behavioral choice" (i.e. growth on either medium) was also in our system a "fitness choice", we additionally assayed growth rates of the fungal isolates independently in another experiment.

Additional References

Drummond A, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214.

Felsenstein J. 1985. Phylogenies and the comparative method. *The American Naturalist* 125: 1–15

Laujeunesse MJ. 2011. On the meta-analysis of response ratios for studies with correlated and multi-group designs. *Ecology* 92: 2049-2055.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Detailed results and R code

Mixed effects linear models (both harvests):

a. mixed effects model that combines phylogenetically corrected total biomass and lrPDA growth values from both sampling points

> **total.biomass<-sign(total.biomass)*sqrt(abs(total.biomass))**

> **logratiobiomass<-sign(logratiobiomass)*sqrt(abs(logratiobiomass))**

> **model1<-lme(logratiobiomass~total.biomass, random=~1|harvest)**

> **summary(model1)**

Linear mixed-effects model fit by REML

Data: NULL

AIC	BIC	logLik
139.2658	147.3672	-65.63289

Random effects:

Formula: ~1 | harvest

	(Intercept)	Residual
StdDev:	2.286191e-05	0.7259467

Fixed effects: logratiobiomass ~ total.biomass

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.2119886	0.09565687	55	2.216136	0.0308
total.biomass	0.2478341	0.09089969	55	2.726457	**0.0086**

Correlation:

	(Intr)
total.biomass	0.084

Standardized Within-Group Residuals:

Min	Q1	Med	Q3	Max
-1.97781836	-0.66100849	0.03304747	0.58814891	3.12025296

Number of Observations: 58

Number of Groups: 2

> **shapiro.test(resid(model1))** ### test non normality of PIC values

Shapiro-Wilk normality test

data: resid(model1)

W = 0.9745, p-value = 0.2588

b. mixed effects model that combines total biomass and lrPDA growth values from both sampling points (without phylogenetic correction)

> totbiomass<-totbiomass^2

> model2<-lme(totpref~totbiomass, random=~1|harvest)

> summary(model2)

Linear mixed-effects model fit by REML

Data: NULL

AIC	BIC	logLik
91.29803	99.5398	-41.64901

Random effects:

Formula: ~1 | harvest

	(Intercept)	Residual
StdDev:	0.1203502	0.4454112

Fixed effects: totpref ~ totbiomass

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.03284720	0.17111382	57	0.1919611	0.8485
totbiomass	0.03467985	0.01179548	57	2.9400959	**0.0047**

Correlation:

(Intr)
totbiomass -0.8

Standardized Within-Group Residuals:
Min Q1 Med Q3 Max
-3.75968315 -0.65246381 0.06626618 0.68971264 2.33466027

Number of Observations: 60
Number of Groups: 2

c. mixed effects model that combines phylogenetically corrected growth rates and lrPDA growth values from both sampling points

> summary(model3<-lme(picPref.all~picGrowth.all, random=~1|harvest))
Linear mixed-effects model fit by REML
Data: NULL
AIC BIC logLik
54.05011 60.60046 -23.02506

Random effects:
Formula: ~1 | harvest
(Intercept) Residual
StdDev: 9.193739e-06 0.3899882

Fixed effects: picPref.all ~ picGrowth.all
Value Std.Error DF t-value p-value
(Intercept) 0.06404137 0.11264875 37 0.568505 0.5731
picGrowth.all 0.06602946 0.01859497 37 3.550932 **0.0011**
Correlation:
(Intr)
picGrowth.all -0.837

Standardized Within-Group Residuals:

Min Q1 Med Q3 Max

-2.3030176 -0.7194411 0.1729375 0.6707805 1.8126261

Number of Observations: 40

Number of Groups: 2

> shapiro.test(resid(model3)) # check deviation from normality

Shapiro-Wilk normality test

data: resid(model3)

W = 0.9623, p-value = 0.2009

d. mixed effects model that combines growth rates and lrPDA growth values from both sampling points (without phylogenetic correction)

> summary(lme(totpref ~ growth.rate, random=~1|harvest))

Linear mixed-effects model fit by REML

Data: NULL

AIC BIC logLik

94.54024 102.6416 -43.27012

Random effects:

Formula: ~1 | harvest

(Intercept) Residual

StdDev: 1.303923e-05 0.4365217

Fixed effects: totpref ~ biomass.all

Value Std.Error DF t-value p-value

(Intercept) 0.4685167 0.06777800 55 6.912518 0.0000

growth.rate -0.0000502 0.00012005 55 -0.418151 **0.6775**

Correlation:

(Intr)
growth.rate -0.534

Standardized Within-Group Residuals:
Min Q1 Med Q3 Max
-2.6919591 -0.5873843 0.1182959 0.7278497 2.0610720

Number of Observations: 58
Number of Groups: 2

Correlation tests (individual harvests)

a. relationship between log-response biomass and biomass 1st harvest

> cor.test(biomass, pref, method="pearson")

Pearson's product-moment correlation

data: biomass and pref
t = 1.4383, df = 28, p-value = 0.1614
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
-0.1081887 0.5688200
sample estimates:
cor
0.2623043

b. relationship between log-response biomass and biomass 2nd harvest

> cor.test(biomass, pref, method="pearson")

Pearson's product-moment correlation

data: biomass and pref
t = 3.8258, df = 28, p-value = 0.000669
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.2860171 0.7812668
sample estimates:
      cor
0.5859121

c. relationship between log-response biomass and growth rate 1st harvest

> cor.test(pref, growth, method="pearson")

        Pearson's product-moment correlation

data: pref3 and growth
t = 0.8819, df = 27, p-value = 0.3856
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.2121957 0.5029832
sample estimates:
      cor
0.1673228

d. relationship between log-response biomass and growth rate 2nd harvest

> cor.test(growth, pref, method="kendall")

        Kendall's rank correlation tau

data: growth and pref3

T = 225, p-value = 0.4233
alternative hypothesis: true tau is not equal to 0
sample estimates:
tau
0.1083744

e. relationship between log-response density and biomass 1st harvest

> cor.test(biomass, density, method="pearson")

Pearson's product-moment correlation

data: biomass and density
t = 1.0296, df = 28, p-value = 0.312
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
-0.1817830 0.5157737
sample estimates:
cor
0.1909946

f. relationship between log-response density and biomass 2nd harvest

> cor.test(biomass, density, method="pearson")

Pearson's product-moment correlation

data: biomass and density
t = 1.4317, df = 28, p-value = 0.1633
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
-0.1093796 0.5680042

sample estimates:

cor

0.2611817

g. relationship between PICs (phylogenetically corrected values) of log-response biomass and biomass 1st harvest

> cor.test(biomass, pref, method="kendall")

Kendall's rank correlation tau

data: biomass2 and pref2

T = 241, p-value = 0.1608

alternative hypothesis: true tau is not equal to 0

sample estimates:

tau

0.1871921

h. relationship between PICs (phylogenetically corrected values) log-response biomass and biomass 2nd harvest

> cor.test(growth, pref, method="pearson")

Pearson's product-moment correlation

data: growth2 and pref4

t = 3.5056, df = 26, p-value = 0.001672

alternative hypothesis: true correlation is not equal to 0

95 percent confidence interval:

0.2453016 0.7756643

sample estimates:

cor

0.5665316

i. relationship between PICs (phylogenetically corrected values) log-response biomass and growth rate 1st harvest

> cor.test(pref, growth, method="pearson")

Pearson's product-moment correlation

data: pref4 and growth2
t = 6.9675, df = 26, p-value = 2.131e-07
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
0.6208102 0.9069937
sample estimates:
cor
0.806982

j. relationship between PICs (phylogenetically corrected values) log-response biomass and growth rate 2nd harvest

> cor.test(growth, pref, method="pearson")

Pearson's product-moment correlation

data: growth2 and pref4
t = 3.5056, df = 26, p-value = 0.001672
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
0.2453016 0.7756643
sample estimates:
cor

0.5665316

k. relationship between PICs (phylogenetically corrected values) log-response density and biomass 1st harvest

> cor.test(biomass, density, method="kendall")

Kendall's rank correlation tau

data: biomass2 and density2
T = 227, p-value = 0.3813
alternative hypothesis: true tau is not equal to 0
sample estimates:
tau
0.1182266

l. relationship between PICs (phylogenetically corrected values) log-response density and biomass 2nd harvest

> cor.test(biomass, density, method="kendall")

Kendall's rank correlation tau

data: biomass2 and density2
T = 252, p-value = 0.06853
alternative hypothesis: true tau is not equal to 0
sample estimates:
tau
0.2413793

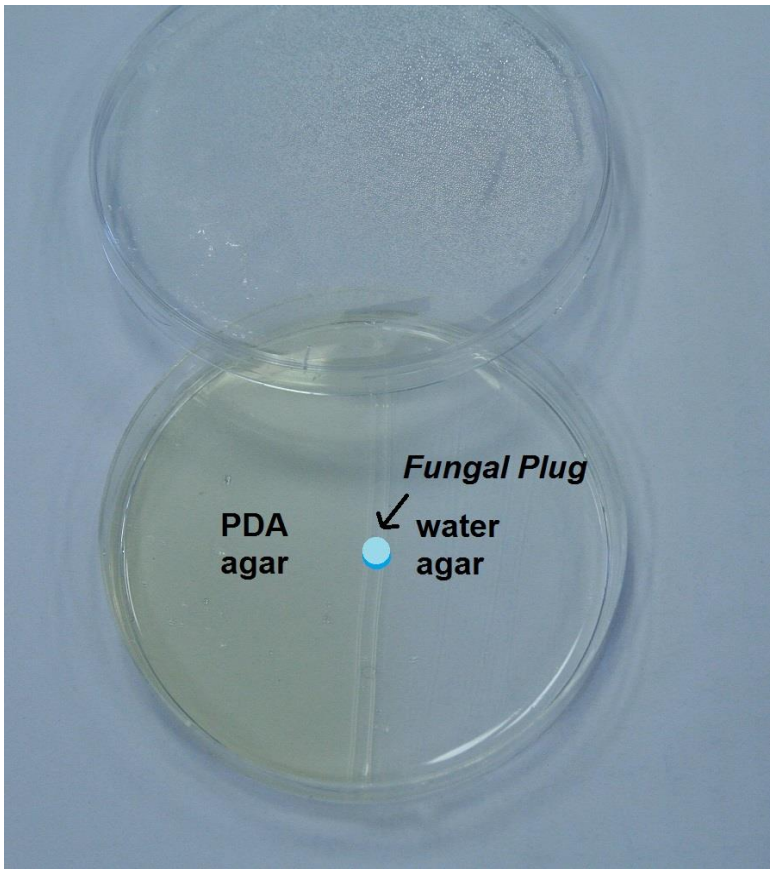


Fig. S2.1 Image of a characteristic compartmentalized plate that was used for the first experiment. Half of the plate is covered with potato dextrose agar (PDA; as rich medium that was poured first and removed aseptically from half of the plate with a scalpel) and half with water agar (as poor medium, poured a day before initiation of the experiment to constrain nutrient diffusion). We also highlight the location on the plate where we added the plugs of the 32 isolates. It is often challenging to select nutrient-rich and nutrient-poor habitats for fungi. For our study we applied the convention often found in mycological studies that Potato-Dextrose Agar (PDA) is a representative nutrient rich growth-environment whereas water-agar a nutrient-deficient medium and assumed that across media of comparable fertility relative fungal growth-rates change only moderate

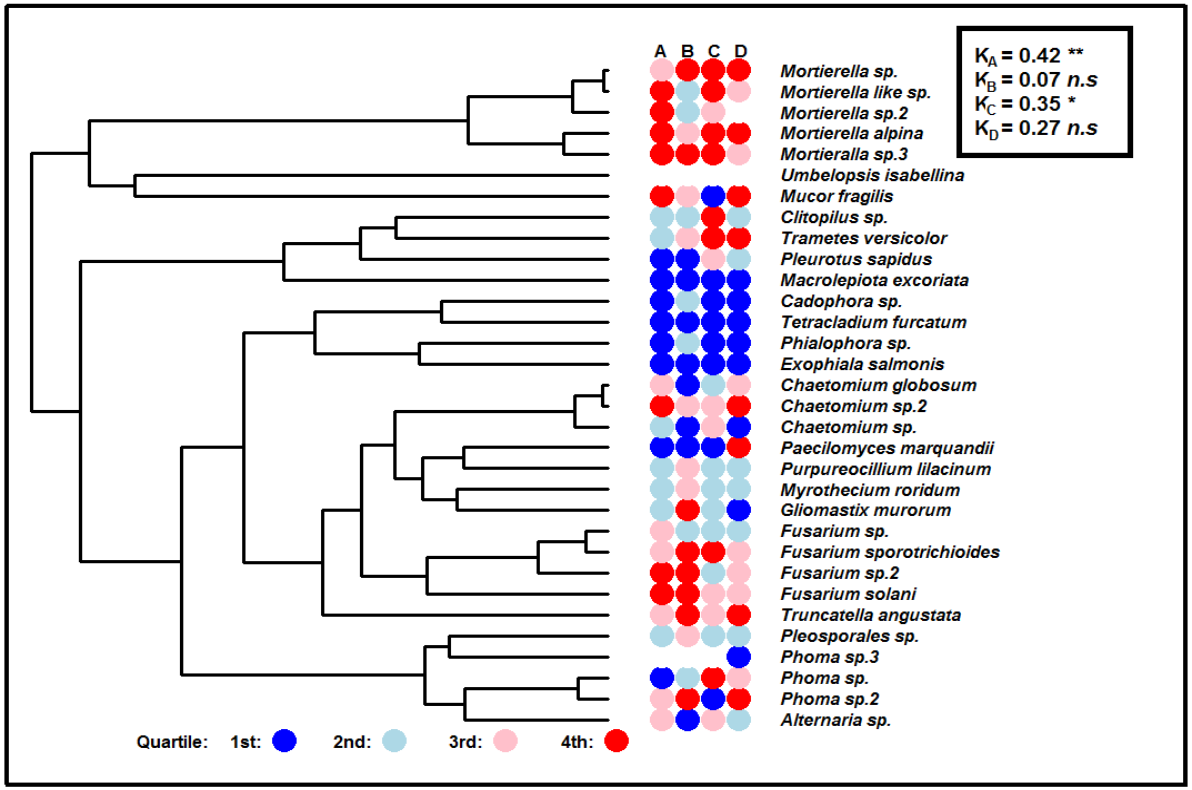


Fig. S2.2 Phylogenetic relationships of fungal isolates and relative values (quartiles – color gradient from dark blue (low relative values) to dark red (high relative values)) of relative growth-preference (lrPDA-growth) (A), lrPDA-density (B) and biomass (C) from the second harvest and growth-rates (D) from the dedicated experiment. Blomberg K values and their significance are displayed in the insert. Missing values are displayed in white.

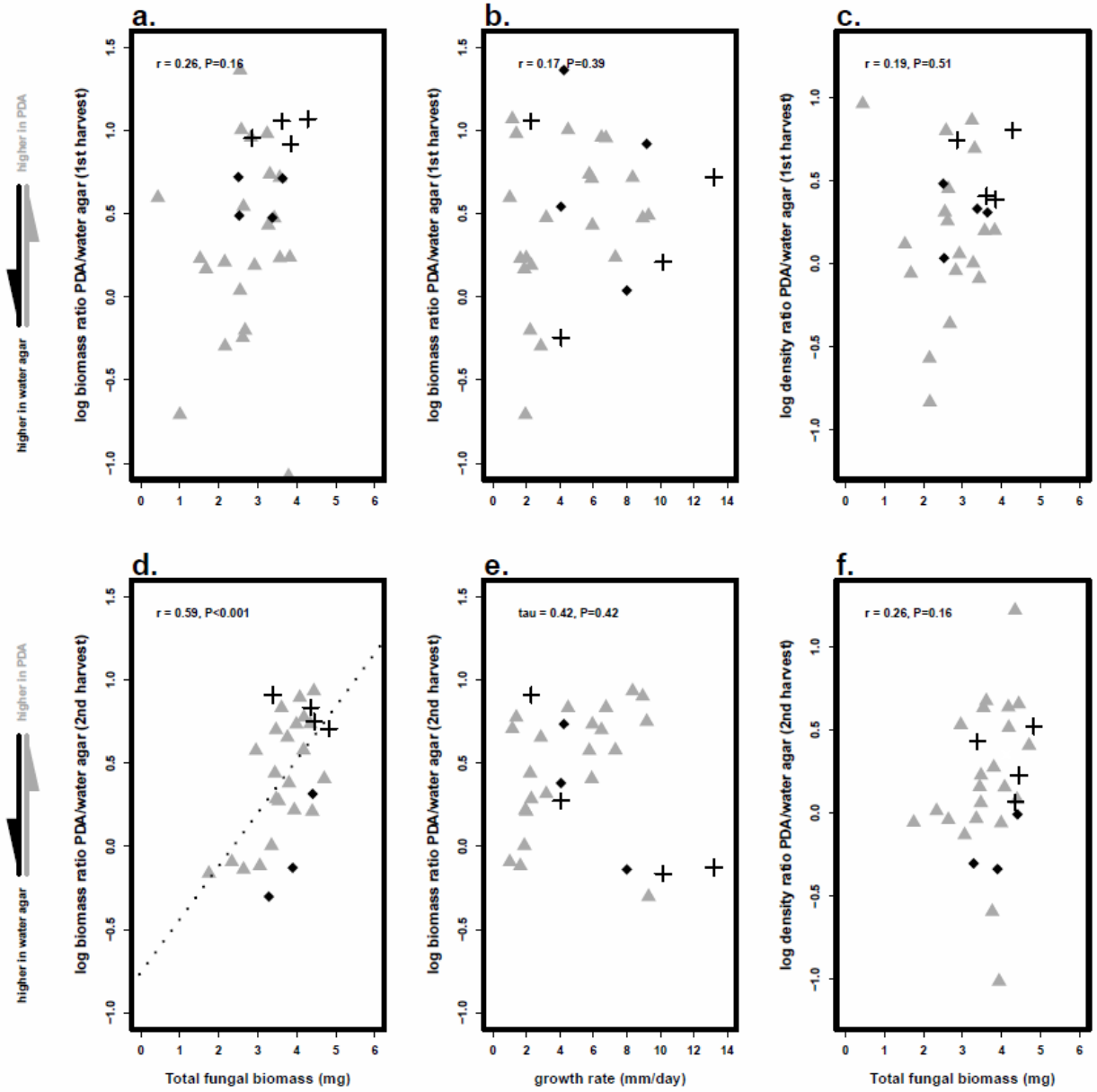

a.
b.
c.
d.
e.
f.
r = 0.26, P=0.16
r = 0.17, P=0.39
r = 0.19, P=0.51
r = 0.59, P<0.001
tau = 0.42, P=0.42
r = 0.26, P=0.16
higher in PDA
higher in water agar
log biomass ratio PDA/water agar (1st harvest)
log density ratio PDA/water agar (1st harvest)
log biomass ratio PDA/water agar (2nd harvest)
log density ratio PDA/water agar (2nd harvest)
Total fungal biomass (mg)
growth rate (mm/day)

Fig. S2.3 Relationships between the log biomass ratio PDA/water agar (lrPDA growth) and total fungal biomass (a - first harvest; d - second harvest) or the growth rate of the isolates independently assayed in the second experiment (b - first harvest; e - second harvest) and between mycelial area density and total fungal biomass (c - first harvest; f - second harvest). Statistics are integrated in the panels and total biomass - lrPDA growth and growth rate - lrPDA growth relationships were significant after correcting for phylogenetic signal (Fig. S4). By contrast we found no relationship between total biomass and lrPDA density in any of our tests. The discontinuous line is a best fit line. Grey triangles stand for Ascomycota isolates whereas black rhombuses and crosses for Basidiomycota and Mucoromycotina isolates, respectively. We present raw data (uncorrected for phylogeny) in this figure. Non-parametric Kendal-tau tests were only used when the assumptions of Pearson correlations were not met.

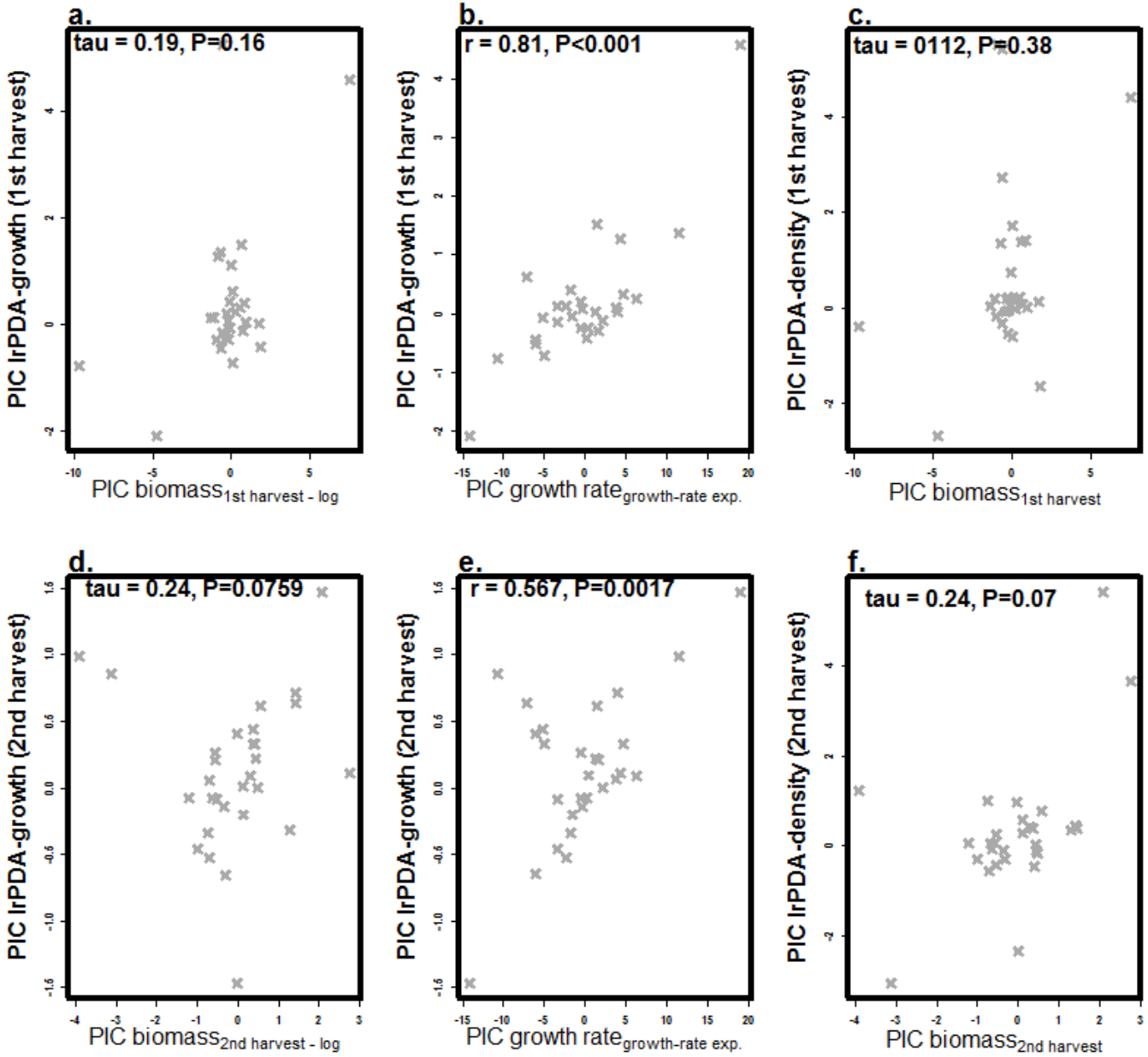

a.
tau = 0.19, P=0.16
PIC lrPDA-growth (1st harvest)
PIC biomass1st harvest - log
b.
r = 0.81, P<0.001
PIC lrPDA-growth (1st harvest)
PIC growth rategrowth-rate exp.
c.
tau = 0112, P=0.38
PIC lrPDA-density (1st harvest)
PIC biomass1st harvest
d.
tau = 0.24, P=0.0759
PIC lrPDA-growth (2nd harvest)
PIC biomass2nd harvest - log
e.
r = 0.567, P=0.0017
PIC lrPDA-growth (2nd harvest)
PIC growth rategrowth-rate exp.
f.
tau = 0.24, P=0.07
PIC lrPDA-density (2nd harvest)
PIC biomass2nd harvest

Fig. S2.4 Relationships between phylogenetically-corrected relative-growth (log-response ratio of mean per isolate biomasses in the PDA sector over the water-agar sector of the plate - lrPDA-growth) values and either biomass (a, d) or growth-rates (b, e) and between lrPDA-density and biomass (c, f) for the 1st (a, b, c) and 2nd (d, e, f) harvest. Note that the relationship between PIC biomass and PIC lrPDA-growth is no longer significant but it remains significant if the data from the two harvests are analyzed together in a mixed effects linear model with harvest being a random effects factor. Statistics are included in the panels. PIC stands for phylogenetic independent contrasts.

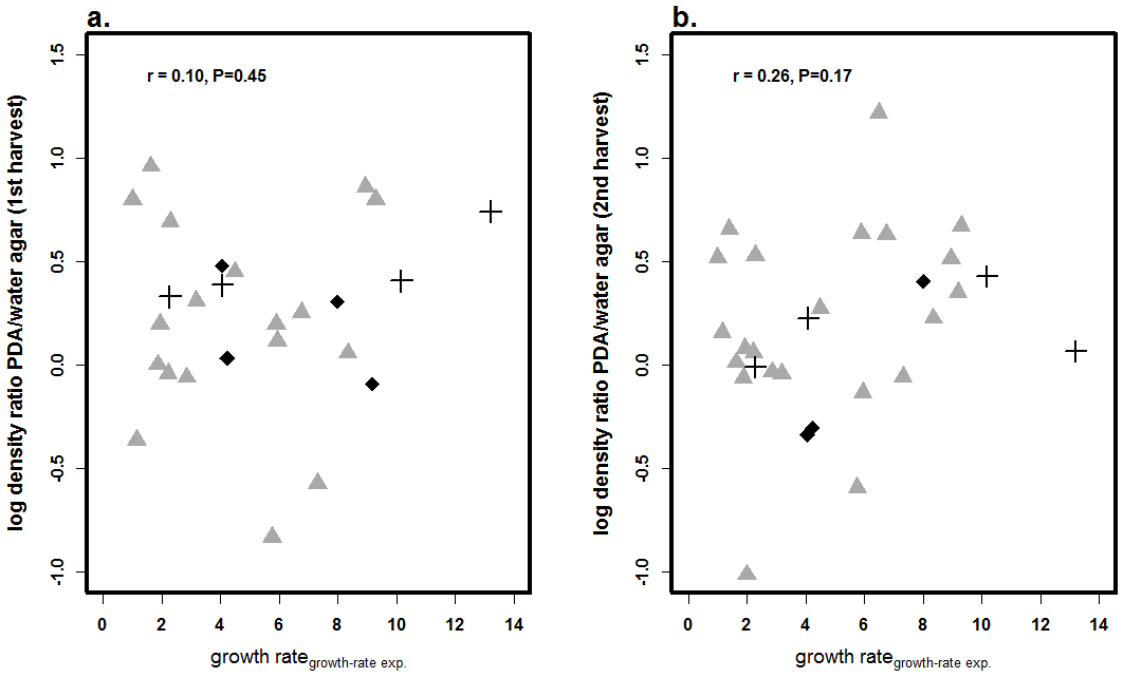


Fig. S2.5 Relationships between the log density ratio PDA/water agar (lrPDA density) and the growth rate in harvest 1 (a) and harvest 2 (b). We observed a lack of relationship between density and growth rate in agreement to what we observed with biomass. We noted however that the growth rates of fungi growing in water-agar were higher than in earlier experiments where we had grown the isolates exclusively in water agar suggesting nutrient transfer from the PDA sector to the water-agar sector of the plate. Moreover, we did not observe any herringbone-pattern-topology in our water agar sectors.

Table S2.1 Information aboutOrder, GenBank and DSMZ accession numbers of the 30 fungal strains used in this study.

Genus species	Order	Phylum	NCBI	DSMZ*
Phoma sp. 3	Pleosporales	Ascomycota	KT582065	DSM 100327
Myrothecium roridum	Hypocreales	Ascomycota	KT582090	DSM 101519
Chaetomium sp. 2	Sordariales	Ascomycota	KT582096	DSM 100400
Tetracladium furcatum	Helotiales	Ascomycota	KT582084	DSM 100330
Truncatella angustata	Xylariales	Ascomycota	KT582088	DSM 100284
Phoma like sp.	Pleosporales	Ascomycota	KT582091	DSM 100401
Cadophora sp.	Helotiales	Ascomycota	KT582085	DSM 100323
Chaetomium sp.	Sordariales	Ascomycota	KT582086	DSM 100326
Fusarium sp.2	Hypocreales	Ascomycota	KT582097	DSM 100403
Phoma sp. 2	Pleosporales	Ascomycota	KT582077	DSM 100404
Alternaria sp.	Pleosporales	Ascomycota	KT582078	DSM 100286
Chaetomium globosum	Sordariales	Ascomycota	KT582079	DSM 100405
Phoma sp.	Pleosporales	Ascomycota	KT582082	DSM 101518
Fusarium sp.	Hypocreales	Ascomycota	KT582068	DSM 100287
Fusarium solani	Hypocreales	Ascomycota	KT582073	DSM 100290
Phialophora sp.	Chaetothyriales	Ascomycota	KT582074	DSM 100328
Exophiala salmonis	Chaetothyriales	Ascomycota	KT582075	DSM 100291
Gliomastix murorum	Hypocreales	Ascomycota	KT582083	DSM 100292
Purpureocillium lilacinum	Hypocreales	Ascomycota	KT582081	DSM 100329
Fusarium sporotrichioides	Hypocreales	Ascomycota	KT582087	DSM 100325
Paecilomyces marquandii	Hypocreales	Ascomycota	KT582066	DSM 100410
Fusarium oxysporum	Hypocreales	Ascomycota	KT582095	DSM 100409
Mortierella like sp.	Mortierellales	Mucoromycotina	KT582092	DSM 100402
Umbelopsis isabellina	Mucorales	Mucoromycotina	KT582093	DSM 100331
Mortierella sp. 2	Mortierellales	Mucoromycotina	KT582094	DSM 100322
Mortierella sp. 3	Mortierellales	Mucoromycotina	KT582070	DSM 100289

Mortierella sp.	Mortierellales	Mucoromycotina	KT582072	DSM 100407
Mucor fragilis	Mucorales	Mucoromycotina	KT582076	DSM 100293
Mortierella alpina	Mortierellales	Mucoromycotina	KT582067	DSM 100285
Clitopilus sp.	Agaricales	Basidiomycota	KT582089	DSM 100324
Macrolepiota excoriata	Agaricales	Basidiomycota	KT582069	DSM 100288
Trametes versicolor	Polyporales	Basidiomycota	KT582071	DSM 100406
Pleurotus sapidus	Agaricales	Basidiomycota	KT582080	DSM 100408

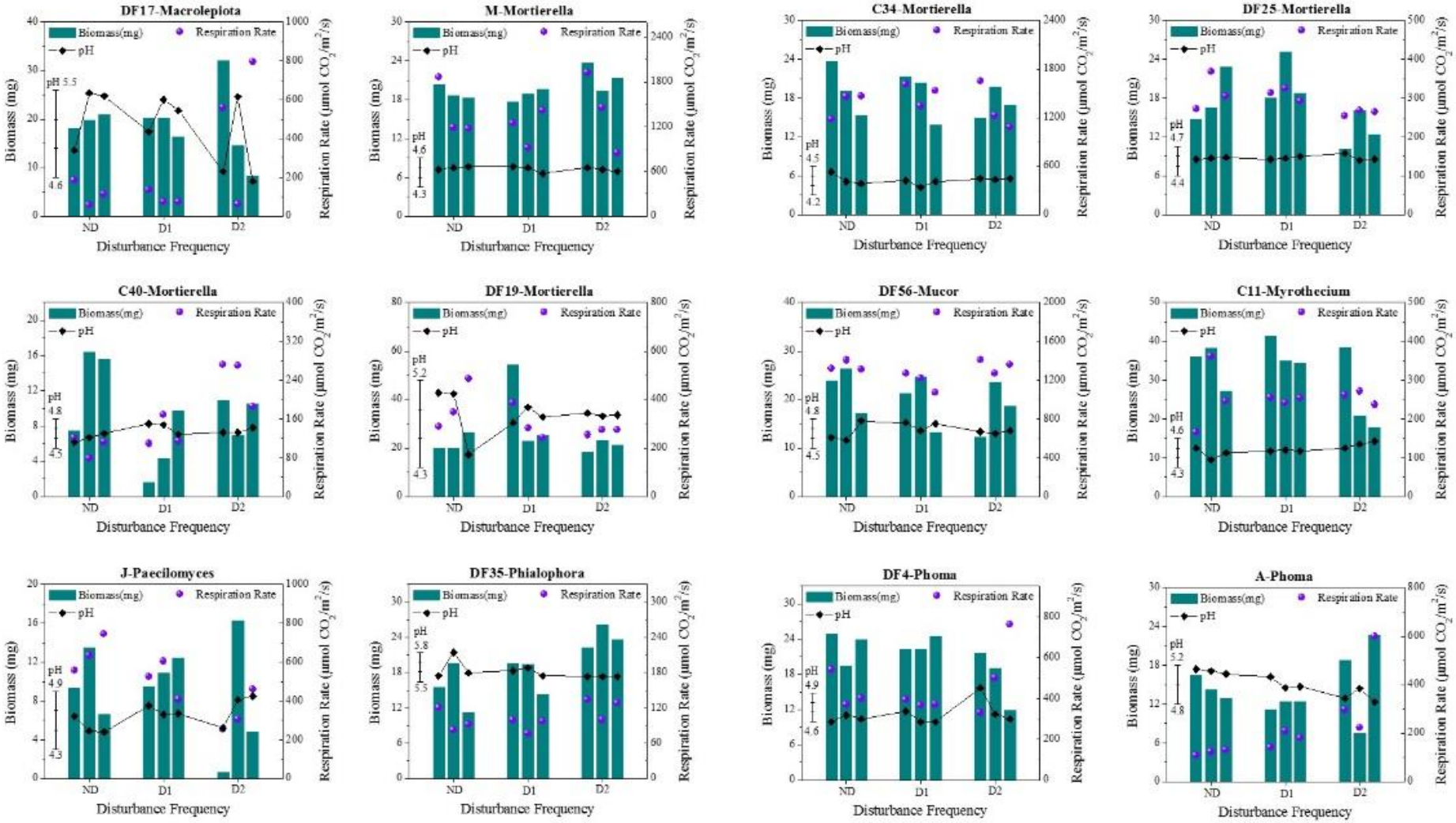
*The accession number is from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellculturen GmbH

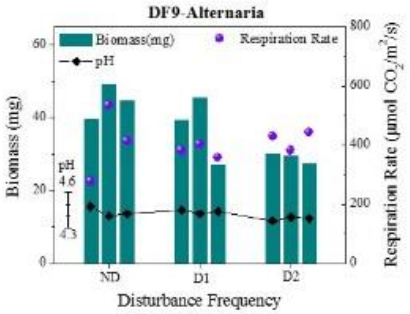
Appendix 3

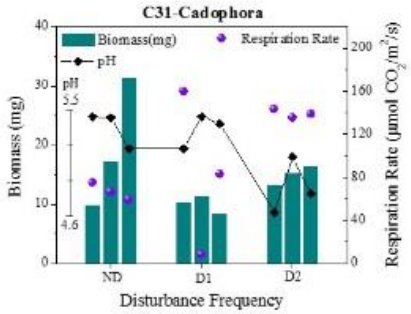
Table S3.1 Information about Order, GenBank and DSMZ accession numbers, Strain IDs and growth rate (Fungal biomass of 8-days incubation, mg) of the 30 fungal strains used in this study.

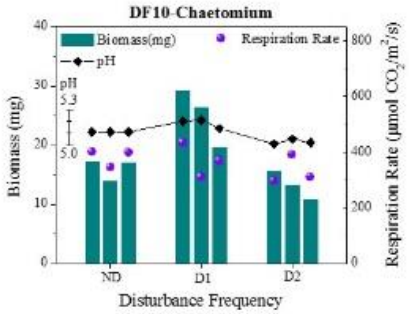
Genus species	Order	Phylum	NCBI	DSMZ*	Growth rate[1]	Strain ID
Phoma sp. 3	Pleosporales	Ascomycota	KT582065	DSM 100327	14.5	A
Myrothecium roridum	Hypocreales	Ascomycota	KT582090	DSM 101519	33.77	C11
Chaetomium sp. 2	Sordariales	Ascomycota	KT582096	DSM 100400	26.8	C13
Tetracladium furcatum	Helotiales	Ascomycota	KT582084	DSM 100330	21.03	C21
Truncatella angustata	Xylariales	Ascomycota	KT582088	DSM 100284	39.17	C23
Phoma like sp.	Pleosporales	Ascomycota	KT582091	DSM 100401	14.3	C29
Cadophora sp.	Helotiales	Ascomycota	KT582085	DSM 100323	19.4	C31
Chaetomium sp.	Sordariales	Ascomycota	KT582086	DSM 100326	20.8	C33
Fusarium sp.2	Hypocreales	Ascomycota	KT582097	DSM 100403	15.3	DF16
Phoma sp. 2	Pleosporales	Ascomycota	KT582077	DSM 100404	22.67	DF04
Alternaria sp.	Pleosporales	Ascomycota	KT582078	DSM 100286	44.43	DF09
Chaetomium globosum	Sordariales	Ascomycota	KT582079	DSM 100405	16	DF10
Phoma sp.	Pleosporales	Ascomycota	KT582082	DSM 101518		
Fusarium sp.	Hypocreales	Ascomycota	KT582068	DSM 100287	16.1	C41
Fusarium solani	Hypocreales	Ascomycota	KT582073	DSM 100290	20.77	DF32
Phialophora sp.	Chaetothyriales	Ascomycota	KT582074	DSM 100328	15.37	DF35
Exophiala salmonis	Chaetothyriales	Ascomycota	KT582075	DSM 100291	3.37	DF36
Gliomastix murorum	Hypocreales	Ascomycota	KT582083	DSM 100292	24.7	DF37
Purpureocillium lilacinum	Hypocreales	Ascomycota	KT582081	DSM 100329	4.13	DF58
Fusarium sporotrichioides	Hypocreales	Ascomycota	KT582087	DSM 100325	22.67	Disc

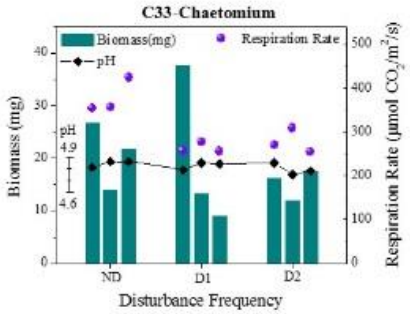
Paecilomyces marquandii	Hypocreales	Ascomycota	KT582066	DSM 100410	9.8	J
Fusarium oxysporum	Hypocreales	Ascomycota	KT582095	DSM 100409		
Mortierella like sp.	Mortierellales	Mucoromycotina	KT582092	DSM 100402	19.37	C34
Umbelopsis isabellina	Mucorales	Mucoromycotina	KT582093	DSM 100331	14.4	C35
Mortierella sp. 2	Mortierellales	Mucoromycotina	KT582094	DSM 100322	13.13	C40
Mortierella sp. 3	Mortierellales	Mucoromycotina	KT582070	DSM 100289	22	DF19
Mortierella sp.	Mortierellales	Mucoromycotina	KT582072	DSM 100407	18.03	DF25
Mucor fragilis	Mucorales	Mucoromycotina	KT582076	DSM 100293	22.47	DF56
Mortierella alpina	Mortierellales	Mucoromycotina	KT582067	DSM 100285	19.07	M
Clitopilus sp.	Agaricales	Basidiomycota	KT582089	DSM 100324	24.8	C28
Macrolepiota excoriata	Agaricales	Basidiomycota	KT582069	DSM 100288	19.6	DF17
Trametes versicolor	Polyporales	Basidiomycota	KT582071	DSM 100406	12.97	DF24
Pleurotus sapidus	Agaricales	Basidiomycota	KT582080	DSM 100408	40.3	DF42

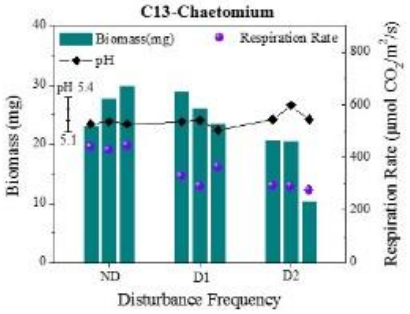

DF17-Macrolepiota
M-Mortierella
C34-Mortierella
DF25-Mortierella
C40-Mortierella
DF19-Mortierella
DF56-Mucor
C11-Myrothecium
J-Paecilomyces
DF35-Phialophora
DF4-Phoma
A-Phoma
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
Disturbance Frequency
ND
D1
D2

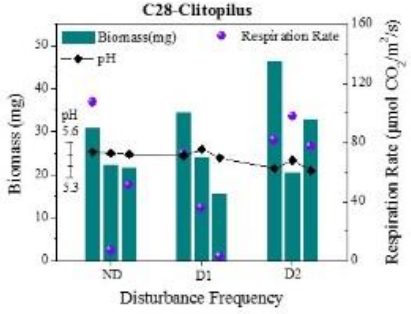

DF9-Alternaria
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 4.6
4.3
ND
D1
D2
Disturbance Frequency

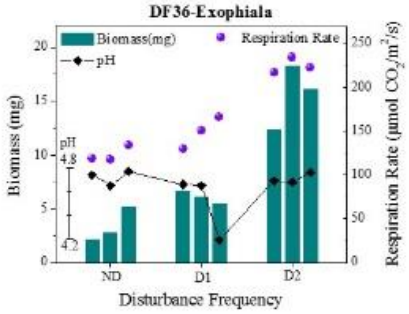

C31-Cadophora
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 5.5
4.6
ND
D1
D2
Disturbance Frequency

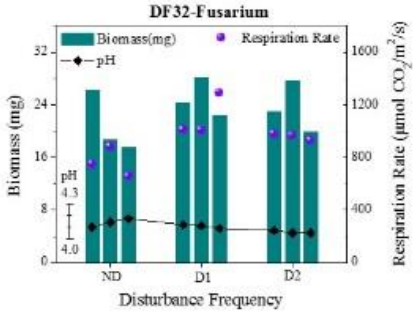

DF10-Chaetomium
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 5.3
5.0
ND
D1
D2
Disturbance Frequency

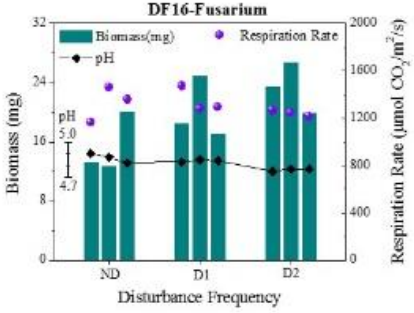

C33-Chaetomium
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 4.9
4.6
ND
D1
D2
Disturbance Frequency

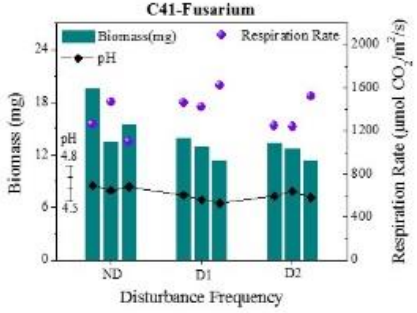

C13-Chaetomium
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 5.4
5.1
ND
D1
D2
Disturbance Frequency

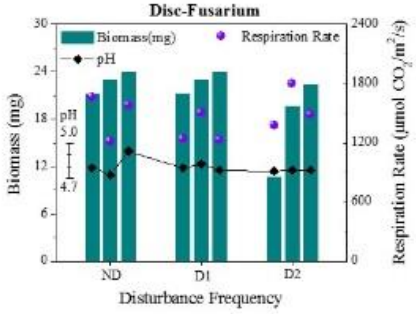

C28-Clitopilus
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 5.6
5.3
ND
D1
D2
Disturbance Frequency

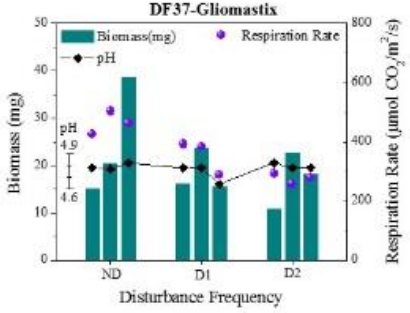

DF36-Exophiala
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 4.8
4.2
ND
D1
D2
Disturbance Frequency


DF32-Fusarium
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 4.3
4.0
ND
D1
D2
Disturbance Frequency


DF16-Fusarium
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 5.0
4.7
ND
D1
D2
Disturbance Frequency


C41-Fusarium
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 4.8
4.5
ND
D1
D2
Disturbance Frequency


Disc-Fusarium
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 5.0
4.7
ND
D1
D2
Disturbance Frequency


DF37-Gliomastix
Biomass(mg)
Respiration Rate
pH
Biomass (mg)
Respiration Rate (μmol CO2/m2/s)
pH 4.9
4.6
ND
D1
D2
Disturbance Frequency

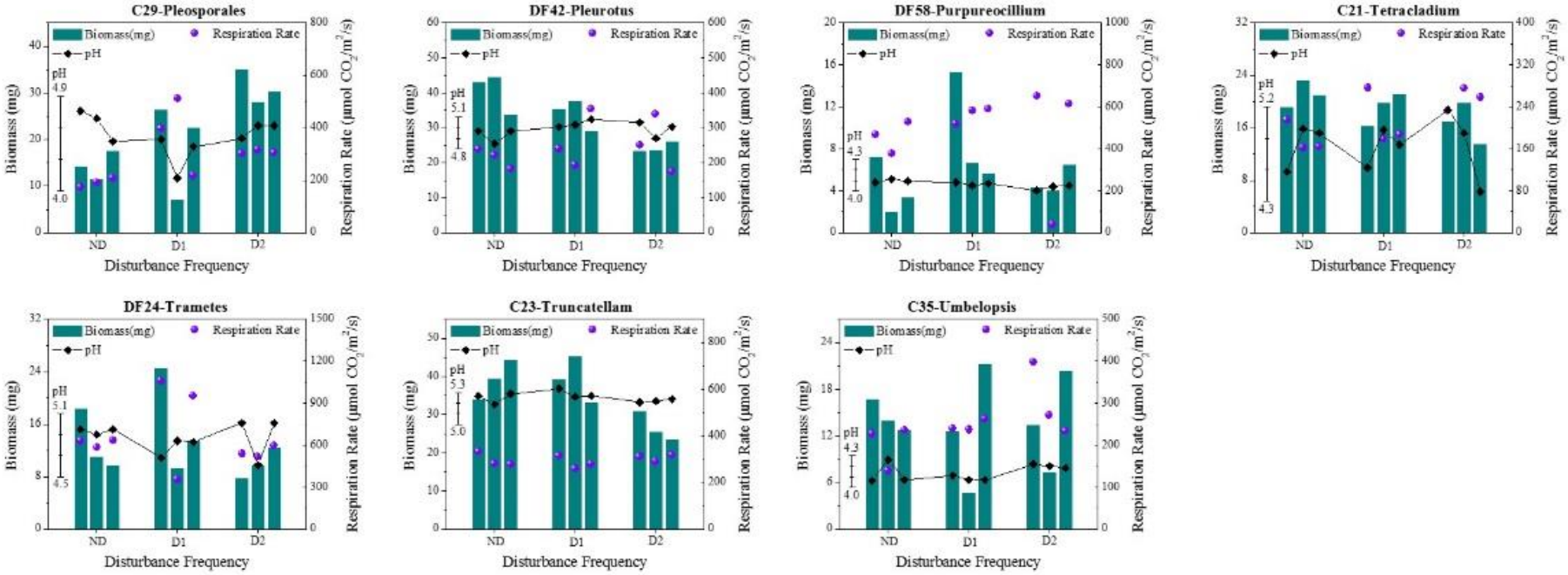


Fig. S3.1. Growth information for the 30 fungal isolates at all three disturbances.

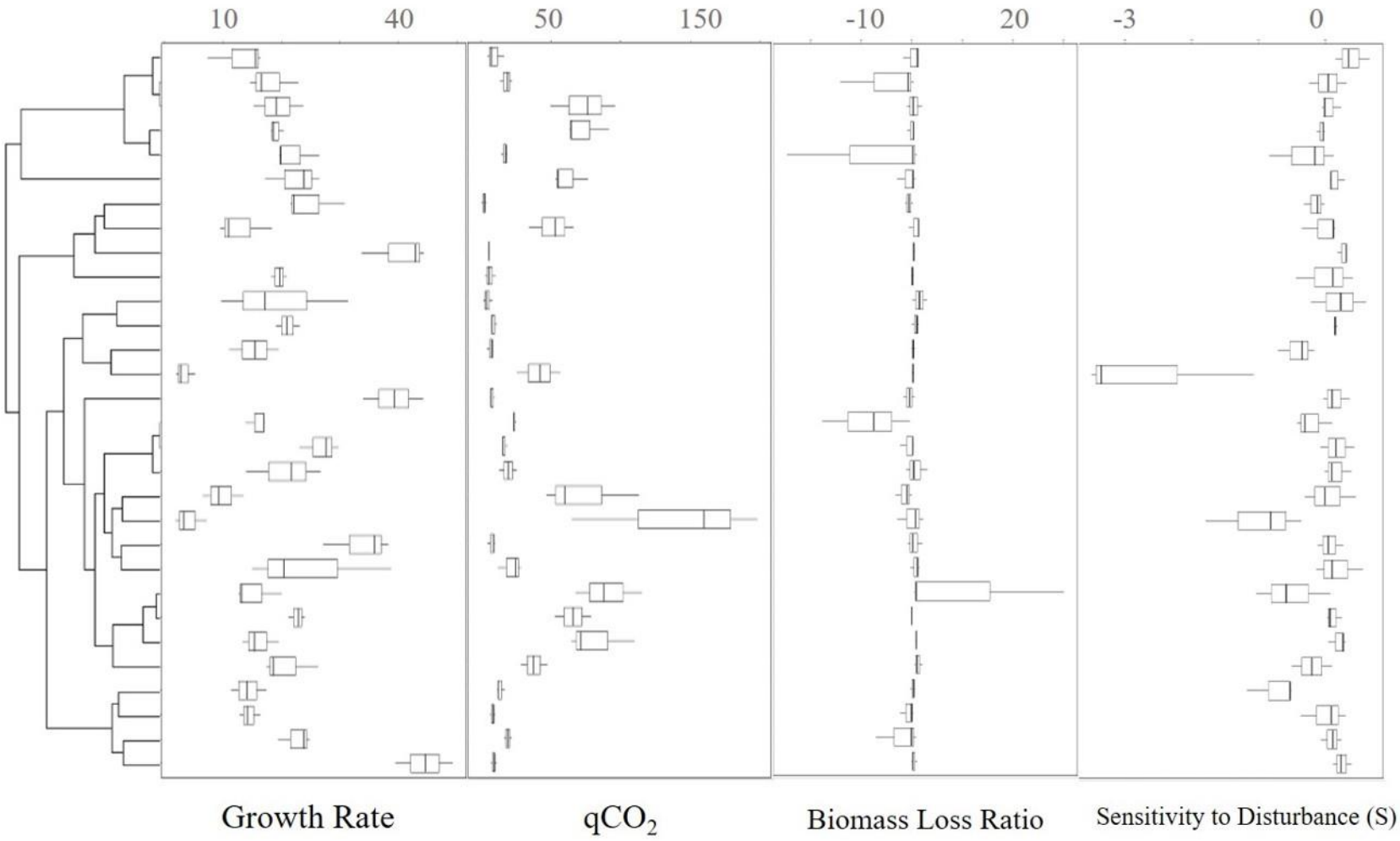


Fig. S3.2. Four fungal ruderal traits for the 30 fungal isolates at all three disturbances. Mean growth rate (assayed by fungal biomass at harvest), qCO2 (assayed for a proxy of Carbon Use Efficiency), Biomass Loss Response Ratio (assayed by the ratios of biomass loss in single versus iterative disturbances), and sensitivity to disturbance (assayed by mean biomass loss of single and iterative disturbances).

Appendix 4

Appendix 4.1 – Handling skewness in modelling growth optima

Filamentous fungi present left-skewed growth rate curves with temperature: growth rates decline faster for temperatures higher than the optimum than for those lower (e.g. Fig. S4.1a). A way to address issues of skewness is to transform the predictor. The fit of left-skewed distribution improves considerably when square-transformed.

We extracted data temperature – growth rate data from Carreiro and Koske (1992). We chose this paper because (i) it presents growth data for a broad range of fungi; (ii) because of the choice of the isolates these vary broadly in regards to their temperature optima. To extract the information from the figures we used PlotDigitizer v2.6.4 (Huwaldt and Steinhorst 2014). We extracted information for ten filamentous fungi which were presented in the main article. In all cases following square transformation of temperature we yielded Gaussian like distributions (Fig. S4.1).

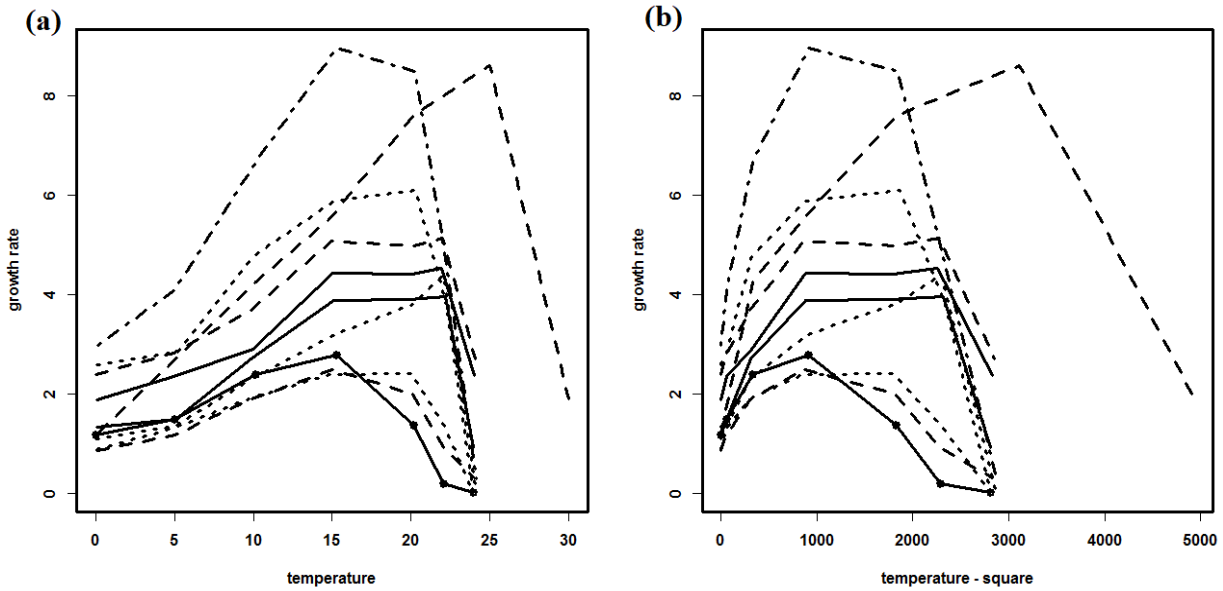


Fig. S4.1. Untransformed (a) and square transformed (b), growth rate – temperature curves for the isolates presented in Carreiro and Koske (1992). Curves in (a) are left skewed, whereas those in (b) are Gaussian like.

References:

Carreiro MM, Koske RE. 1992. Room temperature isolations can bias against selection of low temperature microfungi in temperate forest soils. *Mycologia* 84: 886-900.

Huwaldt JA, Steinhorst S. 2014 Plot Digitizer version 2.6.4. http://plotdigitizer.sourceforge.net/

Appendix 4.2. Inclusion criteria for our models

The modelling approach we used yielded estimates for the parameters (i.e. optimal temperature and standard deviation of the optimal temperature), standard errors for these parameters and goodness of fit values (i.e. summarized with the Akaike Information Criterion – AIC).
The majority of the models maintained a good fit (i.e. negative AIC values or s.e. values for optimal temperature below 10), whereas two models clearly performed worse than the others (i.e. did not meet this criterion – isolates DF35 and DF36) – Fig. S4.2. We excluded these fungi from any further tests.

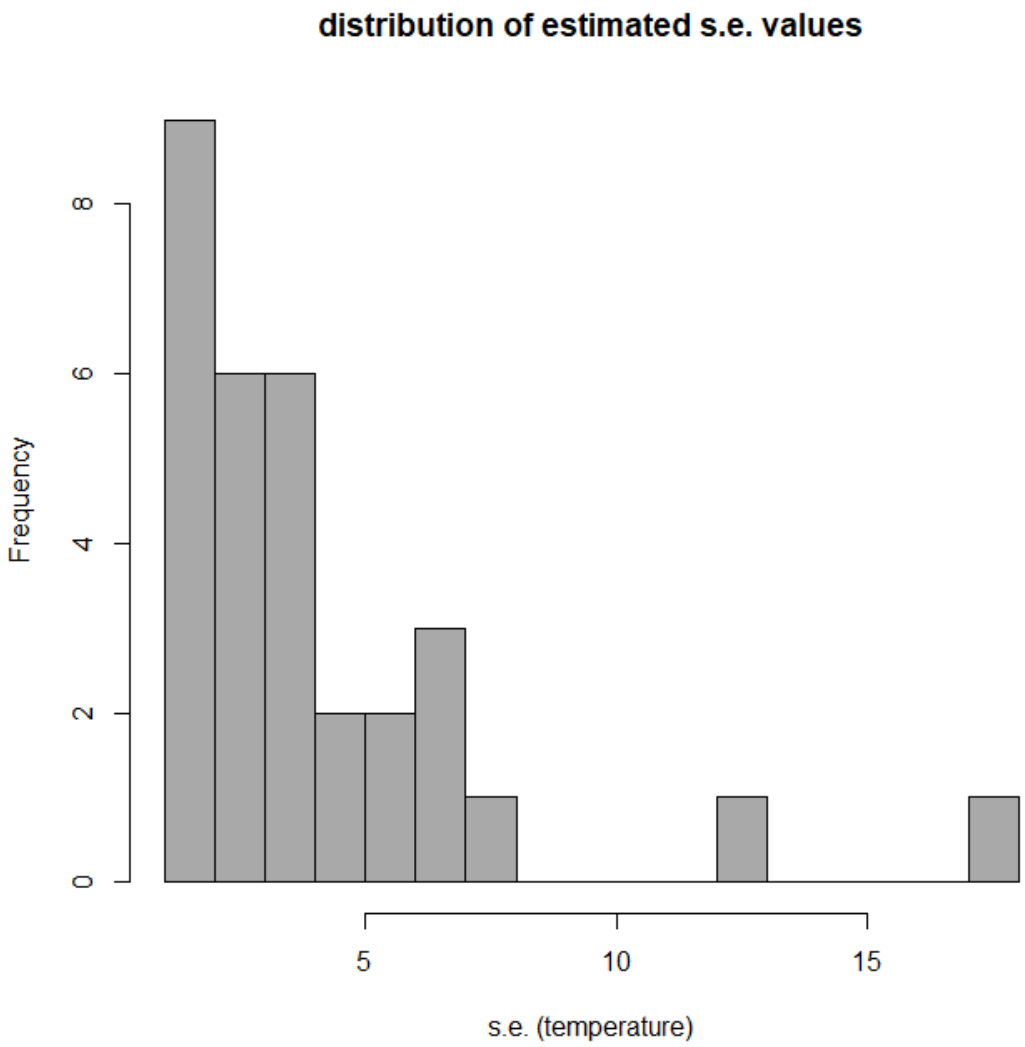


Fig. S4.2. Distribution of standard errors of the parameter optimal temperatures in the models. Note the right skewness we observed in the data, meaning that the majority of models had a good fit but a small subset of models have a considerably worse fit. In our analysis we accepted models

with a standard error for the optimal temperature below ten. Note that temperature values had been square-transformed in calculating the standard errors and it is difficult to scale back these values in regards to optimal temperatures.

Appendix 4.3- phenotypic plasticity of traits parameters

To quantify the magnitude of phenotypic plasticity of traits (specific growth rate, final colony size and growth time) in response to increasing temperature, we calculated the following two metrics: (i) a coefficient of variation (CV) and (ii) phenotypic plasticity index (PIv) (Bloor and Grubb, 2004; Zhang et al., 2016). Four CV values were calculated using data from four replicate plates, with each CV value calculated using the mean values from each species value. CV was calculated as follows:

$$CV_X = \frac{\sigma_X}{\overline{X}} \qquad \ldots(4.4)$$

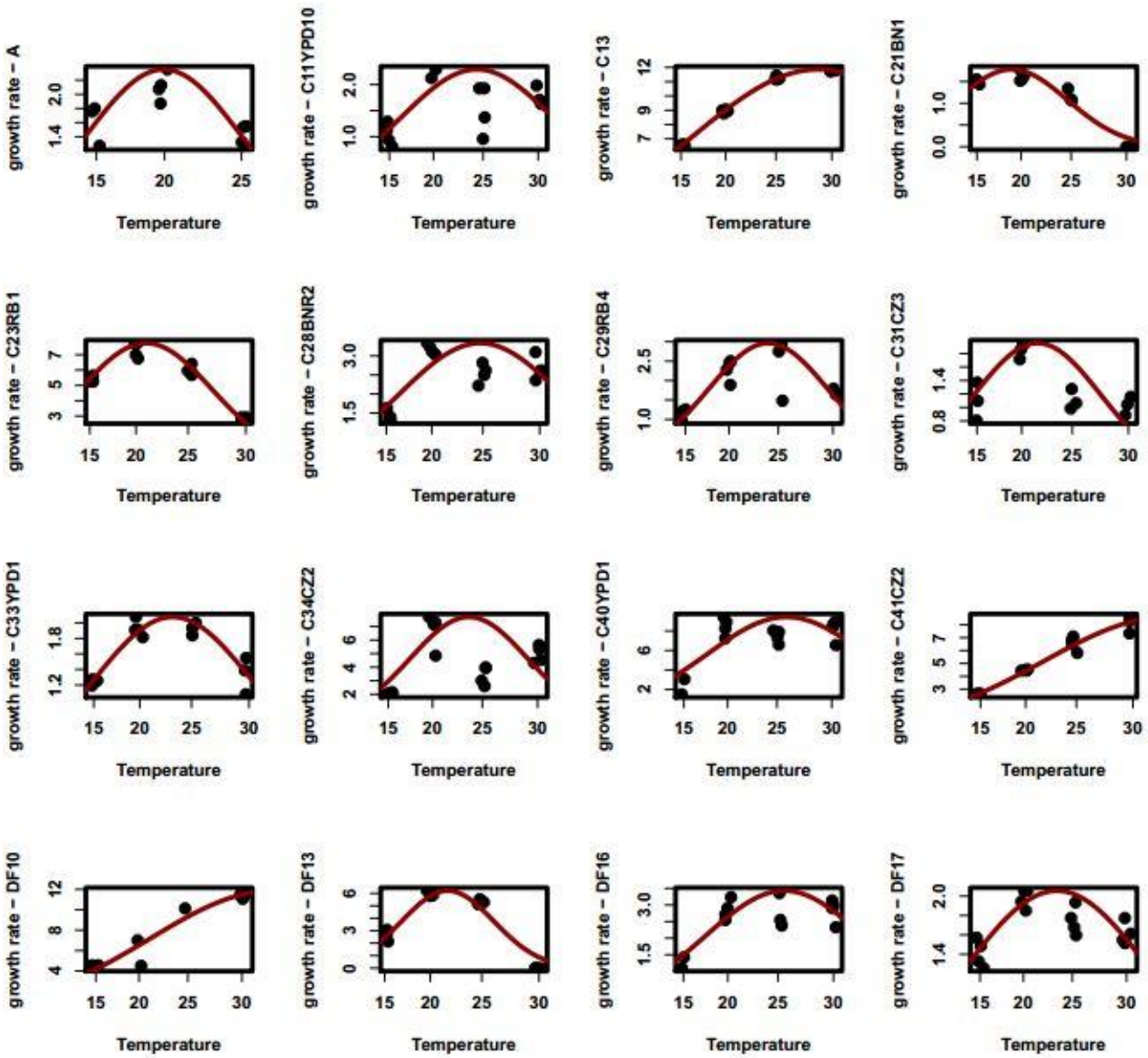
Where σ is the standard deviation of the four-temperature treatment means of trait X and $\overline{X}$ the grand mean of the temperature treatment means, as described elsewhere. To calculated PIv values of each trait, the maximum value and minimum value of this trait were obtained from four temperature levels in one species, with PIv being calculated as

$$PI_v(X) = \frac{X_{\max} - X_{\min}}{X_{\max}} \qquad \ldots(4.5)$$

Where PIv(X) is the phenotypic plasticity value for trait X, while X_{max} and X_{min} are the maximum and minimum values that we measured for trait X. Thus, PIv values are influenced by the magnitude of extreme values collected from species growth in favorable and stressful conditions.

In our experiment, we used colony extension rate as a proxy for body size. This is because the diameter of the hyphae and their branching differ across species and depend a lot on the habitat in which they grow.

Appendix 4.4 - Detailed results



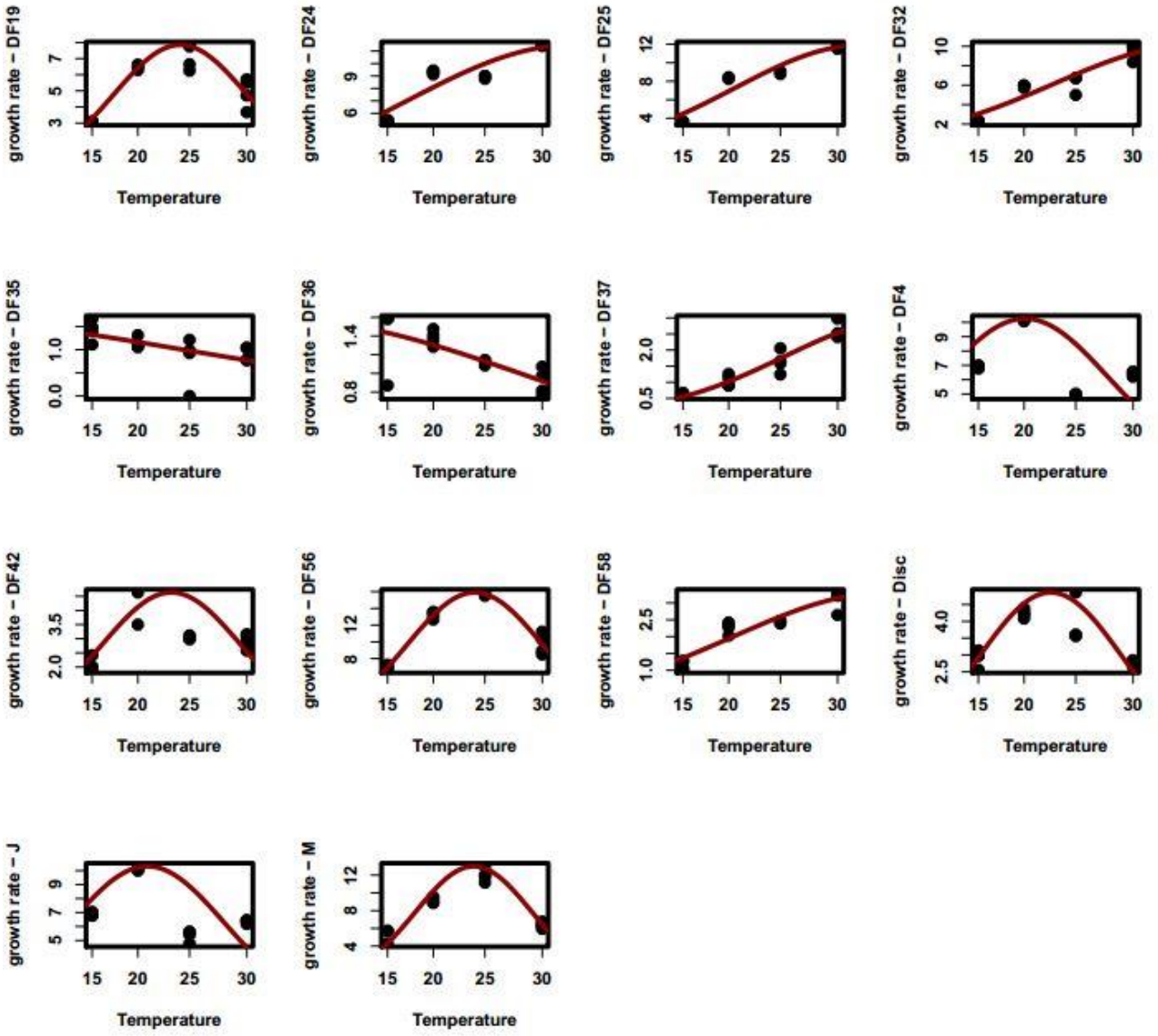


Fig. S4.3. Estimation of optimal temperatures by growth rates of 30 fungal isolates (4 replicates) at square root corrected temperature of 15°C, 20°C, 25°C & 30°C.

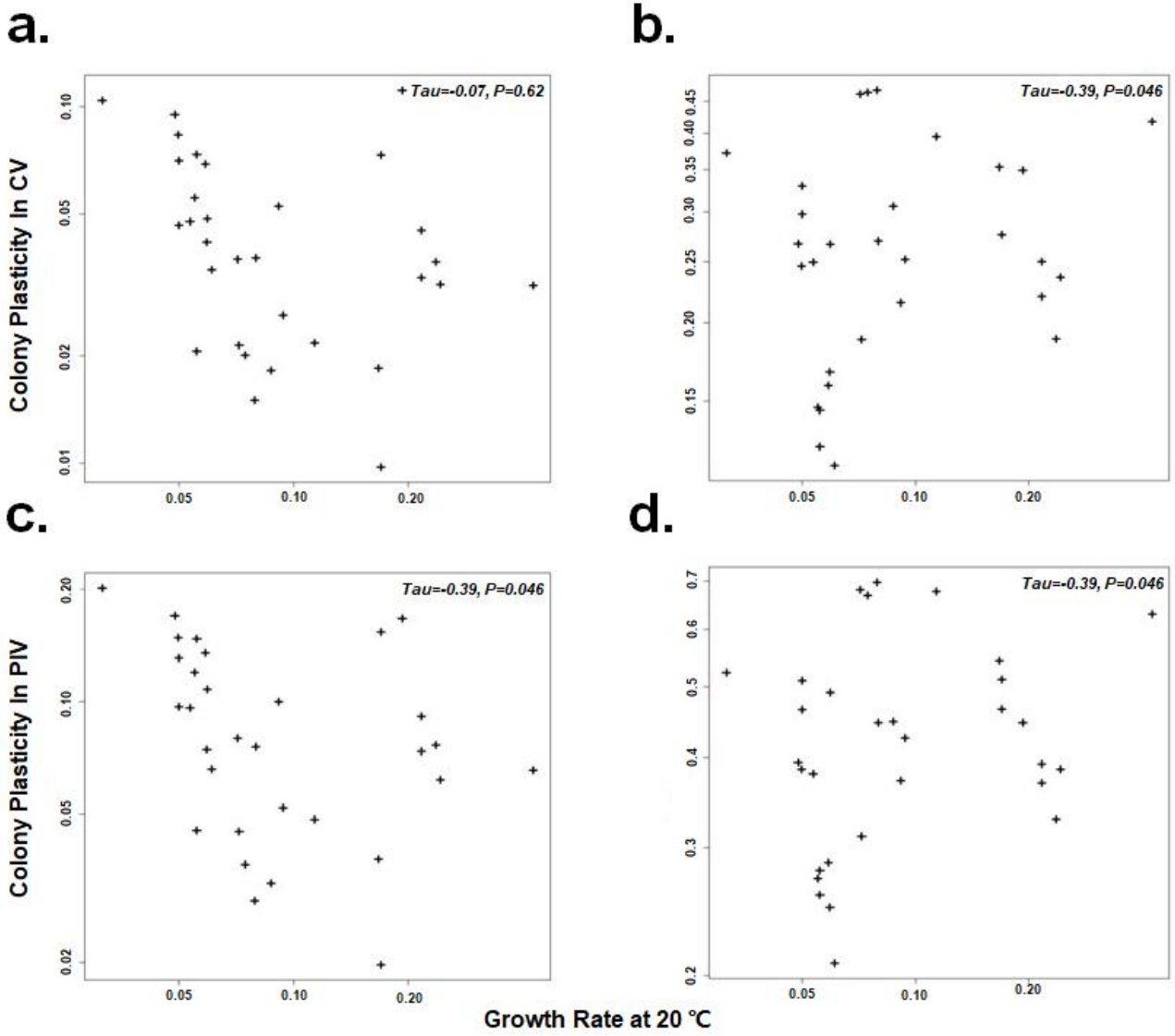


Fig. S4.4. Relationships between the colony size plasticity of CV and PIv (a, c) or the growth rate plasticity (b, d) and specific growth rate at 20°C.

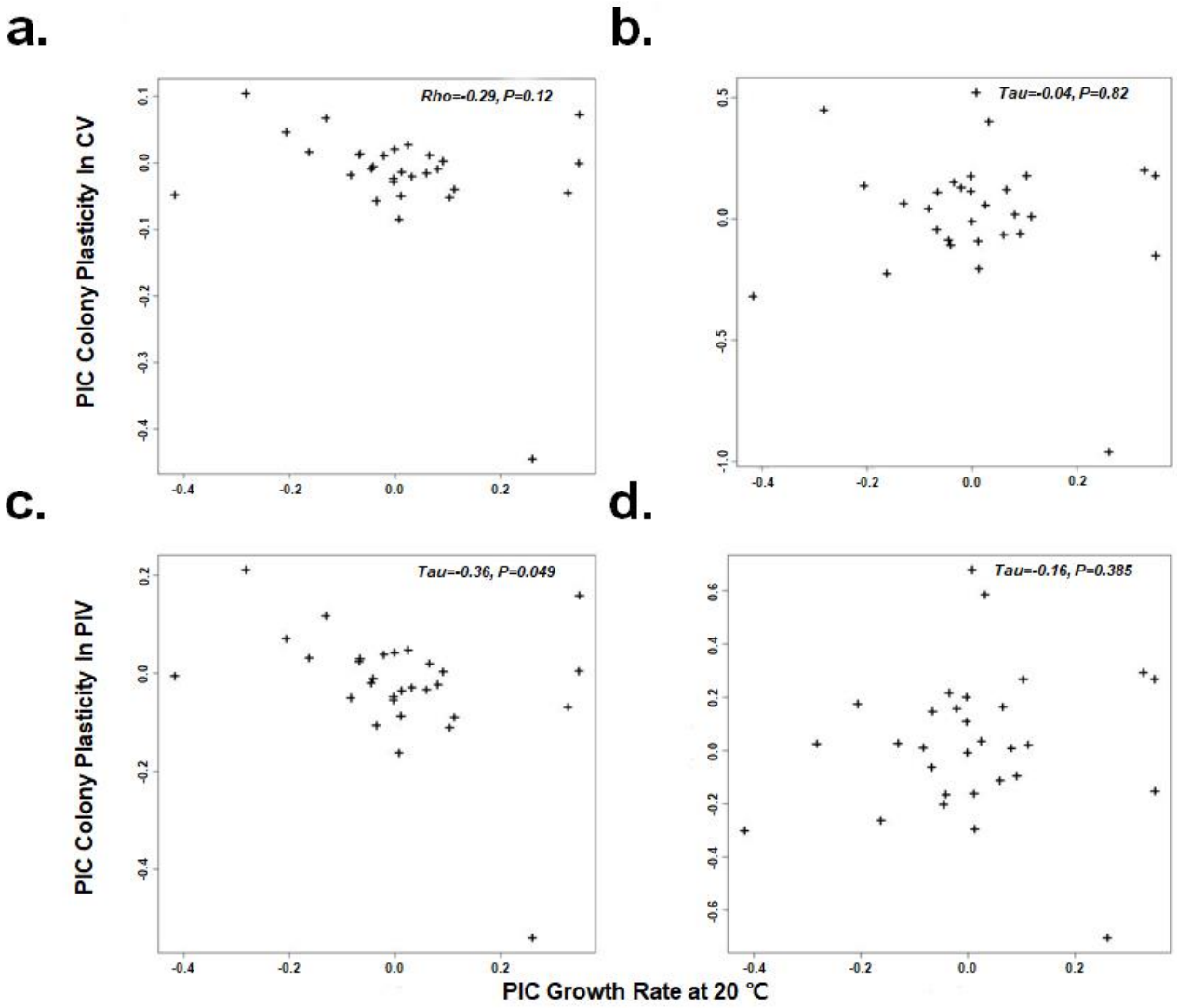


Fig. S4.5. Relationships between either phylogenetically-corrected colony size plasticity values of CV and PIv (a, c) or growth-rate (b, d) and specific growth rate at 20°C.

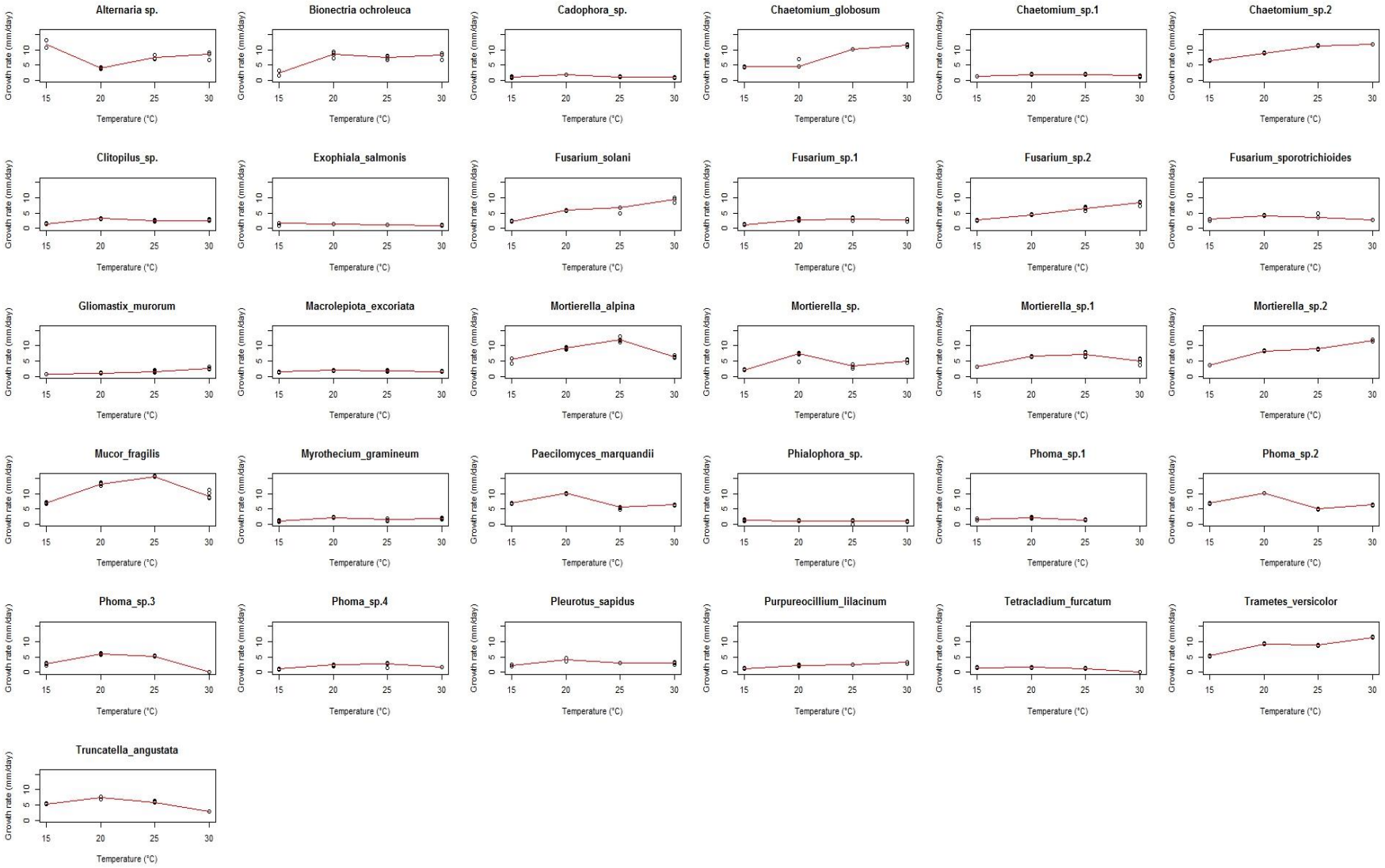


Fig. S4.6. Growth information for the 30 fungal isolates at all four temperatures.

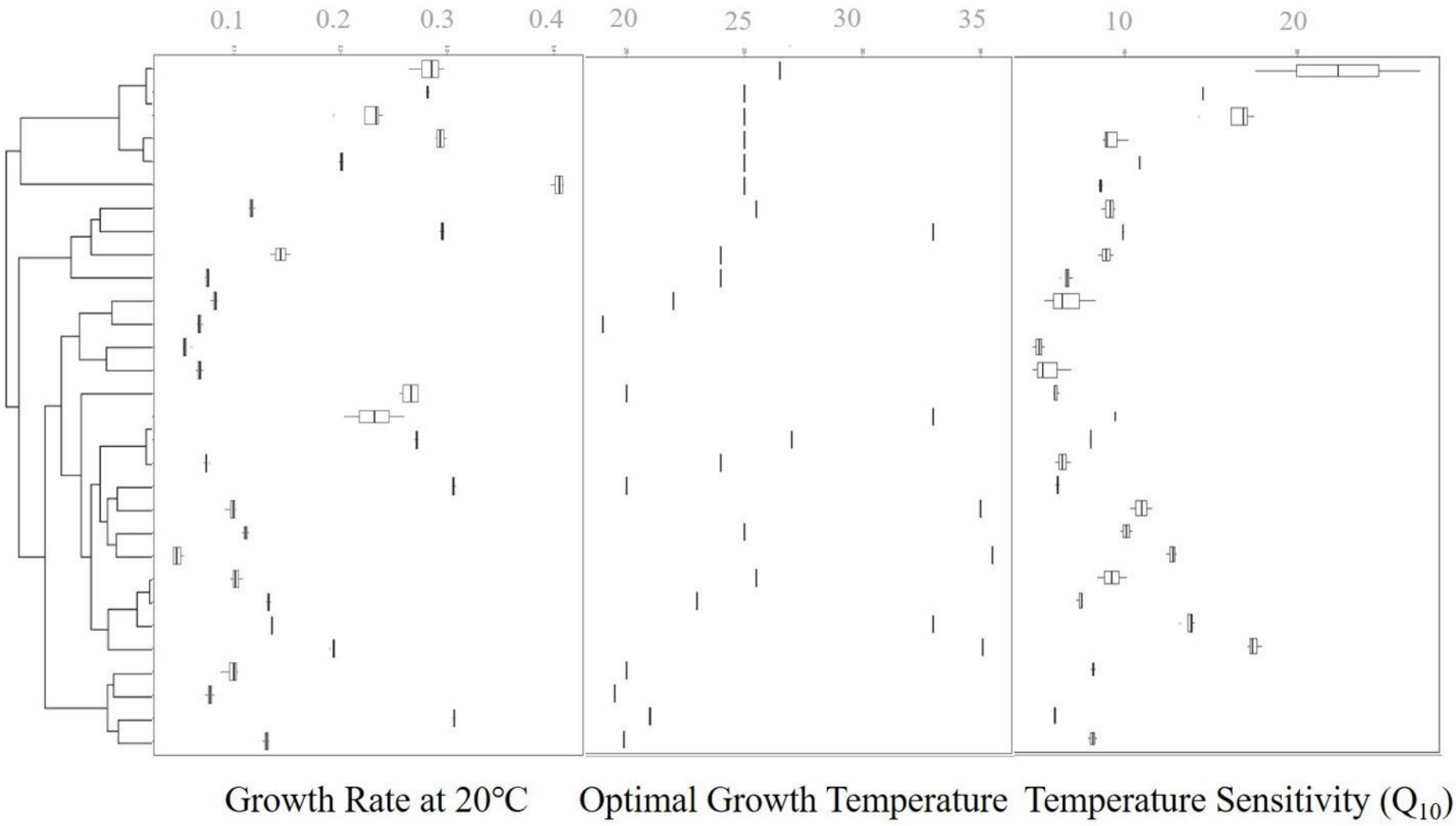


Fig. S4.7. fungal stress traits for the 30 fungal isolates at all four temperatures. For radial extension rate, I standardized for temperature by comparing performance at 20℃ which represents a temperature that most of the isolates should regularly experience at the grassland where they were isolated. I also assessed optimal growth Temperature and Temperature sensitivity. I detected a significant phylogenetic signal in temperature sensitivity.

Table S4.1. Estimates of the goodness of fit of the models (AIC values).

Species	Label	Mean value	AIC(minus)	SE
Phoma sp. 3	A	19.93999942	13.6817982	1.849314513
Myrothecium roridum	C11YPD10	24.41371377	3.600326293	4.032794107
Chaetomium sp. 2	C13	28.84581643	102.6277095	1.226622195
T. furcatum	C21BN1	18.98690848	18.0978389	2.161401699
Truncatella angustata	C23RB1	21.02179837	38.67196298	1.308020087
Clitopilus sp.	C28BNR2	24.75472297	9.000212682	2.965900876
Pleosporales sp.	C29RB4	24.01537323	7.06545922	2.679184186
Cadophora sp.	C31CZ3	21.61343343	1.34853929	3.062890602
Chaetomium sp.	C33YPD1	23.25068956	30.54061452	1.618203339
Mortierella like sp.	C34CZ2	23.70542619	12.66257464	3.616993205
Mortierella sp. 2	C40YPD1	25.89974669	3.689418443	3.647325489
Fusarium sp. 2	C41CZ2	32.89047059	50.51022685	3.23665751
C. globosum	DF10	32.68188548	24.60616986	5.214081381
Phoma sp.	DF13	21.75473395	21.4142293	1.503184174
Fusarium sp.	DF16	25.6025584	11.22604677	2.934539485
Macrolepiota	DF17	23.485699	25.69203003	2.366724637

excoriata				
Mortierella sp. 3	DF19	24.14818723	23.00174879	1.77738627
Trametes versicolor	DF24	32.5990751	22.31061869	7.60613964
Mortierella sp.	DF25	32.10547364	12.05048689	6.987914051
Fusarium solani	DF32	34.79557542	19.92439563	6.249968529
Phialophora sp.	DF35	NA	3.973623048	17.885976
Exophiala salmonis	DF36	NA	14.69068536	12.36799258
Gliomastix murorum	DF37	35.49906191	26.72786185	5.051255515
Phoma sp. 2	DF4	20.08684742	3.257579483	4.768349179
Pleurotus sapidus	DF42	23.30285997	0.391923857	3.124077133
Mucor fragilis	DF56	24.05895081	40.27798653	1.075244678
Purpureocillium lilacinum	DF58	34.06413679	23.12212131	6.689308042
Fusarium sporotrichioides	Disc	22.64261184	19.47902701	2.063997756
Paecilomyces marquandii	J	20.96126449	0.222363755	3.55528536
Mortierella alpina	M	23.95648661	30.91897013	1.386935739

Table S4.2. Correlation tests (without PICs).

Trait relationships	P values	z	tau
CV(growth)~ specific growth rate at 20°C	0.44	2.3293	-0.101
CV(colony)~ specific growth rate at 20°C	0.004	-3.384	-0.36
PIv(growth)~ specific growth rate at 20°C	0.54	2.6199	-0.08
PIv(colony)~ specific growth rate at 20°C	0.006	-3.1928	-0.35
Q10~ specific growth rate at 20°C	0.62	1.5619	-0.06878307
Q10~optimal temperature	0.0001	6.9237	0.59

Table S4.3. Correlation tests (with PICs).

Trait relationships	P values	S	rho
CV(growth)~ specific growth rate at 20°C	0.82	4300	-0.04
CV(colony)~ specific growth rate at 20°C	0.119	5804	0.2912125
PIv(growth)~ specific growth rate at 20°C	0.385	3758	0.16396
PIv(colony)~ specific growth rate at 20°C	0.049	6126	-0.3628476
Q10~ specific growth rate at 20°C	0.046	4550	-0.3888889
Q10~optimal temperature	0.006	1572	0.5201465

Appendix 5

R code used to produce the CSR distribution that are depicted in Fig. 5.1. In all cases we use normal distributions of 30 hypothetical species. To constrain the distributions to positive values we allow the standard deviation to only be a quarter of the mean. In the case of predictable distributions, we introduce noise equivalent to a tenth of the existing variance (or noise of a zero mean and a standard deviation equivalent to a tenth of the expected standard deviation in the data).

```
# Case of completely random distribution #

a<-round(runif(1, 1, 30),0)

b<-round(runif(1, 1, 30),0)

c<-round(runif(1, 1, 30),0)

Q10<-rnorm(30, a, a/4)

comp<-rnorm(30, b, b/4)

dist<-rnorm(30, c, c/4)


# Case of collinearity #

a<-round(runif(1, 1, 30),0)

b<-round(runif(1, 1, 30),0)

Q10<-rnorm(30, a, a/4)

comp<-rnorm(30, b, b/4)
```

```
dist<-Q10 + rnorm(30, 0, a/40)


# Case of a trade-off #
a<-round(runif(1, 1, 30),0)
b<-round(runif(1, 1, 30),0)
Q10<-rnorm(30, a, a/4)
comp<-rnorm(30, b, b/4)
dist<-max(Q10) - Q10 + rnorm(30, 0, a/40)
```

Table S5.1 Information about Order, GenBank and DSMZ accession numbers, Strain IDs, CSR classification and growth rate (Fungal colony radial growth rate, mm/d) of the 30 fungal strains used in this study.

Genus species	Order	Phylum	NCBI	DSMZ*	Growth rate[2]	CSR	Strain ID
Phoma sp. 3	Pleosporales	Ascomycota	KT582065	DSM 100327	0.08		A
Myrothecium roridum	Hypocreales	Ascomycota	KT582090	DSM 101519	0.11	S	C11
Chaetomium sp. 2	Sordariales	Ascomycota	KT582096	DSM 100400	0.27	C	C13
Tetracladium furcatum	Helotiales	Ascomycota	KT582084	DSM 100330	0.06		C21
Truncatella angustata	Xylariales	Ascomycota	KT582088	DSM 100284	0.27	C	C23
Phoma like sp.	Pleosporales	Ascomycota	KT582091	DSM 100401	0.1	C	C29
Cadophora sp.	Helotiales	Ascomycota	KT582085	DSM 100323	0.08	SR	C31
Chaetomium sp.	Sordariales	Ascomycota	KT582086	DSM 100326	0.74	S	C33
Fusarium sp.2	Hypocreales	Ascomycota	KT582097	DSM 100403	0.10	SR	DF16
Phoma sp. 2	Pleosporales	Ascomycota	KT582077	DSM 100404	0.31	C	DF04
Alternaria sp.	Pleosporales	Ascomycota	KT582078	DSM 100286	0.13	CR	DF09
Chaetomium globosum	Sordariales	Ascomycota	KT582079	DSM 100405	0.23	CR	DF10
Phoma sp.	Pleosporales	Ascomycota	KT582082	DSM 101518			
Fusarium sp.	Hypocreales	Ascomycota	KT582068	DSM 100287	0.14	C	C41
Fusarium solani	Hypocreales	Ascomycota	KT582073	DSM 100290	0.19	C	DF32
Phialophora sp.	Chaetothyriales	Ascomycota	KT582074	DSM 100328	0.05	SR	DF35
Exophiala salmonis	Chaetothyriales	Ascomycota	KT582075	DSM 100291	0.07	SR	DF36
Gliomastix murorum	Hypocreales	Ascomycota	KT582083	DSM 100292	0.047	S	DF37
Purpureocillium lilacinum	Hypocreales	Ascomycota	KT582081	DSM 100329	0.1	SR	DF58
Fusarium sporotrichioides	Hypocreales	Ascomycota	KT582087	DSM 100325	0.13	C	Disc
Paecilomyces marquandii	Hypocreales	Ascomycota	KT582066	DSM 100410	0.31	R	J
Fusarium oxysporum	Hypocreales	Ascomycota	KT582095	DSM 100409			

Mortierella like sp.	Mortierellales	Mucoromycotina	KT582092	DSM 100402	0.22	S	C34
Umbelopsis isabellina	Mucorales	Mucoromycotina	KT582093	DSM 100331			C35
Mortierella sp. 2	Mortierellales	Mucoromycotina	KT582094	DSM 100322	0.28	SR	C40
Mortierella sp. 3	Mortierellales	Mucoromycotina	KT582070	DSM 100289	0.2	R	DF19
Mortierella sp.	Mortierellales	Mucoromycotina	KT582072	DSM 100407	0.28	C	DF25
Mucor fragilis	Mucorales	Mucoromycotina	KT582076	DSM 100293	0.4	C	DF56
Mortierella alpina	Mortierellales	Mucoromycotina	KT582067	DSM 100285	0.29	C	M
Clitopilus sp.	Agaricales	Basidiomycota	KT582089	DSM 100324	0.12	S	C28
Macrolepiota excoriata	Agaricales	Basidiomycota	KT582069	DSM 100288	0.075	S	DF17
Trametes versicolor	Polyporales	Basidiomycota	KT582071	DSM 100406	0.3	CR	DF24
Pleurotus sapidus	Agaricales	Basidiomycota	KT582080	DSM 100408	0.14	C	DF42

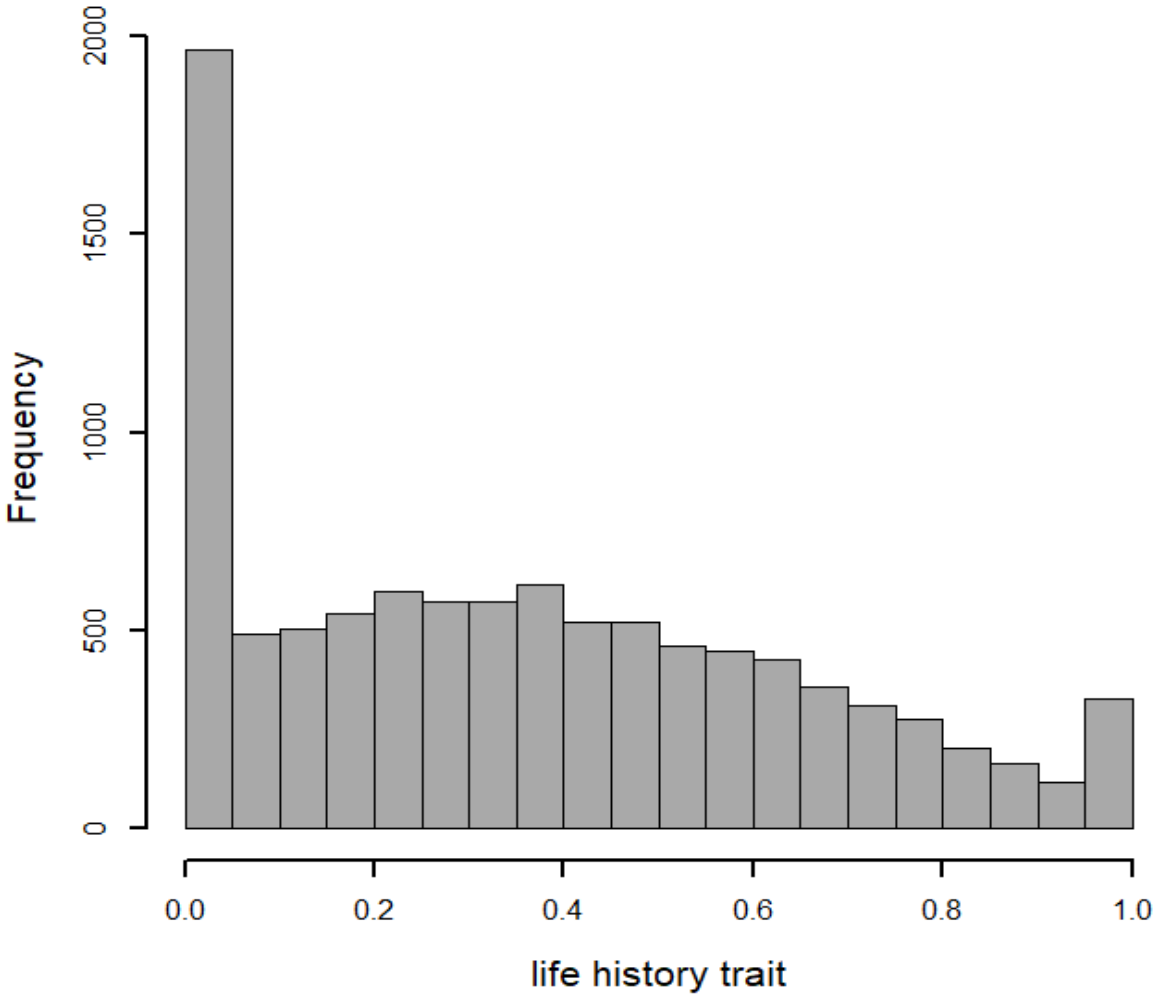


Fig. S5.1 Depiction of the CSR trait distribution after truncation of the values above one and below zero. Note that the distribution is tri-modal.

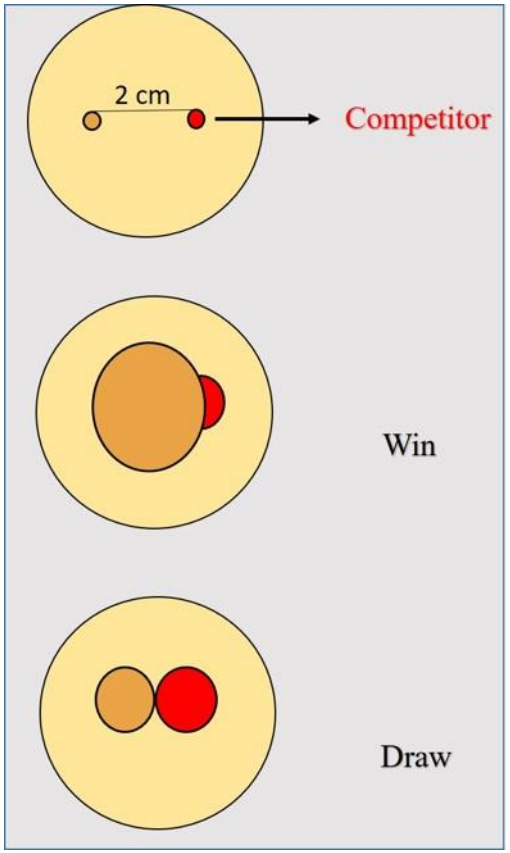


Fig. S5.2 Competitive experiment design for 30 fungal isolates. For each fungal strain, the competitive ability was calculated as percentage of the number of wins or draws the target species could accomplish against all possible 29 competitors.

Appendix 6

Table S6.1 Information about Genus, Phylum and DSMZ accession numbers, Strain IDs, Optimal Growth Temperature, CSR classification and growth rates (1 is the fungal biomass of 8-days incubation, mg and 2 is radial growth rate of fungal colony, mm/d)of the 30 fungal strains used in this study.

Genus species	**Phylum**	**DSMZ***	**Growth rate[1]**	**Growth rate[2]**	**T_{opt}**	**CSR**	**Strain ID**
Phoma sp. 3	Ascomycota	DSM 100327	14.5	0.08	19.94		A
Myrothecium roridum	Ascomycota	DSM 101519	33.77	0.11	24.41	S	C11
Chaetomium sp. 2	Ascomycota	DSM 100400	26.8	0.27	28.85	C	C13
Tetracladium furcatum	Ascomycota	DSM 100330	21.03	0.06	18.99		C21
Truncatella angustata	Ascomycota	DSM 100284	39.17	0.27	21.02	C	C23
Phoma like sp.	Ascomycota	DSM 100401	14.3	0.1	24.01	C	C29
Cadophora sp.	Ascomycota	DSM 100323	19.4	0.08	21.61	SR	C31
Chaetomium sp.	Ascomycota	DSM 100326	20.8	0.74	23.25	S	C33
Fusarium sp.2	Ascomycota	DSM 100403	15.3	0.10	25.6	SR	DF16
Phoma sp. 2	Ascomycota	DSM 100404	22.67	0.31	20.09	C	DF04
Alternaria sp.	Ascomycota	DSM 100286	44.43	0.13	NA	CR	DF09
Chaetomium globosum	Ascomycota	DSM 100405	16	0.23	32.68	CR	DF10
Phoma sp.	Ascomycota	DSM 101518					
Fusarium sp.	Ascomycota	DSM 100287	16.1	0.14	32.89	C	C41
Fusarium solani	Ascomycota	DSM 100290	20.77	0.19	34.8	C	DF32
Phialophora sp.	Ascomycota	DSM 100328	15.37	0.05	NA	SR	DF35
Exophiala salmonis	Ascomycota	DSM 100291	3.37	0.07	NA	SR	DF36

Gliomastix murorum	Ascomycota	DSM 100292	24.7	0.047	35.5	S	DF37
Purpureocillium lilacinum	Ascomycota	DSM 100329	4.13	0.1	34.06	SR	DF58
Fusarium sporotrichioides	Ascomycota	DSM 100325	22.67	0.13	22.64	C	Disc
Paecilomyces marquandii	Ascomycota	DSM 100410	9.8	0.31	20.96	R	J
Fusarium oxysporum	Ascomycota	DSM 100409					
Mortierella like sp.	Mucoromycotina	DSM 100402	19.37	0.22	23.7	S	C34
Umbelopsis isabellina	Mucoromycotina	DSM 100331	14.4				C35
Mortierella sp. 2	Mucoromycotina	DSM 100322	13.13	0.28	25.9	SR	C40
Mortierella sp. 3	Mucoromycotina	DSM 100289	22	0.2	24.15	R	DF19
Mortierella sp.	Mucoromycotina	DSM 100407	18.03	0.28	32.11	C	DF25
Mucor fragilis	Mucoromycotina	DSM 100293	22.47	0.4	24.06	C	DF56
Mortierella alpina	Mucoromycotina	DSM 100285	19.07	0.29	23.96	C	M
Clitopilus sp.	Basidiomycota	DSM 100324	24.8	0.12	24.75	S	C28
Macrolepiota excoriata	Basidiomycota	DSM 100288	19.6	0.075	23.49	S	DF17
Trametes versicolor	Basidiomycota	DSM 100406	12.97	0.3	32.6	CR	DF24
Pleurotus sapidus	Basidiomycota	DSM 100408	40.3	0.14	23.3	C	DF42

Publikationen

[1] Rillig MC, … **Wang DW** … et al., Myristate and the ecology of AM fungi: significance, opportunities, applications and challenges. *New Phytologist* (in press).

[2] **Wang DW**, Veresoglou SD, Lehmann A and Rillig MC. A trait-based framework to understand the life history of filamentous fungi. *In prep*.

[3] **Wang DW**, Veresoglou SD, Forstreuter M and Rillig MC. Fast-growing fungi have a higher sensitivity to a mechanical disturbance but tolerate better its reoccurrence. *In prep*.

[4] Veresoglou SD, **Wang DW**, Andrade-Linares DR Hempel S and Rillig MC. 2018. Fungal Decision to exploit or explore depends on growth rate. *Microbial ecology* 75 (2): 289-292.

[5] Huang Y, **Wang DW**, Cai JL and Zheng WS. 2011. Review of glomalin-related soil protein and its environmental function in the rhizosphere, *Chinese Journal of Plant Ecology* 35 (2): 232-236.

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[10] Huang Y, Cai JL, **Wang DW**, Su Y. 2009. Aquatic ecological function regionalization at watershed scale and its key issues. *Ecology and Environmental Sciences* 18 (5): 1995-2000.

[11] The module 8 of Regional Integrated Environmental Assessment (IEA), Global Environment, Outlook United Nations Environment Program (Translation).

Curriculum Vitae

For reasons of data protection, the Curriculum Vitae is not included in the online version.

Eidesstattliche Erklärung

Hiermit erkläre ich, die vorliegende Dissertation selbstständig und ohne Verwendung unerlaubter Hilfe angefertigt zu haben. Die aus fremden Quellen direkt oder indirekt übernommenen Inhalte sind als solche kenntlich gemacht. Die Dissertation wird erstmalig und nur an der Freie Universität Berlin eingereicht. Weiterhin erkläre ich, nicht bereits einen Doktortitel im Fach Biologie zu besitzen. Die dem Verfahren zu Grunde liegende Promotionsordnung ist mir bekannt.

Dongwei Wang

Berlin, den 31. Mai 2020