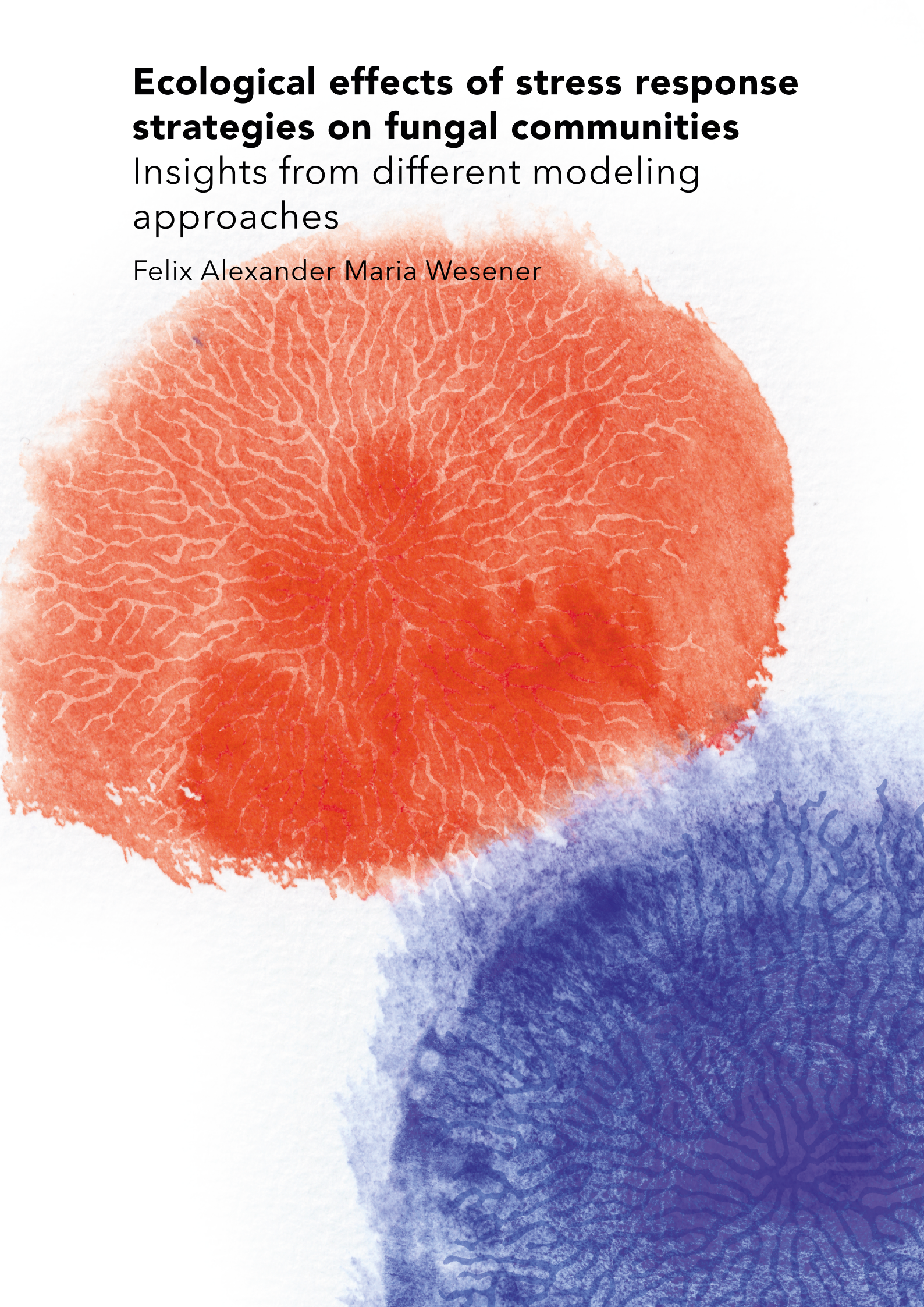
Ecological effects of stress response strategies on fungal communities

Insights from different modeling approaches

Felix Alexander Maria Wesener



Ecological effects of stress response strategies on fungal communities: insights from different modeling approaches

Inaugural-Dissertation
to obtain the academic degree
Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry, Pharmacy
of Freie Universität Berlin

by

Felix Alexander Maria Wesener

2021

This thesis was conducted between October 2016 and June 2021, under the supervision of Prof. Dr. Britta Tietjen (Freie Universität Berlin) and Prof. Dr. Matthias C. Rillig (Freie Universität Berlin). The work was carried out at Freie Universität Berlin.

1st Reviewer: Prof. Dr. Britta Tietjen

2nd Reviewer: Prof. Dr. Matthias Rillig

Date of defense: 21.09.2021

The Dissertation is based on the following publications and manuscripts:

1. Wesener, F.[1] and Tietjen, B.[1,2] (2019) **Primed to be strong, primed to be fast: modeling benefits of microbial stress responses.** *FEMS Microbiol Ecol* **95**: 1–9.

   DOI: https://doi.org/10.1093/femsec/fiz114

   Licence: CC BY (https://creativecommons.org/licenses/by/4.0/)

   Author contributions: FW and BT conceived the idea and design of the study. FW developed and evaluated the ODE models, FW and BT discussed and interpreted the results, FW wrote the first draft of the manuscript, BT and FW reviewed and revised the manuscript.

2. Wesener, F.[1], Szymczak[3], A., Rillig, M.C.[2,3], and Tietjen, B.[1,2] (2021) **Stress priming affects fungal competition - evidence from a combined experimental and modelling study.** *Environ Microbiol* **00**: 1–12.

   DOI: https://doi.org/10.1111/1462-2920.15418

   Licence: CC BY-NC (https://creativecommons.org/licenses/by-nc/4.0/)

   Author contributions: FW and BT conceived the idea and design of the study. FW developed the cellular automaton model, MR and AS designed the laboratory experiments, AS carried out the experiments, FW statistically analyzed the data obtained from the Rillig group, FW and BT discussed and interpreted the data, FW wrote the first draft of the manuscript, FW, BT and MR reviewed and revised the manuscript

3. Wesener, F.[1] A., Rillig, M.C.[2,3], and Tietjen, B.[1,2] (in preparation)

   Author contributions: FW and BT conceived the idea and design of the study. FW adapted the PDE model and calibrated it with data obtained from the Rillig group, FW and BT discussed and interpreted the results, FW wrote the first draft of the manuscript, all authors reviewed and revised the manuscript

[1]*Freie Universität Berlin, Institute of Biology, Theoretical Ecology, Königin-Luise-Str. 2/4, Gartenhaus, 14195 Berlin, Germany*

[2]*Berlin Brandenburg Institute of Advanced Biodiversity Research (BBIB), 14195 Berlin, Germany*

[3]*Freie Universität Berlin, Institute of Biology, Ecology of Plants, Altensteinstraße 6, 14195 Berlin, Germany*

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

First and foremost, I want to thank my supervisor Prof. Dr. Britta Tietjen for making this work possible by giving me the opportunity to become a member in her group. I want to thank you for your constant support and for always finding the time to listen to my questions and suggesting brilliant solutions. Thank you for all the great input, for your guidance through the depths of academia, and for entrusting me with responsible and stimulating tasks. I learned a lot from you.

I want to thank Prof. Dr. Matthias C. Rillig and Prof. Dr. Jens Rolff not only for accepting to supervise my work, but also for making time for fruitful discussion and giving me great input during my supervisory meetings.

Thanks to Aleksandra Szymczak, who shared all her data with me and even spent extra days in the laboratory to provide me with additional data that I had asked for.

Of course I would like to express my gratitude to the whole Tietjen group, who created an inspiring and fun environment dominated by interesting discussions and, most of all, delicious (vegan) cakes. Thanks especially to my fellow PhD students Selina Baldauf, Sebastian Fiedler and Katja Irob for laid-back and funny coffee breaks. I thank Selina for our motivating discussions about coding and R packages, and for supporting me whenever I was facing a problem. Thank you, Sebastian, for your honesty and for always taking time for an exchange when I needed one. Also, I want to thank former members of the group. Thanks to Yvonne Buchholz and Hanna Weise who shared a cozy office in “the Villa” with me – I will not forget those days. Thank you, Katharina Ziegler, who always found a way to brighten up everyone’s mood with silly jokes.

I am very thankful to Sandra Kries, who is incredibly organized and happily showed me a way through the jungle of paperwork. Thanks to Dr. Tobias Otte, who not only organized great retreats within the SFB973 but also readily assisted with any administrative problems.

Last but not least, I thank my family and friends for supporting me during the last years, for listening and giving me emotional support. Many thanks to Sophia and Sonny for helping me making the mycelial artwork. I want to thank Sören for encouraging me to pursue this project.

ZUSAMMENFASSUNG

Pilze leben in einer sich ständig ändernden Umwelt und haben viele Strategien entwickelt, um verschiedenste Stressfaktoren zu bewältigen. Induzierte Stressantworten erlauben es ihnen, eine Abwehr erst dann aufzubauen, wenn sie einem Stress ausgesetzt sind, während sie Kosten sparen, wenn eine Abwehr nicht benötigt wird. Viele Mikroben können ihre Abwehr auch mit einer Strategie verbessern, die als „Priming“ bekannt ist. Diese Strategie beschreibt die Vorbereitung auf einen bevorstehenden Stress nach dem Erleben eines Umweltreizes (genannt „Primingstimulus“), was zu einer effektiveren Stressabwehr führt. So zeigen zum Beispiel manche Bodenpilze eine höhere Fitness unter Hitzestress, wenn sie vorher eine milde Temperaturerhöhung erlebt haben. Bisher wurde Priming jedoch, so wie die meisten induzierten Stressantworten, hauptsächlich in Isolation untersucht. Wie die mikrobielle Artengemeinschaft den Nutzen von Priming verändert und umgekehrt, wie Priming die Dynamiken der Gemeinschaft verändert, ist unklar. In dieser Arbeit habe ich verschiedene Simulationsmodelle genutzt, um das Zusammenspiel zwischen der Artengemeinschaft und induzierten Hitzestressantworten zu untersuchen. Hierfür habe ich verschiedene Primingstrategien in mikrobiellen Populationen und Gemeinschaften untersucht (Kapitel II) und mich dann auf den Nutzen von Hitzepriming in Gemeinschaften von Bodenpilzen (Kapitel III) sowie den Effekt von induzierten Hitzestressantworten im Allgemeinen auf Pilzinteraktionen (Kapitel IV) konzentriert.

In Kapitel II habe ich ein gewöhnliches Differentialgleichungsmodell benutzt, um den Nutzen von verschiedenen Primingstrategien zu bewerten (dabei verglich ich Strategien, die eine frühere, eine schnellere oder eine stärkere Stressantwort erlauben) und um zu untersuchen, wie sich dieser Nutzen für verschiedene Primingkosten, Stressdauern und unter Konkurrenz verändert. Meine Resultate zeigten, dass sich abhängig von der Stressdauer verschiedene Strategien als am vorteilhaftesten erweisten. Ein früherer Aufbau einer Stressantwort erhöhte die Fitness und Überlebenswahrscheinlichkeit für kurze Stressdauern, während lange Stressdauern am effektivsten mit einer stärkeren Antwort gekontert wurden. In einer mikrobiellen Gemeinschaft erwies sich Priming im Allgemeinen und insbesondere die frühere Primingstrategie als effektiver als in Isolation.

ZUSAMMENFASSUNG

Um den Effekt von Priming im Wettkampf um Terrain zwischen Pilzen zu untersuchen, habe ich in Kapitel III einen zellulären Automaten entwickelt, der radiales Wachstum und räumliche Trennung von Pilzkolonien in einer Petrischale unter Hitzestress mit und ohne Primingstimulus simulieren kann. Die Resultate des Modells zeigten, dass der Nutzen von Priming im Vergleich zur Isolation von den Eigenschaften der Gemeinschaftsmiglieder, wie zum Beispiel deren Wachstumsrate, Hitzeresistenz, oder vom Alter der Gemeinschaft abhängte. Das zeigte, dass Priming unter den Bedingungen der Gemeinschaft in der Tat einen größeren Nutzen aufweisen konnte als in Isolation, aber dass dieser Nutzen sehr variabel war und den Wettkampf zwischen zwei Pilzen zugunsten des einen oder des anderen beeinflussen konnte.

Das Ziel in Kapitel IV war es, den Effekt einer induzierten Stressantwort auf die Konkurrenz und die spezifischen Interaktionstypen zwischen Pilzen zu verstehen. Hierfür nutze ich ein partielles Differentialgleichungsmodell, um die Prozesse, die der Konkurrenz und Hitzestressantwort von Pilzen zugrundeliegen, zu untersuchen. Hierbei handelt es sich um die Produktion von antimykotischen Inhibitoren, die das Wachstum von Konkurrenten stoppen, sowie von Hitzestressproteinen, die vor zellulären Hitzeschäden schützen. Die Einbeziehung von Dynamiken jenseits der phänomenologischen Ebene offenbarte, dass ein hitzeinduzierter Wachtsumsstopp in Pilzen den Zeitraum erhöhte, in dem sich Hemmstoffe ansammeln konnten. Daher konnte ein Hitzestress zu veränderter Inhibitorverteilung und veränderten Interaktionstypen führen, z.B. einer Veränderung von partiellem Überwachsen zu gegenseitiger Hemmung. Dieser Wachtsumsstopp konnte nicht nur Interationstypen verändern, sondern auch Konkurrenz zwischen Pilzen zugunsten von langsameren Spezies verschieben, die eine Abwehr gegen schnelle wachsende Konkurrenten aufbauen oder Terrain, dass sie nicht schnell bewachsen können, blockieren.

In dieser Arbeit habe ich mit verschiedenen Herangehensweisen das Zusammenspiel von mikrobieller Konkurrenz und induzierter Stressantwort untersucht. Meine Resultate offenbarten, dass Erkenntnisse über Spezies in Isolation nicht direkt auf den Gemeinschaftskontext übertragen werden können, da der Nutzen von Stressantworten stark von den Eigenschaften aller Gemeinschaftsmitglieder abhängt. Zudem zeigte ich, dass sich auch Aspekte der Artengemeinschaft, wie etwa die Entstehung und die Zusammensetzung, durch den Einfluss induzierter Abwehrmechanismen verändern können. Diese Arbeit stellt somit eine Verbindug zwischen den Effekten einer induzierten Stressantwort auf Arten- und Gemeinschaftsebene her. Induzierte Stressantworten werden hier als wichtiger Treiber von

Gemeinschaftsdynamiken identifiziert, was die Wichtigkeit von mikrobieller Stressökologie für ein besseres Verständnis von Gemeinschaftsfunktionen unterstreicht.

SUMMARY

Fungi live in highly fluctuating environments and have developed many different strategies to cope with various stressors. Induced stress responses allow them to mount defenses upon experiencing a stress, while saving costs when defense mechanisms are not needed. Many microbes can enhance their defenses using a strategy known as “priming”, which describes the preparation for an upcoming stress after experiencing an environmental cue (called “priming stimulus”) leading to a more effective stress defense. Some soil fungi, for example, perform better under heat stress if they have previously experienced a temperature stimulus. However, just like most induced defenses in microbes, priming has so far been mainly investigated in isolation. How the community context changes the benefit of priming, and vice versa, how priming can change community dynamics, remains unclear. In this thesis, I used several simulation modeling approaches to assess the interplay of the community and induced heat stress defenses. I investigated different priming strategies in microbial populations and communities (Chapter II), and then concentrated on the benefit of heat priming in communities of soil fungi (Chapter III). Finally, I analyzed the effect of induced heat stress defenses in general on fungal interactions (Chapter IV).

In Chapter II, I used an ordinary differential equation model to assess how the benefit of the individual priming strategies (namely a strategy granting an earlier, faster or stronger response) changed for varying priming costs and stress durations, as well as under competition. The results showed that the benefit of the different priming strategies for a population highly depended on the stress duration. An early build-up of a stress response enhanced performance and survivability for short stresses, whereas prolonged periods of heat were most efficiently countered with a stronger response, i.e. a higher response level. In the community, priming in general and the early primed stress response in particular were more beneficial than in isolation.

I developed a cellular automaton in Chapter III to investigate priming under fungal competition for space. The model simulated radial growth and spatial segregation of fungal colonies growing in a petri dish under heat stress with and without preceding priming cue. The model results showed that compared to isolation, the benefit of priming was dependent on the traits of the different community members, such as their growth rate, heat stress susceptibility or primeability, as well as the time point of community buildup. Therefore, priming could indeed be more beneficial to an organism in the community context, but its benefit was highly variable and could shift competition between two fungi towards either competitor.

In Chapter IV, I aimed to further understand the effect of induced heat stress defenses on fungal competition and fungal interactions. I used a partial differential equation model to account for the processes underlying fungal competition and heat stress defenses, namely the production of antifungal compounds that inhibit competitors, as well as the production of heat shock proteins that protect against cellular damage. Including these dynamics beyond the phenomenological level revealed that a heat stress-induced lag phase increased the time for species to accumulate antifungal compounds. A heat stress could therefore lead to altered inhibitor distributions and changed interaction types, e.g. a shift from partial overgrowth to inhibition. This stress-induced lag could not only change interaction types but could also affect competition in favor of slower growing species, which could mount defenses against faster competitors or block territory with inhibitors.

In this thesis, I used different modeling approaches to assess the interplay of microbial induced stress defenses and competition. My results revealed that findings from species in isolation cannot be directly transferred to the community context, because the benefit of induced defenses highly depends on the traits of community members. Moreover, I showed that also different aspects of the community, such as community assembly and composition, can change under the effect of induced defenses. With this work, I achieved to establish a link between the effect of stress responses at the species level and at the community level. The results identify induced stress defenses as an important driver of community dynamics, highlighting the importance of microbial stress ecology for a better understanding of community functioning.

CHAPTER I

General introduction

Soil fungi are a highly diverse group (Tedersoo *et al.*, 2014) fundamental to the terrestrial ecosystem, as they fulfill numerous important ecological functions, such as soil carbon cycling, mediation of plant nutrition or formation of soil structure (Boddy, 2001; Ritz and Young, 2004; Treseder and Lennon, 2015). It has been shown that soil functions such as decomposition depend on local-scale processes of soil fungal communities rather than regional phenomena like climate (Bradford *et al.*, 2014; Wagg *et al.*, 2014), and hence a knowledge of the dynamics of fungal communities is vital to understanding the response of soil ecosystem functions to environmental changes.

Because fungal colonies are sessile, soil fungal communities are spatially structured and competition for nutrients between community members also entails the competition for space. Therefore, fungal communities are shaped by various antagonistic interactions (Boddy, 2000; Kolesidis *et al.*, 2019). In addition, environmental fluctuations, such as periods of heat, change community dynamics (Allison and Martiny, 2008; Shade *et al.*, 2012; Hiscox, Clarkson, *et al.*, 2016), as they affect community members differently depending on their degree of resilience and their stress defense strategy. Both fungal stress defenses and competition are therefore an important factor to take into account when investigating soil fungal community dynamics.

Fungal stress defense strategies

Soil fungi are sessile organisms and lack, apart from the production of mobile spores, the ability to escape from disturbances in search for better conditions. They have consequently developed a multitude of defense strategies to fend off various environmental stressors.

Constitutive defenses are permanently active and include the expression of different chemicals, such as bitter compounds that protect against predators (Kusari *et al.*, 2012) or melanin that shields the organism from harsh environmental conditions like UV light (Eisenman and Casadevall, 2012).

A non-constitutive defense is the synthesis of compounds that is initiated only upon an encounter with a stressor. Here, I will refer to this mechanism as *induced direct defense*. Many induced direct defenses are expressed constitutively at a low level and upregulated when

encountering a stress (for example, the production of heat shock proteins in fungi; Mohsenzadeh *et al.*, 1998; Tereshina, 2005). Another type of induced direct defenses are wound-induced defenses, which usually describe the enzymatic conversion of already present, inactive precursors to active defense molecules (Spiteller, 2008). In general, induced direct defenses are activated on demand and are a cost-saving alternative to constitutive defenses, especially in fluctuating and unpredictable environments (Harvell, 1990).

Another induced but indirect defense mechanism that has also been observed in fungi is *priming* (Alvarez-Peral *et al.*, 2002; Berry and Gasch, 2008; Rangel *et al.*, 2008; Mitchell and Pilpel, 2011; Diana R. Andrade-Linares *et al.*, 2016; Guhr *et al.*, 2017), which describes the enhanced defense against a stressor due to a previously experienced environmental stimulus. This prior stimulus (called "priming stimulus", Hilker *et al.* (2016)) does not directly provide a defense, but prepares for a possible future stress event (called the "triggering stress"). Some soil fungi, for example, perform better after a heat pulse if they have previously experienced a mild temperature stimulus (Diana R. Andrade-Linares *et al.*, 2016). The preparation can result in a more effective response compared to an induced direct defense, but is mostly efficient for predictable stresses, as a priming stimulus that is not followed by a stress would render the invested preparation costs futile (Mitchell and Pilpel, 2011).

Both induced direct defenses and priming have been the focus of numerous molecular studies, many of which have aimed at isolating bioactive compounds for the development of new drugs (Spiteller, 2008; Keller, 2019), or at optimizing cultivation procedures (Guhr *et al.*, 2017). However, the effect at the ecological level, such as the benefit of induced defenses in the community context, is poorly understood. Because community dynamics are heavily influenced by the interactions between community members (Hiscox 2017b), one has to take into account these interactions and their interplay with stress defenses to understand the effect of induced defenses.

Fungal interactions

Because fungal competition for resources is realized by the competition for space, fungal exploitation competition (sequestering of resources and consequently reducing their availability for others) usually entails interference competition (inhibiting competitors and limiting their access to nutrients). This makes the distinction between these types of competition less straightforward than for other organisms (Boddy, 2000). Another way of classification of fungal competition is primary resource capture, which describes the colonization of unoccupied

territory, and secondary resource capture, which is the colonization of territory already occupied by another fungus. Primary resource capture is most effective for fast growing species that can cover a large territory with their colony (Lee and Magan, 2000; Prospero *et al.*, 2003). Secondary resource capture is often achieved via overgrowth, leading to competitive exclusion of the overgrown competitor (Maynard *et al.*, 2017). Other ways of competition for nutrients include the secretion of antifungal compounds such as mycotoxins (Knowles *et al.*, 2019; Pfliegler *et al.*, 2020), that inhibit competitor's at a distance, or morphological or chemical alterations of the own mycelium to stop the competitors growth on contact. These different interaction types are influencing competition between fungi and are hence an important factor determining community structure (Boddy, 2001).

Periods of heat have been shown to change competitive outcomes in fungal cultures (Heilmann-Clausen and Boddy, 2005; Toljander *et al.*, 2006; A'Bear *et al.*, 2012). Different fungi are affected by heat in distinct ways and exhibit a variety of stress defenses, and a possible shift in dominance due to a stress might play an important role in community buildup and coexistence between competitors in a community. Moreover, also the qualitative interaction types between competitors described above have been shown to change with temperature (Schoeman *et al.*, 1996; Hiscox, Clarkson, *et al.*, 2016). Why competition and interaction types in a fungal community change under heat stress, remains to be answered. Interestingly, apart from stress defenses influencing community dynamics, vice versa, the community context influences induced stress defenses as well: Rillig *et al.* (2015) used a simulation model to show that the benefit of priming is higher under competition than in isolation for a species that is negatively impacted by a competitor. In such simulation models, different environmental conditions and trait combinations of community members can be assessed systematically, which makes them a useful tool to disentangle the interactions between stress defenses and competition in a community.

Simulation models of fungi

An individual fungal hypha exhibits rather straightforward behavioral patterns that can be readily represented with simple mathematical models. The entirety of hyphae and hyphal tips, however, forms a complex network, which is the base for a dynamic and multifaceted fungal mycelium. This is a great example of emergent behavior and has, in my opinion, led to an interest of theoreticians in modeling the dynamics of single hyphae with ordinary differential equation (ODE) models. ODE models have been used to describe the extension and elasticity

of hyphal walls (Saunders and Trinci, 1979), and the branching of hyphae (Prosser and Trinci, 1979; Yang *et al.*, 1992), while more general models used ODE systems to describe the growth and shape of hyphal tips (Bartnicki-Garcia *et al.*, 1989; Goriely and Tabor, 2008).

Another common use of ODEs in fungal biology is the description of well-mixed or spatially discrete systems, for example yeast cultures in flowers (Letten *et al.*, 2018). ODEs are also a useful tool to illustrate cellular processes (Klipp, 2007) or investigate isolated dynamics or ecological concepts, as well as cost-benefit analyses (Mitchell and Pilpel, 2011).

Spatial heterogeneity is an important aspect to be considered in ecology in general (Levin *et al.*, 1997), and for fungal community dynamics in particular (Kiziridis *et al.*, 2020). Partial differential equation (PDE) models are a valuable tool to represent fungal growth, because they can explicitly extend ODEs with a continuous spatial dimension, and thus expand the idea of isolated hyphae by applying rules of hyphal extension and branching to the mycelium. Edelstein (1982) has developed some of the first PDE models, simulating different fungal growth strategies by including anastomosis and branching of hyphae, and later also nutrient uptake (Edelstein and Segel, 1983) in one or two spatial dimensions (Edelstein-Keshet and Ermentrout, 1989).

These models inspired several other PDE models of fungal mycelia, some of them extending the original models (Stacey *et al.*, 2001), and others reducing the detail of the hyphal growth and rather modeling the radial diffusion of biomass (Davidson *et al.*, 1996, 1997; Jabed *et al.*, 2018). Other studies used an intermediate approach by modeling radially extending biomass, but differentiating between tips and hyphal biomass (Boswell *et al.*, 2002, 2003; Falconer *et al.*, 2005, 2007), which also gave rise to models that could simulate interactions between two fungal colonies (Falconer *et al.*, 2008; Boswell, 2012).

A third class of models used to simulate fungi are cellular automata (CA), which simulate the state of individual cells on a two-dimensional grid (Halley *et al.*, 1994; Bown *et al.*, 1999; Gerlee and Anderson, 2007). By attributing simple behavioral rules to single cells, one can easily simulate radially extending biomass, which in many aspects resembles a fungal mycelium. Some studies also developed hybrid models, using mathematical rules derived from PDE models on cellular automata (Boswell *et al.*, 2007; Falconer *et al.*, 2011).

Despite the large variety of models simulating fungal colonies, I could not find a model that has implemented heat stress or induced defenses in fungi, let alone assessed the effect of stress defenses on fungal growth or fungal interactions.

Aim of this study

The aim of this study was to put induced heat stress defenses, such as priming, in an ecological perspective, as most studies focused on molecular mechanisms and priming in isolation only. How the community context changes the benefit of induced defenses and, vice versa, how an induced stress defense may change community composition, remains unclear. Emanating from the study by Rillig *et al.* (2015), I aimed to find out whether priming was beneficial for different stress durations, for populations exhibiting heterogeneous stress response strategies, and with and without competition. I furthermore focused on soil fungi with different degrees of primeability and stress resistance, and on spatially structured communities of fungi exhibiting typical fungal characteristics such as competition for space and different interaction types. To do so, I have developed the first simulation models that assess different priming strategies and the effect of heat stress defenses on fungal competition for space and on fungal interactions.

With my research, I want to promote an understanding of fungal priming in particular, and induced defenses in general, beyond the molecular level. This study assessed the costs and benefits of induced defenses of microbes under different conditions, and transferred the results from species in isolation to competition. This transfer provides an understanding of how processes at the species level are linked to the community level, therefore leading to a better understanding of microbial community dynamics under stress conditions.

Study outline

To investigate induced stress defenses in fungal communities, I used three different simulation model approaches and increased the complexity of the overall approach stepwise by adding more aspects that influence the interplay of induced defenses and competition (Figure 1).

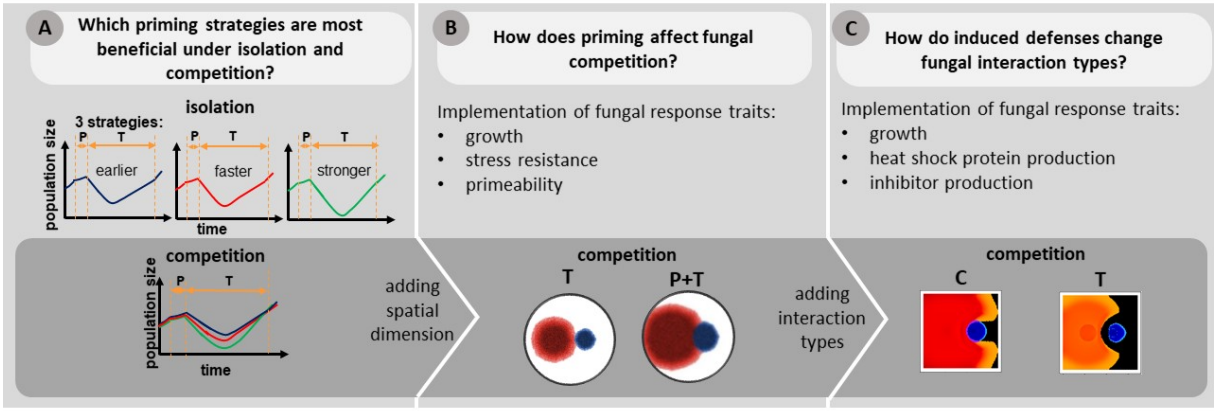


Figure 1 Study outline and overview of the three studies conducted within this thesis. **A** Chapter II: An ODE model was used to compare three different priming response strategies in isolation and under competition. **B** Chapter III: A cellular automaton model simulated the effect of priming on fungal competition for space. **C** Chapter IV: A PDE model was used to assess the effect of heat stress defense and inhibitor production on fungal combat & interaction types. C= control treatment, P = priming treatment, T = triggering stress treatment, P+T= priming and triggering treatment

I started the investigation in **Chapter II** by focusing on priming in microbes in general rather than fungi to accomplish a universal cost-benefit analysis (Figure 1A). I analyzed three different isolated strategies of priming (i.e. a strategy granting an earlier, a faster or a stronger induced defense) and their benefit for a microbial population. I developed an ODE model representing species with different primed response strategies but otherwise similar traits in a well-mixed community with constant and limited resources. I varied priming costs and stress duration to assess which strategy was most successful under different stress conditions. Moreover, I added stochastic population dynamics to assess the efficacy of different stress responses in reducing the extinction risk. I then transferred the results to the community context and compared them with the results from isolation. In order to focus only on the priming strategies, I did not include additional factors that might influence the cost-benefit ratio like the spatial structure of the community, interference competition or nutrient heterogeneity. With this approach, I could evaluate which factors influenced the benefit of a primed stress response strategy, and whether the results from the population level were robust towards the community level.

In **Chapter III**, I added several new components to the analysis: To take into account the sessile nature of soil fungal communities and to incorporate spatial segregation between fungal competitors, I used a cellular automaton simulating competition between two fungi in a Petri dish (Figure 1B). I calibrated and validated the model with empirical growth data of fungi. I then assessed trait diversity between community members by including different degrees of primeability, different susceptibilities to heat stress and different growth rates. These traits I varied to allow a systematic assessment of the effect of heat priming on the individual benefit of a fungus and on competition for different trait combinations. With this, I further investigated priming on the community level and determined how traits of community members influenced its benefit.

Finally, in **Chapter IV**, I further specified the spatial structure of a fungal community by including different interaction types depending on the production of inhibitory compounds into my research (Figure 1C). For reasons of computational performance, I did not continue my analysis with the cellular automaton but instead used a PDE model. In addition to the production of inhibitors, I explicitly implemented the processes underlying the induced stress response, i.e. the production and accumulation of heat shock proteins. Again, I assessed different trait combinations of two competing species by varying inhibitor production, heat shock protein production and growth. I therefore took into account the interplay of the stress response and inhibitor production at the process level and then investigated the combined effect of heat resistance and competition at the phenomenological level. This allowed me to assess which factors led to a change of fungal interaction types under heat stress.

References

A'Bear, A.D., Boddy, L., and Hefin Jones, T. (2012) Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola. Glob Chang Biol **18**: 1823–1832.

Allison, S.D. and Martiny, J.B.H. (2008) resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A **105** Suppl: 11512–9.

Alvarez-Peral, F.J., Zaragoza, O., Pedreno, Y., and Argüelles, J.-C. (2002) Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in Candida albicans. Microbiology **148**: 2599–2606.

Andrade-Linares, D.R., Veresoglou, S.D., and Rillig, M.C. (2016) Temperature priming and memory in soil filamentous fungi. Fungal Ecol **21**: 10–15.

Bartnicki-Garcia, S., Hergert, F., and Gierz, G. (1989) Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. Protoplasma **153**: 46–57.

Berry, D.B. and Gasch, A.P. (2008) Stress-activated Genomic Expression Changes Serve a Preparative Role for Impending Stress in Yeast. Mol Biol Cell **19**: 4580–4587.

Boddy, L. (2001) Fungal Community Ecology and Wood Decomposition Processes in Angiosperms : From Standing Tree to Complete Decay of Coarse Woody Debris. Ecol Bull 43–56.

Boddy, L. (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol Ecol **31**: 185–194.

Boswell, G.P. (2012) Modelling combat strategies in fungal mycelia. J Theor Biol **304**: 226–234.

Boswell, G.P., Jacobs, H., Davidson, F.A., Gadd, G.M., and Ritz, K. (2003) A positive numerical scheme for a mixed-type partial differential equation model for fungal growth. Appl Math Comput **138**: 321–340.

Boswell, G.P., Jacobs, H., Davidson, F.A., Gadd, G.M., and Ritz, K. (2002) Functional consequences of nutrient translocation in mycelial fungi. J Theor Biol **217**: 459–477.

Boswell, G.P., Jacobs, H., Ritz, K., Gadd, G.M., and Davidson, F.A. (2007) The development of fungal networks in complex environments. Bull Math Biol **69**: 605–634.

Bown, J.L., Sturrock, C.J., Samson, W.B., Staines, H.J., Palfreyman, J.W., White, N.A., et al. (1999) Evidence for emergent behaviour in the community-scale dynamics of a fungal microcosm. Proc R Soc B Biol Sci **266**: 1947–1952.

Bradford, M.A., Warren, R.J., Baldrian, P., Crowther, T.W., Maynard, D.S., Oldfield, E.E., et al. (2014) Climate fails to predict wood decomposition at regional scales. Nat Clim Chang **4**: 625–630.

Davidson, F.A., Sleeman, B.D., Rayner, A.D.M., Crawford, J.W., and Ritz, K. (1996) Context-dependent macroscopic patterns in growing and interacting mycelial networks. Proc R Soc London Ser B Biol Sci **263**: 873–880.

Davidson, F.A., Sleeman, B.D., Rayner, A.D.M., Crawford, J.W., and Ritz, K. (1997) Travelling waves and pattern formation in a model for fungal development. J Math Biol **35**: 589–608.

Edelstein-Keshet, L. and Ermentrout, B. (1989) Models for Branching Networks in Two Dimensions. SIAM J Appl Math **49**: 1136–1157.

Edelstein, L. (1982) The propagation of fungal colonies: a model for tissue growth. J Theor Biol.

Edelstein, L. and Segel, L.A. (1983) Growth and metabolism in mycelial fungi. J Theor Biol.

Eisenman, H.C. and Casadevall, A. (2012) Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol **93**: 931–940.

Falconer, R.E., Bown, J., White, N., and Crawford, J. (2011) Linking individual behaviour to community scale patterns in fungi. Fungal Ecol **4**: 76–82.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2007) Biomass recycling: a key to efficient foraging by fungal colonies. Oikos **116**: 1558–1568.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2005) Biomass recycling and the origin of phenotype in fungal mycelia. Proc R Soc B Biol Sci **272**: 1727–1734.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2008) Modelling interactions in fungi. J R Soc Interface **5**: 603–15.

Gerlee, P. and Anderson, A.R.A. (2007) Stability analysis of a hybrid cellular automaton model of cell colony growth. Phys Rev E - Stat Nonlinear, Soft Matter Phys **75**: 1–8.

Goriely, A. and Tabor, M. (2008) Mathematical modeling of hyphal tip growth. Fungal Biol Rev **22**: 77–83.

Guhr, A., Horn, M.A., and Weig, A.R. (2017) Vitamin B 2 (Riboflavin) increases drought tolerance of Agaricus bisporus. Mycologia **109**: 860–873.

Halley, J.M., Comins, H.N., Lawton, J.H., and Hassell, M.P. (1994) Competition, Succession and Pattern in Fungal Communities: Towards a Cellular Automaton Model. Oikos **70**: 435.

Harvell, C.D. (1990) The ecology and evolution of inducible defenses. Q Rev Biol **65**: 323–340.

Heilmann-Clausen, J. and Boddy, L. (2005) Inhibition and stimulation effects in communities of wood decay fungi: Exudates from colonized wood influence growth by other species. Microb Ecol **49**: 399–406.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., et al. (2016) Priming and memory of stress responses in organisms lacking a nervous system. Biol Rev **91**: 1118–1133.

Hiscox, J., Clarkson, G., Savoury, M., Powell, G., Savva, I., Lloyd, M., et al. (2016) Effects of pre-colonisation and temperature on interspecific fungal interactions in wood. Fungal Ecol **21**: 32–42.

Jabed, M., M. J. Trevelyan, P., and P. Boswell, G. (2018) A mathematical model of nutrient influence on fungal competition. J Theor Biol **438**: 9–20.

Keller, N.P. (2019) Fungal secondary metabolism: regulation, function and drug discovery. Nat Rev Microbiol **17**: 167–180.

Kiziridis, D.A., Fowler, M.S., and Yuan, C. (2020) Modelling fungal competition for space: Towards prediction of community dynamics. Discret Contin Dyn Syst - Ser B **25**: 4411–4426.

Klipp, E. (2007) Modelling dynamic processes in yeast. Yeast **24**: 943–959.

Knowles, S.L., Raja, H.A., Wright, A.J., Lee, A.M.L., Caesar, L.K., Cech, N.B., et al. (2019) Mapping the fungal battlefield: Using in situ chemistry and deletion mutants to monitor interspecific chemical interactions between fungi. Front Microbiol **10**: 1–15.

Kolesidis, D.A., Boddy, L., Eastwood, D.C., Yuan, C., and Fowler, M.S. (2019) Predicting fungal community dynamics driven by competition for space. Fungal Ecol **41**: 13–22.

Kusari, S., Hertweck, C., and Spiteller, M. (2012) Chemical ecology of endophytic fungi: Origins of secondary metabolites. Chem Biol **19**: 792–798.

Lee, H.B. and Magan, N. (2000) Impact of environment and interspecific interactions between spoilage fungi and Aspergillus ochraceus on growth and ochratoxin production in maize grain. Int J Food Microbiol **61**: 11–16.

Letten, A.D., Dhami, M.K., Ke, P., and Fukami, T. (2018) Species coexistence through simultaneous fluctuation-dependent mechanisms. Proc Natl Acad Sci U S A 1–6.

Levin, S.A., Grenfell, B., Hastings, A., and Perelson, A.S. (1997) Mathematical and computational challenges in population biology and ecosystems science. Science (80-) **275**: 334–343.

Maynard, D.S., Bradford, M.A., Lindner, D.L., van Diepen, L.T.A., Frey, S.D., Glaeser, J.A., and Crowther, T.W. (2017) Diversity begets diversity in competition for space. Nat Ecol Evol **1**: 156.

Mitchell, A. and Pilpel, Y. (2011) A mathematical model for adaptive prediction of environmental changes by microorganisms. Proc Natl Acad Sci U S A **108**: 7271–6.

Mohsenzadeh, S., Saupe-Thies, W., Steier, G., Schroeder, T., Fracella, F., Ruoff, P., and Rensing, L. (1998) Temperature adaptation of house keeping and heat shock gene expression in Neurospora crassa. Fungal Genet Biol **25**: 31–43.

Pfliegler, W.P., Pócsi, I., Győri, Z., and Pusztahelyi, T. (2020) The Aspergilli and Their Mycotoxins: Metabolic Interactions With Plants and the Soil Biota. Front Microbiol **10**:.

Prospero, S., Holdenrieder, O., and Rigling, D. (2003) Primary resource capture in two sympatric Armillaria species in managed Norway spruce forests. Mycol Res **107**: 329–338.

Prosser, J.I. and Trinci, A.P.J. (1979) A Model for Hyphal Growth and Branching. J Gen Microbiol **111**: 153–164.

Rangel, D.E.N., Anderson, A.J., and Roberts, D.W. (2008) Evaluating physical and nutritional stress during mycelial growth as inducers of tolerance to heat and UV-B radiation in Metarhizium anisopliae conidia. Mycol Res **112**: 1362–1372.

Rillig, M.C., Rolff, J., Tietjen, B., Wehner, J., and Andrade-Linares, D.R. (2015) Community priming--effects of sequential stressors on microbial assemblages. FEMS Microbiol Ecol **91**: 1–7.

Ritz, K. and Young, I.M. (2004) Interactions between soil structure and fungi. Mycologist **18**: 52–59.

Saunders, P.T. and Trinci, A.P.J. (1979) Determination of Tip Shape in Fungal Hyphae. J Gen Microbiol **110**: 469–473.

Schoeman, M.W., Webber, J.F., and Dickinson, D.J. (1996) The effect of diffusible metabolites of Trichoderma harzianum on in vitro interactions between basidiomycete isolates at two different temperature regimes. Mycol Res **100**: 1454–1458.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., et al. (2012) Fundamentals of Microbial Community Resistance and Resilience. Front Microbiol **3**: 417.

Spiteller, P. (2008) Chemical Defence Strategies of Higher Fungi. Chem - A Eur J **14**: 9100–9110.

Stacey, A.J., Truscott, J.E., and Gilligan, C.A. (2001) Soil-borne fungal pathogens: Scaling-up from hyphal to colony behaviour and the probability of disease transmission. New Phytol 150: 169–177.

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N.S., Wijesundera, R., et al. (2014) Global diversity and geography of soil fungi. Science (80-) **346**: 1256688.

Tereshina, V.M. (2005) Thermotolerance in fungi: The role of heat shock proteins and trehalose. Microbiology **74**: 247–257.

Toljander, Y.K., Lindahl, B.D., Holmer, L., and Högberg, N.O.S. (2006) Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. Oecologia **148**: 625–631.

Treseder, K.K. and Lennon, J.T. (2015) Fungal Traits That Drive Ecosystem Dynamics on Land. Microbiol Mol Biol Rev **79**: 243–262.

Wagg, C., Bender, S.F., Widmer, F., and Van Der Heijden, M.G.A. (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci U S A **111**: 5266–5270.

Yang, H., King, R., Reichl, U., and Gilles, E.D. (1992) Mathematical model for apical growth, and branching of microorganisms. Biotechnol Bioeng **39**: 49–58.

CHAPTER II

Primed to be strong, primed to be fast: modeling benefits of microbial stress responses

Abstract

Organisms are prone to different stressors and have evolved various defense mechanisms. One such defense mechanism is priming, where a mild preceding stress prepares the organism towards an improved stress response. This improved response can strongly vary, and primed organisms have been found to respond with one of three response strategies: a shorter delay to stress, a faster buildup of their response, or a more intense response. However, a universal comparative assessment, which response is superior under a given environmental setting, is missing.

We investigate the benefits of the three improved responses for microorganisms with an ordinary differential equation model, simulating the impact of an external stress on a microbial population that is either naïve or primed. We systematically assess the resulting population performance for different costs associated with priming and stress conditions. Our results show that independent of stress type and priming costs, the stronger primed response is most beneficial for longer stress phases, while the faster and earlier responses increase population performance and survival probability under short stresses. Competition increases priming benefits and promotes the early stress response. This dependence on the ecological context highlights the importance of including primed response strategies into microbial stress ecology.

Introduction

Microorganisms are subject to stressors of different nature and intensity, and have thus developed various response mechanisms to counteract these stressors. In contrast to constitutive stress defenses, which are always expressed, an induced direct defense is often activated directly upon the encounter of an initial stress. This initial, often milder, stress does not necessarily immediately initiate an active stress defense by, for example, inducing the production of defense molecules. Instead, it can lead to a more efficient defense only upon the occurrence of stronger environmental stress, which has been termed 'priming' (Hilker *et al.*, 2016). Alternative terms for priming include 'stress hardening' (Lou and Yousef, 1997), 'acquired stress response' (Berry and Gasch, 2008; Guan *et al.*, 2012) or 'cross protection', if a mild stress confers enhanced resistance towards a stressor of a different nature (Rangel *et al.*, 2008; Dhar *et al.*, 2013; Hilker *et al.*, 2016). Priming has been found in plants (Baldwin and Schmelz, 1996; Newman *et al.*, 2002; Hulten *et al.*, 2006; Pozo *et al.*, 2009), in the mammalian immune system (Gifford and Lohmann-Matthes, 1987; Hayes and Zoon, 1993; Hayes *et al.*, 1995), and in different groups of microbes like bacteria (Koutsoumanis and Sofos, 2004; Mitchell *et al.*, 2009; Cebrián *et al.*, 2010; Hernández *et al.*, 2012; Diana R Andrade-Linares *et al.*, 2016), fungi (Alvarez-Peral *et al.*, 2002; Berry and Gasch, 2008; Rangel *et al.*, 2008; Mitchell *et al.*, 2009; Diana R. Andrade-Linares *et al.*, 2016), and Archaea (Trent, 1996).

Priming can be a cost-saving strategy in fluctuating, but predictive environments, since the environmental cue (also called priming stimulus) does not require the full commitment of a direct induced defense, but instead improves the defense against a possible future stress. When assessing the effectiveness of an induced stress defense, the cost-benefit-ratio of this response can be used as measure of success, since a certain behavior or physiological process can only be evolutionary persistent if it confers benefits that are higher than the invested costs. The costs of priming have been studied in plants (Hulten *et al.*, 2006) and animals (Krebs and Loeschcke, 1994), however, studies on priming costs in microbes are missing and the molecular basis of priming is poorly understood. In yeast, the genes activated after a mild oxidative stress only partly overlap the genes of direct defense (Kelley and Ideker, 2009), indicating distinct mechanisms. However, the mechanisms of priming vary greatly not only between taxa , but also within a single organism: For example, priming for H_2O_2 tolerance in yeast involves different sets of genes depending on the nature of the priming stimulus (Berry *et al.*, 2011).

Since priming is not the result of one universal molecular or physiological process, also the ecologically observed response of an organism to an impending stress can strongly differ. In the ecological context, it is therefore essential to evaluate the impact of different priming responses on the performance of an organism or population. Four different improved stress responses of a primed organism were described, namely a stronger, a faster, an earlier and a more sensitive response than a naïve organism (Conrath *et al.* 2006, Hilker *et al.* 2016). In the following, we will focus on faster, earlier and stronger primed responses and will briefly introduce the three responses jointly with potential underlying mechanisms at the molecular level.

An *earlier* response would exhibit the same kinetics as a naïve stress response with an induced direct defense, but with a shorter lag phase until the stress response has built up. Therefore, the final defense level will be reached earlier than in the naïve state. Possible underlying molecular mechanisms of the earlier primed response could, for example, be based on the accumulation of transcription factors due to a previous priming stimulus leading to an earlier start of transcription and translation of response proteins after a triggering stress. Primed yeast cells, for example, have been shown react earlier to sudden exposure to fungicidal stress due to predictive translation and transcription (Berry and Gasch, 2008). The dynamics of the *faster* stress response are characterized by a similar lag phase as the naïve response but a steeper slope in the stress defense buildup. This response could be caused by hyperactivation and a faster signaling cascade, leading to a faster build-up of the stress defense. For example, cells of *Saccharomyces cerevisiae* that were repeatedly exposed to NaCl exhibited faster gene-expression if exposed to H_2O_2 afterwards (Guan *et al.*, 2012). A *stronger* stress response initially resembles the naïve response (exhibiting the same lag phase and slope) but eventually reaches a higher final response level than the earlier, the faster and the naïve response. Here, too, hyperactivation and an enhanced gene expression could be responsible and lead to a higher response amplitude. *Bacillus subtilis*, for example, showed a significantly increased survival during heat stress of 52 °C when primed with a 48 °C heat shock beforehand, caused by raised levels of the Spx transcription factor (Runde *et al.*, 2014).

As we observe all three proposed primed response types in nature, the question arises, which response could be most beneficial for an organism. In a systematic analysis of costs and benefits of different priming response strategies Douma *et al.* (2017) addressed this question for a single plant organism suffering from herbivory. However, a universal analysis for microbial

populations and communities is still missing. Here, we examine the benefits of the three priming responses in a highly generalized ordinary differential equation (ODE) model that describes the performance of primed and unprimed microbial populations under stress. Since we expect the benefits of the priming strategies to be highly context dependent, we use the ODE model to quantify the effect of the priming response strategies on population performance under different stress durations and priming costs for species in isolation and in a community, as well as the efficiency of these strategies in preventing extinction of a population.

Methods

We used a descriptive ordinary differential equation model simulating the population size of an arbitrary microbial species to investigate which of three potential primed stress responses (faster, earlier or stronger) is most beneficial compared to a naïve stress response. We assessed different stress conditions and priming costs to determine how these factors affect the different primed response types.

Model Description

The model describes the dynamics of a microbial population (measured in terms of e.g. biomass or colony forming units) growing in isolation and later in competition with constant, but limited resources. The population experiences a triggering stress of a given duration TD, beginning at a certain point in time t_{TS}. We chose stress dynamics to occur at the same time scale as population dynamics, which can range from minutes to days or weeks, and thus refer to a general time unit t. We used the relative difference in size between primed and naïve populations as direct measure of population performance. Thus, we could assess the effectiveness of primeability under different conditions by comparing the size of a primed population with the size of a naïve population after the triggering stress event has ended at $t = t_{TE}$.

The basic model describes a simple exponential function of population size (S) at time (t). The growth model was extended by functions describing the growth rate ($g(P,t)$) dependent on impacts of priming (P) events at a given point of time and an additional mortality rate ($m(T,t)$):

$$\frac{dS}{dt} = g(P,t) \cdot S(t) - m(T,t) \cdot S(t) \quad (1)$$

The costs of priming appear as reduced growth rate during the priming phase for a primeable population, while the naïve population does not exhibit a reduction in growth during this phase. This cost factor reflects additional transcription and translation which are induced by priming, and are expected to exert a constant cost rate (Stoebel *et al.*, 2008) leading to costs proportional to growth (Mitchell and Pilpel, 2011). The subsequent triggering stress is applied directly after the priming phase. Here, we defined adversary effects on microbial populations as disturbance that leads to partial or total destruction of biomass and therefore implemented the triggering stress as additional mortality rate $m(T,t)$ while the stress is lasting (for duration TD). However, triggering does not impact the intrinsic growth rate.

$$g(P,t) = \begin{cases} g_I, & t < t_P \\ g_I \cdot (1 - c_P), & t_P \leq t < t_{TS} \\ g_I, & t \geq t_{TS} \end{cases} \quad (2)$$

A primeable species exhibits a growth rate reduced by c_p during the priming phase. Since we assumed the priming stress to be mild, the growth of a non-primeable species remains at its initial level during this period, i.e. $c_p = 0$. At the beginning of the triggering stress at time t_{TS}, the priming costs c_p are set back to zero, because we assumed the stress defense costs to be equal between naïve and primed response.

$$m(T,t) = \begin{cases} 0, & t < t_{TS} \\ m_I, & t_{TS} \leq t < t_L \\ (-s_R \cdot (t - t_L) + 1) \cdot m_I, & t_L \leq t < t_R \\ m_R, & t_R \leq t \leq t_{TE} \end{cases} \quad (3)$$

A triggering stress instantly leads to an initial high mortality m_I for the duration of a lag phase L, since the organism needs to induce a stress response to counteract the stressor. At time point t_L, the stress response starts building up and linearly reduces the mortality rate with response speed s_R until the maximum response level with mortality m_R is reached, which is not further

improved as long as the stress lasts (until t_{TE}). A primed organism was assumed to exhibit an improved response to the triggering stress, which is, as proposed by Hilker *et al.* (2016), either realized by an earlier, a faster or a stronger stress response. We did not evaluate the additionally proposed more sensitive response, as sensitivity cannot be quantitatively investigated in a similar manner as the other response types without modulating the stress intensity. When the stress vanishes (after stress duration TD at time point t_{TE}), we used population size $S(t = t_{TE})$ as estimation of population fitness and calculate the relative benefits of priming as the relative difference to the population size of a naïve population. Note that in our model, mortality is never lower than the primed growth rate $m_I > g_I \cdot (1 - c_p)$, which always results in a negative effect of m_I.

Baseline scenario for primed responses

The three primed response types were realized as follows: an *earlier* response leads to a shorter lag duration L and thus a lower value of t_L in comparison to a naïve organism (Fig. 2a). The *faster* stress response is characterized by a higher absolute value of the slope s_R, which causes a steeper slope in the decrease of the mortality rate (Fig. 2b) compared to the response of the naïve organism. A *stronger* stress response modulates the final response value m_R (Fig. 2c) that can be reached while the stress lasts.

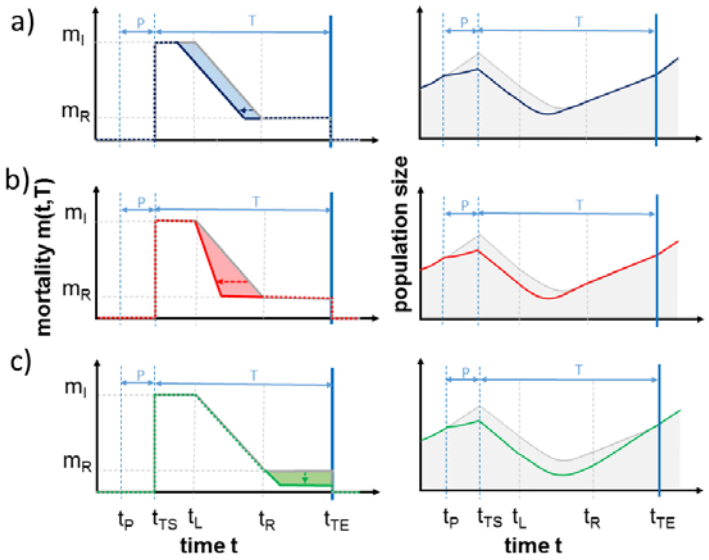


Figure 2 Potential responses of primed and naïve species towards stress impacts on mortality and the resulting population size. Left panels: Mortality of primed and naïve species, having **a)** a stronger, **b)** an earlier and **c)** a faster response. Right panels: the respective population dynamics. In all panels, the naïve stress response is represented by the grey line, and the colored line represents the primed response. Priming costs are not illustrated here, as they do not affect mortality. Abbreviations: $\boldsymbol{P}$= priming, $\boldsymbol{T}$=triggering, $\boldsymbol{m_I}$= initial mortality, $\boldsymbol{m_R}$= final mortality level of the stress response, $\boldsymbol{t_P}$= beginning of the priming phase, $\boldsymbol{t_{TS}}$= beginning of the triggering stress, $\boldsymbol{t_L}$= end of the response lag phase, $\boldsymbol{t_R}$= time point when $\boldsymbol{m_R}$ has been reached, $\boldsymbol{t_{TE}}$= end of triggering stress.

To allow for direct comparison between the three response types, we defined a baseline stress scenario. In this scenario, the specific primed response parameters L_p, s_{Rp} or m_{Rp}, respectively, were adjusted in a way that the benefits of priming exactly compensate its costs. Thus, for each response we chose the value that caused the naïve and the primed population to be of equal size at a specific point in time t_B and for given costs c_{PB}. We used this baseline scenario as starting point for further analyses on the impacts of stress characteristics and the costs associated with preparation for priming. Since organisms might exhibit enhanced stress responses that are a combination of the earlier, faster or stronger responses, we additionally performed an analysis of stress responses that combine these strategies (see Appendix A, section 3).

First, we analytically solved our model to assess the effect of stress duration TD and priming costs c_P on the altered performance of the primed population, given as relative change in population size compared to the naïve population (for calculations, see Appendix A, section 1). Based on these results we evaluated whether a naïve response, or an earlier, stronger or faster response, respectively, is most beneficial for a population at the end of the applied triggering stress (t_{TE}).

To investigate the effect of stress predictability on the benefit of primed response strategies, we analytically assessed different probabilities for a priming cue to correctly predict the occurrence of a triggering stress. Our analysis followed the approach by Mitchell and Pilpel (2011) and is given in Appendix A (section 4).

Table 1 Parameter description and values for a non-primed population. * = the units are system dependent, e.g. biomass (mg) or colony forming unit

Parameter	Description	Default value	Unit
g_I	initial growth rate	0.0488	1/t
K	environmental capacity	10000	*
c_P	priming costs	varied	-
s_R	response speed	0.03	1/t
m_I	initial mortality induced by the triggering stress	0.0976	1/t
m_R	minimal mortality reached by the stress response	$0.25 \cdot m_I$	1/t
t_R	Time when m_R is reached	60	t
L	lag phase duration	5	t
t_L	end of lag phase	35	t
t_P	beginning of the priming phase	30	t
t_{TS}	beginning of the triggering stress	50	t
TD	stress duration	varied	t
t_{TE}	end of the triggering stress and time point of comparison between strategies	varied	t
c_{PB}	priming costs of the baseline scenario, when all three response strategies grant a benefit equal to the naïve response	0.3	-
t_B	time point of the baseline scenario, when all three response strategies grant a benefit equal to the naïve response	75	t

Stochasticity in population performance

If the modelled population is of small size, e.g. after encountering a strong stress, additional stochastic fluctuations might drive the population towards extinction. To determine the likelihood of such stochastic extinction events, we formulated the deterministic ODE model as a stochastic model using the Gillespie stochastic simulation algorithm (SSA, Pineda-Krch 2010). This requires a numerical solution of the model. For parametrization, we assumed a default growth rate of $g = 0.0488t^{-1}$, which corresponds to an approximate growth of 5% per time step. The default mortality is $m_I = 0.0976t^{-1}$, which is double the growth rate and thus leads to a decrease of 5% per time unit (satisfying $g_I - m_I = -g_I$) . The default response lag L is set to $5t$, and the time point t_R, at which the final stress response level is reached, is set to $25t + L$ to allow for stress durations shorter or longer than the buildup of the stress response. The response speed s_R is set accordingly, i.e. to fulfill $t_R = L + \frac{m_I - m_R}{m_I s_R} = 30t$, assuming a final response level of $m_R = 0.25 \cdot m_I$. A parameter overview is given in Table 1, and a more detailed investigation of the sensitivity of the ODE model results towards this choice is part of Appendix A (section 1). For the baseline scenario, we applied an intermediate stress duration of $TD_B = 75t$ and priming costs of $c_{PB} = 0.3$. To our knowledge, there are no studies quantifying the costs a priming stimulus exerts on microbes, so we applied moderate costs based on the costs found by Hulten *et al.* (2006), who observed a growth reduction of around 27% in *Arabidopsis* plants primed with β-Aminobutyric acid. These costs are in the same magnitude as assumed in a population model of microbes experiencing stress of Mitchell & Pilpel (2011).

To implement the SSA, we followed the original method ("direct method") of Gillespie (1977) and implemented a population of which each unit (e.g. cell) has a certain probability to replicate (p_1) or to die (p_2):

$$p_1 = g \cdot S \tag{4}$$

$$p_2 = \begin{cases} \left((-s_R \cdot (t - t_L) + 1) \cdot m_I\right) \cdot S, & t < t_R \\ m_R \cdot S, & t \geq t_R \end{cases} \tag{5}$$

Because we only implemented the phase of stress ($t_{TS} \leq t \leq t_{TE}$), priming costs are realized as different initial values of S, i.e. $S_{np}(t = t_{TS}) > S_p(t = t_{TS})$, which are parametrized to match the difference after the priming phase in the deterministic model with priming costs of

$c_P = 0.3$. The stochastic model was developed and assessed with the R package "GillespieSSA" Version 0.5-4 (Pineda-Krch, 2008, 2010).

To assess the effect of stress intensity and stress duration on stress survival, we systematically varied separately the initial mortality m_I and the stress duration TD, simulated 10,000 runs of each response strategy and compared, which of the different strategies was most beneficial in preventing the population from going extinct. For each response strategy, we recorded the extinction probability as fraction of runs with population extinction.

Species and species interactions under resource limitation

For introducing species competition, we extended the original model by resource limitation expressed by the environmental capacity K leading to logistic growth of a population:

$$\frac{dS}{dt} = g(P,t) \cdot S(t) \cdot \left(1 - \frac{S(t)}{K}\right) - m(T,t) \cdot S(t) \quad (6)$$

First, we numerically investigated the effect of different priming costs and stress durations on the benefits of the three response strategies of a single population. We used the parameter values defined above and the assumption of $K = 10{,}000$ and chose the baseline scenario for $c_p = 0.3$ and $TD = 75t$. The numerical analysis of all ODEs was performed with the R package "deSolve" Version 1.21 (Soetaert *et al.*, 2010).

Afterwards, we investigated if the optimal stress response shifts under competition. For this, we run simulations of communities containing four microbial populations, each population following one of the four analyzed stress responses: one population was naïve, and the other three populations showed an earlier, a faster or a stronger primed response. Competition between populations was included by a generalized Lotka-Volterra model (Smale, 1976). For this, the model of a single population under resource limitation (Eq. 6) was expanded by an interaction parameter $\alpha \in [0,1]$, which describes the strength of competition, and by a joint carrying capacity K for all four populations. Each population S_i of the community was then described as

$$\frac{dS_i}{dt} = g(P,t) \cdot S_i(t) \cdot \left(1 - \frac{S_i(t) + \alpha \cdot \sum_{j \neq i} S_j(t)}{K}\right) - m(T,t) \cdot S_i(t) \quad (7)$$

Similar to the simulations of populations in isolation, we applied a mild priming stimulus and a subsequent strong triggering stress to the community and applied the same set of default parameters. We systematically varied the interaction parameter α between $\alpha = 0$ (no

competition, i.e. same equation as in single species case) and $\alpha = 1$ (high competition intensity).

Results

Comparison of the three stress responses

We analytically assessed which of the three primed response strategies is most beneficial for different stress durations and costs associated with priming (Fig. 3). For short stress durations

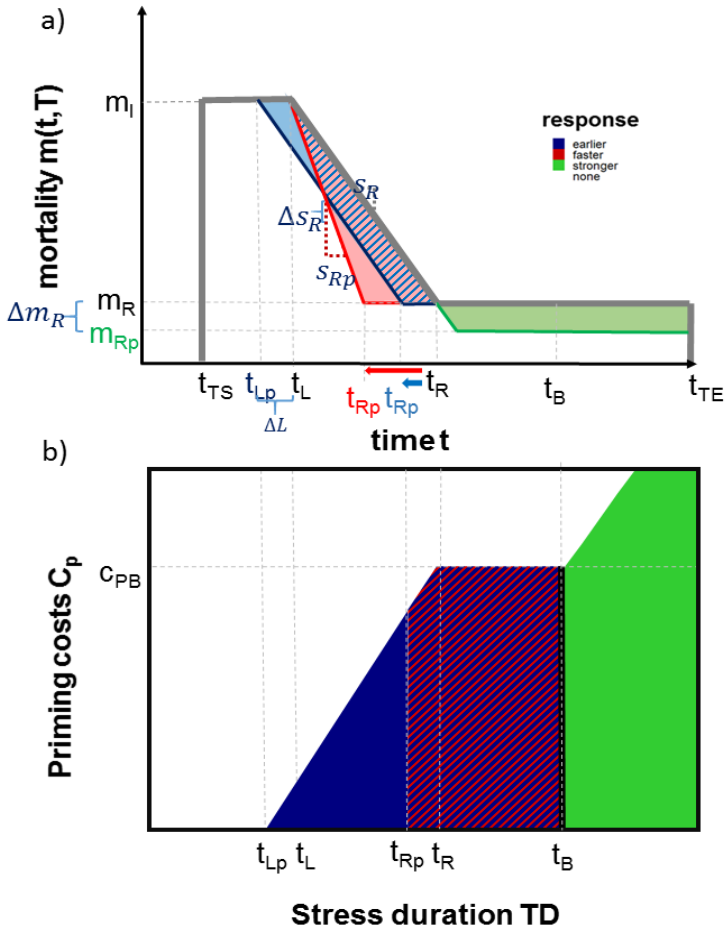


Figure 3 a) Mortality reduction of the three strategies and **b)** analytically determined parameter space favoring the different primed stress responses under exponential growth depending on stress duration and priming costs. Abbreviations: c_{PB}= (baseline) priming costs for which the three responses grant a benefit equal to the naïve response, m_I= initial mortality, m_R= final mortality level of the stress response, m_{Rp}= primed (reduced) m_R (stronger response), t_{TS}= beginning of the triggering stress, t_L= end of the response lag phase, t_{Lp}= end of the primed (shorter) response lag phase (earlier response), t_R= time point when m_R has been reached, t_{Rp}= time point when m_R has been reached with an earlier or faster stress response, t_B=(baseline) stress duration when the three responses grant a benefit equal to the naïve response, t_{TE}= end of triggering stress, s_R= slope of the mortality reduction, s_{Rp}= primed (higher) slope of the mortality reduction (faster response)

($t_{TE} < t_R$) the earlier response is most beneficial, because an early buildup of defense already grants a benefit while other response strategies are still delayed. However, this advantage is compensated for by the faster response for stress durations that are longer than the defense buildup ($t_{TE} \geq t_R$), since both responses reach the same benefit when the final response level m_R has been reached, i.e. at time point t_{Rp}. For both response strategies, only priming costs of the baseline scenario can be balanced, i.e. for priming costs higher than c_{PB}, priming is not beneficial. Higher costs than those of the baseline scenario will lead to a decrease in performance and higher stress durations cannot compensate that decrease, because after t_R both responses do not confer increased growth rate compared to the naïve response. The stronger stress response is the most beneficial response for long stress events (stresses that last longer than our defined baseline scenario $t_{TE} \geq t_B$). The longer the stress, the larger the difference in the integral of the stronger stress response compared to the other ones and thus the overall fitness. However, for the stronger stress response, there is no benefit for stress durations shorter than t_R, independent of the costs. This is because the advantage of the stronger response only starts when the final stress response level m_R is reached, i.e. at t_R (Fig. 2c). At longer stress durations, the benefit increases linearly (green shaded area of Fig. 2c), allowing also for priming costs higher than those of the baseline scenario.

Although the performance of each response type decreases with increasing costs, the fitness rank of the three response types, i.e. which one is most beneficial, is not altered, i.e. priming costs do not affect which response is most beneficial. Moreover, our analytical results show that all response parameters affect population fitness independently of initial mortality m_I or growth rate g, thus the results are robust to different intensities of stress and different growth conditions. None of the response parameters influences the qualitative pattern of Figure 3 (see Appendix A, section 1), while the shape of the region can vary: a generally faster response (i.e. higher s_R) leads to a reduced value of t_R and a smaller parameter space favoring only the early response in Figure 3.

Stochasticity

We evaluated the likelihood that a microbial population following none or one of the three different priming strategies becomes extinct under different stress durations and intensities (Fig. 4). The stochatic simulation approach shows that independent of the mortality rate, all primed responses show a decreased extinction probability compared to the naïve stress response. The reduction in extinction risk is of a robust order across all mortality rates (Fig 4a), with the earlier response providing the lowest risk of extinction, followed by the faster and stronger response. Since the early response decreases the mortality earlier than the other responses, it reduces the risk of driving the population size close to zero, thus reducing extinction probability. The stronger response is less beneficial, as it takes effect later than the other strategies. This pattern changes for longer durations (Fig 4b): longer exposal to a possibly lethal stress dramatically increases the probability of extinction under a faster and earlier stress response, but does less so under a stronger response strategy. Once the stronger response level m_{Rp} is reached, this strategy leads to a lower mortality level and a fitness benefit towards the other response strategies.

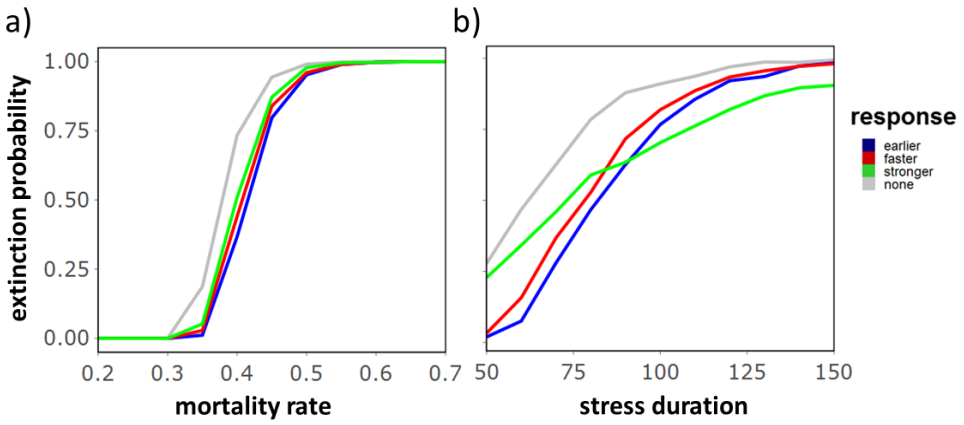


Figure 4 Extinction probability of a population following no or one of the three primed response strategies under **a)** different stress intensities with stress duration $\boldsymbol{TD = 75}$ or **b)** different stress durations with stress intensity $\boldsymbol{m_I = 0.4}$. The extinction probability is approximated by the fraction of 10,000 populations that did not survive until $\boldsymbol{t_{SE}}$ (end of stress).

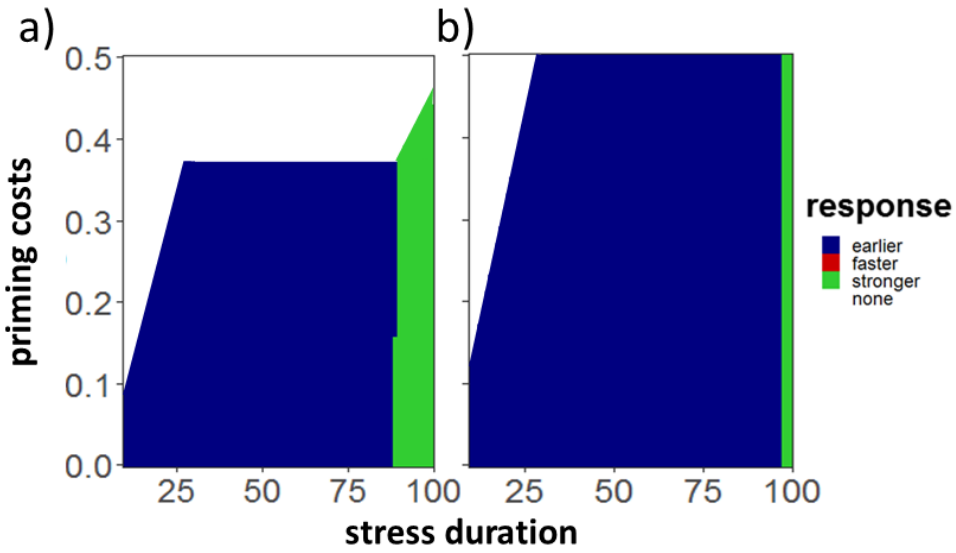


Figure 5 Most beneficial primed response under competition dependent on stress duration and priming costs. Results are given for **a)** medium competition intensity ($\boldsymbol{\alpha = 0.5}$) and **b)** high competition intensity ($\boldsymbol{\alpha = 1}$).

Lastly, we quantified the effect of resource limitation on the benefit of priming for populations in isolation (Fig. A1) and in the community context (Fig. 5). Under the influence of a limiting carrying capacity K, the benefit of the faster response exceeds the earlier response towards the end of the response buildup t_R, as opposed to an equal benefit without K (Fig. A1). As for the unlimited resources scenario, the stronger response is beneficial for longer durations of stress. The increased benefit of the faster response is caused by the additional density-dependent pressure on the population caused by K: The slower (but earlier) buildup of the early response leads to a longer phase where the population following the early response strategy is of increased size, thus subject to increased resource limitation. The faster response, however, exhibits the same reduction of mortality as the earlier response in a shorter amount of time, thus suffering less from resource limitation imposed by the carrying capacity K. Competition for resources between response strategies generally leads to higher benefits of priming, as priming is beneficial even for higher priming costs compared to the isolated case (Fig. 5). Moreover, the earlier response outcompetes the faster response, i.e. for the evaluated scenarios, the faster response is never the most beneficial one. For the stronger response to be most beneficial, the stress has to be of longer duration compared to the single-species case, because the benefit of the early response is outperformed later in time. For low competition (Fig. 5a), the overall parameter space of stress duration and priming costs that benefits priming is smaller than for strong competition, and for strong competition (Fig. 5b), the early primed response grants the highest benefit for most costs/durations, while the stronger response only more beneficial for a

very long stress durations. A visualization of the population dynamics in a community is given in Appendix A (Fig. A2).

Discussion

We used a simple ordinary differential equation model to assess the benefits of primeability for microbes showing an earlier, faster and stronger stress response than naïve organisms dependent on different scenarios. In the first part of the discussion, we focus on the three primed stress response strategies, and extend the discussion to the effects of stress intensity and growth on our results. In the last part, we discuss the priming response types under resource limitation and competition.

Benefits of the three primed stress responses

Our analyses show that the duration of stress has a strong impact on which priming strategy might grant the highest benefit, as hypothesized in the introduction. For short and medium duration of stress, the earlier and faster stress responses are most beneficial. This is in accordance with Douma *et al.* (2017), who analyzed primed responses of the plant *Brassica nigra* suffering from herbivory also using a modeling approach. However, their plant model does not account for a lag phase in the response, therefore, we additionally find a benefit of an earlier response for short durations of stress compared to a fast response. In contrast to our assumptions, they associate a stronger stress response with additional defense costs to account for the maintenance during the stress. Implementing additional maintenance costs is reasonable for many forms of defense, but might not apply to all stress defense strategies (for example, increased production of constitutive defense compounds after wounding in fungi, Spiteller 2008). We thus neglected additional costs of the different responses. However, as long as the maintenance costs are lower than the growth benefit gained by the stronger response, there will always be a net performance gain for longer stress durations, producing results that are qualitatively the same. Another assumption of the model is that the elevated level of the stronger response is maintained as long as the stress lasts, leading to a linearly increasing benefit with stress duration. If we, however, reduced the primed response level back to the naïve response level m_R at a specific point in time (e.g. because of increased gene expression leveling off or degradation of excess defense molecules), the benefit would not further increase. If this point

in time was after until $t > t_B$, i.e. the time point when the benefit of the faster and earlier responses is compensated for, the stronger response would still be the most beneficial strategy for longer responses, and results would not change qualitatively.

We found that priming costs do not exhibit control over which stress defense type is most beneficial. This is because the costs affect all response types in the same way, leading to the same decrease in the benefit of priming for all types. For a given stress duration, however, only a certain amount of priming costs can be compensated for, and if costs are too high or the stress duration is too short, it is more profitable not to invest into any type of priming**.** Here, we implement priming costs as costs that are directly linked to the buildup of the preliminary stress response and do only occur after a priming stimulus. Still, successful priming also requires more general investment in certain mechanisms, for example the retention of information about a past stress stimulus. Potential memory mechanisms have been discussed in different organisms, such as yeasts (Acar *et al.*, 2005; Zacharioudakis *et al.*, 2007; Guan *et al.*, 2012), prokaryotes (Casadesús and D'Ari, 2002; Wolf *et al.*, 2008; Lambert *et al.*, 2014) and filamentous fungi (Diana R. Andrade-Linares *et al.*, 2016). This investment constitutes additional costs of priming, which might reduce its overall benefit but affect the three primed responses equally and are thus not expected to change the observed pattern. The effect of memory and a decrease of the primed defense over time could be implemented in our model, e.g. by assuming a linear relationship between the decay or dilution of primed proteins and a reduction of the primed defense level. Assuming that the three response strategies are equally reduced in their efficacy by decreasing memory, the qualitative results of this study, i.e. which response is most beneficial, would still hold. If, however, one of the primed response strategies was associated with a shorter memory of the priming event than the other stress responses, it might lose its benefits. As the underlying physiological processes of response strategies are very diverse, we cannot make a general assumption on whether one primed response strategy exhibits a more sustained memory than another.

The effect of different stress intensities

In our analyses, we assess the impacts of stress implemented as increased mortality on microbes. Stressors of different intensity can be simulated by our model by assuming different mortality rates m_I: Intense or multifactorial forms of stress, like fungivory (Döll *et al.*, 2013; Ortiz *et al.*, 2013) or low pH (Koutsoumanis and Sofos, 2004) lead to the destruction of biomass and can be realized by $m_I > g_I$, i.e. an overall decrease of the population size. But also moderate stress

that does not reduce biomass of a population but instead lowers growth can be implemented by values of $g_I \cdot (1 - c_p) < m_I < g_I$, representing moderately damaging stresses, while $m_I = g_I$ would simulate growth halting stress. This particular case has for example been shown for hydrogen peroxide concentrations as high as 20 mM, that exhibited a fungistatic and not fungicidal effect on *Metarhizium anisopliae* (Rangel *et al.*, 2008).

Our model suggests that the stress intensity does not influence the benefit of the priming responses, as the resulting response pattern is the same (see Appendidx A for an analytical investigation of the effect of stress intensity m_I on the primed responses). While the intensity of the stress can affect whether priming at all would pay off, which of the three responses is most beneficial depends on how much time the organisms has to build up a stress response. However, the observed pattern changes for stress intensities that are high enough to drive a population to extinction. Under the pressure of a possibly lethal stress, the relative benefit of the early stress response increases, because it is the first response to take effect and thus most likely to prevent the population from dying out. If a severe stress is of longer duration and a population survives the initial, the stronger response pays off, as it reduces the stress impact further than the other responses and increases chances for survival.

Priming under resource limitation and competition

If we introduce a carrying capacity *K* into the model, the benefit of the faster response is increasing and surpassing the earlier response for intermediate stress durations. Here, the advantage of the faster response is an increase in defense in a relatively shorter amount of time compared to the earlier response, which leads to a more efficient exploitation of the environmental limitations. Because the final response level and the resulting mortality is the same for both responses, the benefit of both strategies converges for longer stress durations. However, this benefit shifts under competition between species: We found that the community context can alter the costs and benefits of induced defenses, as the benefit of priming is increasing and even high costs can be compensated for. This is in line with the results of Rillig *et al.* (2015), who found that competition enhances the payoff arising from priming. Under competition, the early response leads to a priority effect (Kennedy *et al.*, 2009) and thus provides a larger benefit than in the single-species context: A population reacting earlier to a stress can acquire nutrients and space before the population following a different response type has started building up its defense. Therefore, even for low competition (expressed by a low value of α, see Eq. 6), the early response outperforms the faster response in all cases. For a

better understanding of the underlying community dynamics, we added two timelines of community development to Appendix A (Fig. A2). The stronger the competition between species (high value of α), the higher are the benefits of priming. Therefore, in the community context priming is beneficial under higher costs than for isolated species. As shifts in the composition of microbial communities after disturbance are common (Schimel *et al.*, 2007), priming might not only influence the short-term physiological responses of the community members, but also the overall composition of a community. Favoring primeable species following a certain strategy more than others, priming could thus change the effect of disturbance legacy (Jurburg, Nunes, Brejnrod, *et al.*, 2017) and have a long-lasting effect on ecosystem process rates and community function (Allison and Martiny, 2008).

Priming uncertainty and combination of different priming responses

Our results are based on the assumption that the priming cue predicts the upcoming stress without error. In reality, however, the benefit of priming will be greatly reduced by the degree of the predictability of the disturbance (Mitchell and Pilpel, 2011; Katz and Springer, 2016; Douma *et al.*, 2017). Therefore, we analytically investigated the effect of predictability on the benefit of primed response strategies. While predictability influences whether priming at all is beneficial compared to the naïve response, it does not impact which of the three priming response types is the most beneficial for a given stress duration, i.e. the observed pattern is robust even under unpredictable environments (see Appendix A).

So far, we have only discussed the primed response types under the assumption that they are mutually exclusive. It is to be expected, however, that organisms exhibit mixed responses to increase their defense. Soil fungi that were temperature primed and exposed to severe heat, for example, showed an earlier re-growth and higher overall growth than naïve fungi (Diana R. Andrade-Linares *et al.*, 2016). We analytically investigated the benefit of primed responses incorporating two strategies and showed that for shorter durations, the combination of fast and early, while for longer stress durations the fast and strong primed response is most beneficial (See Appendix A, section 3, and Fig. A1b), because with a faster buildup, the final response level m_R is reached quickly and the stronger benefit takes effect earlier.

Our theoretical approach has provided novel insights into the benefits of different priming responses dependent on species traits, such as specific priming costs, and stress characteristics, i.e. the stress intensity and duration. Although the level of abstraction in our model approach is high, we could relate the findings to empirical studies and propose, which priming responses

are most beneficial and thus most likely to find under a given set of conditions. We could show that the stronger primed response is most beneficial for longer stress phases, while the faster and earlier responses increase performance under short durations of stress. More fatal levels of stress that might drive populations to the edge of extinction are best met with early defense strategies. Thus, at the ecological level, the dynamics of priming can be highly variable and the benefits of different priming responses depend strongly on abiotic and biotic environmental factors. We therefore expect to find different priming responses to co-occur under varying stress conditions, while a more homogenous stressor (e.g. in terms of stress duration) might favor similar priming strategies across populations. That is, we hypothesize primed stress responses to be more diverse under diverse stressors.

Priming in the community context has a higher significance than in isolation, and disturbance (i.e. a trigger stress) will benefit certain primed response strategies stronger than other strategies, thus shifting community composition. In systems prone to frequent disturbances and a high degree of competition, timing of colonization is vital and the early primed response is most beneficial. For systems experiencing longer stress durations, the stronger response gains in significance.

With our study, we would like to stimulate a discussion of priming effects that goes beyond the molecular basis of priming, but that considers priming in the ecological context. Our work shows that priming effects vary between the community context and between stress characteristics, but that some patterns are robust across environmental settings. These theoretical findings now need to be complemented with empirical studies and should find their way into stress ecology in general.

Acknowledgements

We are grateful to the German Research Foundation (DFG) for funding our Collaborative Research Centre 973 ‘Priming and Memory of Organismic Responses to Stress’ (www.sfb973.de).

References

Acar M, Becskei A, Van Oudenaarden A. Enhancement of cellular memory by reducing stochastic transitions. Nature 2005, DOI: 10.1038/nature03524.

Allison SD, Martiny JBH. resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A 2008;**105** Suppl:11512–9.

Alvarez-Peral FJ, Zaragoza O, Pedreño Y et al. Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in Candida albicans. Microbiology 2002;**148**:2599–606.

Andrade-Linares DR, Lehmann A, Rillig MC. Microbial stress priming: a meta-analysis. Environ Microbiol 2016;**18**:1277–88.

Andrade-Linares DR, Veresoglou SD, Rillig MC. Temperature priming and memory in soil filamentous fungi. Fungal Ecol 2016;**21**:10–5.

Baldwin I, Schmelz E. Immunological “memory” in the induced accumulation of nicotine in wild tobacco. Ecology 1996;**77**:236–46.

Berry DB, Gasch AP. Stress-activated Genomic Expression Changes Serve a Preparative Role for Impending Stress in Yeast. Mol Biol Cell 2008;**19**:4580–7.

Berry DB, Guan Q, Hose J et al. Multiple means to the same end: The genetic basis of acquired stress resistance in yeast. PLoS Genet 2011;**7**, DOI: 10.1371/journal.pgen.1002353.

Casadesús J, D’Ari R. Memory in bacteria and phage. BioEssays 2002;**24**:512–8.

Cebrián G, Sagarzazu N, Pagán R et al. Development of stress resistance in Staphylococcus aureus after exposure to sublethal environmental conditions. Int J Food Microbiol 2010;**140**:26–33.

Conrath U, Beckers GJM, Flors V et al. Priming: Getting Ready for Battle. Mol Plant-Microbe Interact 2006;**19**:1062–71.

Dhar R, Sägesser R, Weikert C et al. Yeast Adapts to a Changing Stressful Environment by Evolving Cross-Protection and Anticipatory Gene Regulation. Mol Biol Evol 2013;**30**:573–88.

Döll K, Chatterjee S, Scheu S et al. Fungal metabolic plasticity and sexual development mediate induced resistance to arthropod fungivory. Proc R Soc B Biol Sci 2013;**280**, DOI: 10.1098/rspb.2013.1219.

Douma JC, Vermeulen PJ, Poelman EH et al. When does it pay off to prime for defense? A modeling analysis. New Phytol 2017;**216**:782–97.

Gifford GE, Lohmann-Matthes ML. Gamma interferon priming of mouse and human macrophages for induction of tumor necrosis factor production by bacterial lipopolysaccharide. J Natl Cancer Inst 1987;**78**:121–4.

Gillespie DT. Exact Stochastic Simulation of Coupled Chemical Reactions. 1977;**81**:2340–61.

Guan Q, Haroon S, Bravo DG et al. Cellular memory of acquired stress resistance in Saccharomyces cerevisiae. Genetics 2012;**192**:495–505.

Hayes MP, Freeman SL, Donnelly RP. IFN-[gamma] priming of monocytes enhances LPS-induced TNF production by augmenting both transcription and mRNA stability. Cytokine 1995;**7**:427–35.

Hayes MP, Zoon KC. Priming of human monocytes for enhanced lipopolysaccharide responses: Expression of alpha interferon, interferon regulatory factors, and tumor necrosis factor. Infect Immun 1993;**61**:3222–7.

Hernández SB, Cota I, Ducret A et al. Adaptation and preadaptation of Salmonella enterica to bile. PLoS Genet 2012;**8**, DOI: 10.1371/journal.pgen.1002459.

Hilker M, Schwachtje J, Baier M et al. Priming and memory of stress responses in organisms lacking a nervous system. Biol Rev 2016;**91**:1118–33.

Hulten M van, Pelser M, Loon LC van et al. Costs and benefits of priming for defense in Arabidopsis. Proc Natl Acad Sci 2006;**103**:5602–7.

Jurburg SD, Nunes I, Brejnrod A et al. Legacy Effects on the Recovery of Soil Bacterial Communities from Extreme Temperature Perturbation. Front Microbiol 2017;**8**:1832.

Katz Y, Springer M. Probabilistic adaptation in changing microbial environments. PeerJ 2016;**4**, DOI: 10.7717/peerj.2716.

Kelley R, Ideker T. Genome-wide fitness and expression profiling implicate Mga2 in adaptation to hydrogen peroxide. PLoS Genet 2009;**5**, DOI: 10.1371/journal.pgen.1000488.

Kennedy PG, Peay KG, Bruns TD. Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? Ecology 2009;**90**:2098–107.

Koutsoumanis KP, Sofos JN. Comparative acid stress response of Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella Typhimurium after habituation at different pH conditions. Lett Appl Microbiol 2004;**38**:321–6.

Krebs RA, Loeschcke. Costs and benefits of activation of the heat-shock response in Drosophila melanogaster. Funct Ecol 1994;**8**:730–7.

Lambert G, Kussell E, Heuveling J et al. Memory and Fitness Optimization of Bacteria under Fluctuating Environments. Matic I (ed.). PLoS Genet 2014;**10**, DOI: 10.1371/journal.pgen.1004556.

Lou Y, Yousef AE. Adaptation to sublethal environmental stresses protects Listeria monocytogenes against lethal preservation factors. Appl Environ Microbiol 1997;**63**:1252–5.

Mitchell A, Pilpel Y. A mathematical model for adaptive prediction of environmental changes by microorganisms. Proc Natl Acad Sci U S A 2011;**108**:7271–6.

Mitchell A, Romano GH, Groisman B et al. Adaptive prediction of environmental changes by microorganisms. Nature 2009;**460**:220–4.

Newman MA, Von Roepenack-Lahaye E, Parr A et al. Prior exposure to lipopolysaccharide potentiates expression of plant defenses in response to bacteria. Plant J 2002;**29**:487–95.

Ortiz SC, Trienens M, Rohlfs M. Induced Fungal Resistance to Insect Grazing : Reciprocal Fitness Consequences and Fungal Gene Expression in the Drosophila - Aspergillus Model System. PLoS One 2013;**8**:1–10.

Pineda-Krch M. GillespieSSA : Implementing the Stochastic Simulation Algorithm in R. J Stat Softw 2008;**25**, DOI: 10.18637/jss.v025.i12.

Pineda-Krch M. GillespieSSA: Gillespie's stochastic simulation algorithm (SSA). 2010.

Pozo MJ, Verhage A, García-Andrade J et al. Priming plant defence against pathogens by arbuscular mycorrhizal fungi. Mycorrhizas - Funct Process Ecol Impact 2009:123–35.

Rangel DEN, Anderson AJ, Roberts DW. Evaluating physical and nutritional stress during mycelial growth as inducers of tolerance to heat and UV-B radiation in Metarhizium anisopliae conidia. Mycol Res 2008;**112**:1362–72.

Rillig MC, Rolff J, Tietjen B et al. Community priming-Effects of sequential stressors on microbial assemblages. FEMS Microbiol Ecol 2015;**91**, DOI: 10.1093/femsec/fiv040.

Runde S, Molière N, Heinz A et al. The role of thiol oxidative stress response in heat-induced protein aggregate formation during thermotolerance in Bacillus subtilis. Mol Microbiol 2014;**91**:1036–52.

Schimel J, Balser TC, Wallenstein M. Microbial Stress-Response Physiology and Its Implications for Ecosystem Function. Ecology 2007;**88**:1386–94.

Smale S. On the Differential Equations of Species in Comptetition. J Math Biol 1976;**7**:5–7.

Soetaert K, Petzoldt T, Setzer WR. Solving Differential Equations in R : Package deSolve. J Stat Softw 2010;**33**.

Spiteller P. Chemical defence strategies of higher fungi. Chem - A Eur J 2008;**14**:9100–10.

Stoebel DM, Dean AM, Dykhuizen DE. The cost of expression of Escherichia coli lac operon proteins is in the process, not in the products. Genetics 2008;**178**:1653–60.

Trent JD. A review of acquired thermotolerance, heat-shock proteins, and molecular chaperones in archaea. FEMS Microbiology Reviews. Vol **18**. 1996, 249–58.

Wolf DM, Fontaine-Bodin L, Bischofs I et al. Memory in Microbes: Quantifying History-Dependent Behavior in a Bacterium. PLoS One 2008;**3**:e1700.

Zacharioudakis I, Gligoris T, Tzamarias D. A Yeast Catabolic Enzyme Controls Transcriptional Memory. Curr Biol 2007;**17**:2041–6.

CHAPTER III

Stress priming affects fungal competition – evidence from a combined experimental and modelling study

Abstract[1]

Priming, an inducible stress defence strategy that prepares an organism for an impending stress event, is common in microbes and has been studied mostly in isolated organisms or populations. How the benefits of priming change in the microbial community context and, vice versa, whether priming influences competition between organisms, remains largely unknown. In this study, we grew different isolates of soil fungi that experienced heat stress in isolation and pairwise competition experiments and assessed colony extension rate as a measure of fitness under priming and non-priming conditions. Based on this data, we developed a cellular automaton model simulating growth of the ascomycete *Chaetomium angustispirale* competing against other fungi and systematically varied fungal response traits to explain similarities and differences observed in the experimental data. We showed that competition changes the priming benefit compared to isolated growth, and that it can even be reversed dependent on the competitor's traits such as growth rate, primeability and stress susceptibility. With this study, we transfer insights on priming from studies in isolation to competition between species. This is an important step towards understanding the role of inducible defences in microbial community assembly and composition.

[1] In contrast to the rest of this thesis, this chapter is written in British English, as it is based on a publication in a British journal.

Introduction

Priming is a stress defence mechanism that enables an organism to remember an environmental cue and to build up an enhanced stress response to a potentially stronger future stress. Primed defence mechanisms have been observed across many microbial taxa (see meta-analysis by Andrade-Linares, Lehmann and Rillig 2016), most of which have focused on the molecular processes that underlie priming. Complementary to research on priming processes, understanding the role of priming in stress ecology is an important step to comprehend how priming might change the effect of stressors on species fitness and community development. At the ecological level, it is still unclear how the ability of an individual to be primed, termed primeability, might influence competitive interactions and thus the community development and, vice versa, how the community context affects the benefits of priming.

Microbial priming is a defence strategy found in bacteria (Koutsoumanis and Sofos, 2004; Mitchell *et al.*, 2009; Cebrián *et al.*, 2010; Hernández *et al.*, 2012), archaea (Trent, 1996), as well as fungi (Alvarez-Peral *et al.*, 2002; Berry and Gasch, 2008; Rangel *et al.*, 2008; Mitchell *et al.*, 2009; Guhr *et al.*, 2017). Especially fungi are suitable model organisms to study the effects of priming under different conditions, as many isolates exhibit varying degrees of primeability (Szymczak *et al.*, 2020) and memory length (Diana R. Andrade-Linares *et al.*, 2016). In nature, isolated growth of fungi is rare, usually occurring only when new territory is colonized (Boddy, 2000), and fungi normally live in highly complex communities of different species that compete for space and display a broad range of mostly antagonistic interactions (Boddy, 2000; Toljander *et al.*, 2006; Hiscox and Boddy, 2017), which influence community composition (Boddy, 2000, 2001). Several studies have shown that fungal combative ability is not only dependent on the species that interact, but also on environmental factors such as resource availability (Stahl and Christensen, 1992; Falconer *et al.*, 2008) or temperature (Boddy *et al.*, 1985; Schoeman *et al.*, 1996; Toljander *et al.*, 2006; Hiscox, Clarkson, *et al.*, 2016), and temperature changes can even lead to reversed competitive outcomes (Crowther *et al.*, 2012). Therefore, we expect that heat priming, which affects species of distinct heat tolerance, but also distinct primeability differently in their response to heat stress, has an impact on fungal community development.

Experimental research on priming usually requires time-intensive multifactorial setups, in which organisms experience, apart from control conditions, a stress with and without

preceding priming cue, as well as a priming cue without subsequent stress (Hilker *et al.*, 2016). Here, simulation models can complement laboratory experiments by testing different environmental factors and species traits, e.g. imitating conditions or species combinations that could not be investigated empirically. A modelling approach thus allows a systematic investigation of distinct costs and benefits of priming for an organism. Using a mathematical model of microbes in competition, Rillig *et al.* (2015) could show that priming is beneficial more often under community conditions compared to species investigated under isolation. A follow-up study (Wesener and Tietjen, 2019), additionally showed that different strategies to reach an enhanced stress response are of different benefit. Especially the stress duration determined if an early or fast build-up of the response was most beneficial or a stronger response. However, a general understanding of how the benefit of priming can change under competition and thus influences community structure is still missing. To fill this gap, we carried out an experiment to collect dedicated data and developed a cellular automaton model simulating the growth of fungal colonies in isolation and in pairwise interactions. Our model is based on experimental data of the ascomycete *Chaetomium angustispirale* as focal species and various competitors of *C. angustispirale*, experiencing a mild temperature stimulus and/or heat stress. It can successfully reproduce the growth dynamics of two competing soil fungi under primed or non-primed conditions. To gather a general understanding of priming impacts on fungal competition, we systematically varied several traits of the species competing with *C. angustispirale* and observed how the priming benefit of *C. angustispirale* depends of the traits of the competitor. The specific aims of the study are i) to identify fungal traits that affect the pay-off of priming by comparing the benefit of *C. angustispirale* in dual cultures with various competitors and ii) to assess the influence of priming on competitive success.

Methods

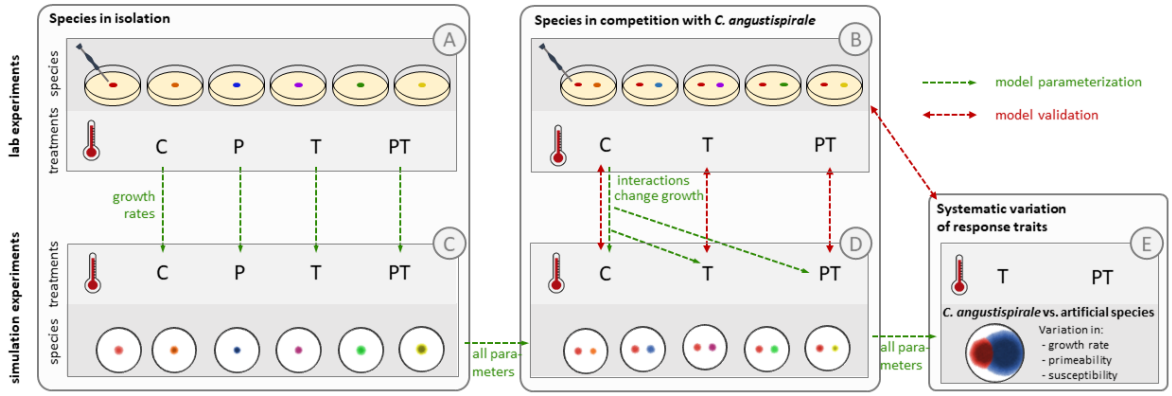


Figure 6 Schematic overview of the experimental setup. Boxes show the performed experiments with empirical work in the upper row and simulation experiments in the lower row. Links between Parts A-E are indicated by arrows. Information on each part is given in the main text of the Methods description: (**A**) sections *Experimental setup* and *Determination of colony extension rates*; (**B**) section *Competition experiments* (**C**) sections *Simulation model*; (**D**) section *Implementation of competition;* and (**E**) section *Simulation experiments*. Abbreviations C (control), P (priming), T (triggering), PT (priming and triggering) refer to our treatment scheme.

We carried out a laboratory experiment on six soil fungal species that have been taken from the top 10 cm soil of a grassland site in Brandenburg, Germany and are thus stemming from the same soil communities. The species were grown in isolation and in dual-species mixtures to determine how the growth of species is altered by heat stress and by priming towards this stress (Figure 6, Panel A and B). We used parts of the data to parameterize and validate growth rates of the model (Figure 6, Panel C and D). Finally, we used the validated model to systematically assess the effect of fungal species traits and of competition on the benefits of priming as well as the influence of priming on competition between species (Figure 6, Panel E).

Laboratory experiment of priming effects

Experimental setup

First, six soil fungal species were grown in isolation, and then *C. angustispirale* was grown in competition with the other five species. All single and dual cultures were grown in a Petri dish of 90 mm diameter on potato dextrose agar (PDA) in a full-factorial design of the following treatments with six replicates per fungus and treatment: i) A control treatment (C) at constant conditions of 22 °C with no disturbance, ii) a priming-only treatment (P), in which a fungus experienced a priming stimulus of 35 °C after one day of undisturbed growth, iii) a triggering-only treatment (T), in which a triggering heat stress of 45 °C was applied, and iv) a primed

stress treatment (PT), in which the priming stimulus was immediately followed by the triggering stress. After the priming and/or triggering stimulus, the temperature was set back to 22 °C. For details on the fungal species and the choice of treatment temperatures, see Appendix B.

The colony area was approximated by the measured diameter once per day to determine species-specific colony extension rates. Measurements were taken until the colonies reached the edges of the Petri dish or, for slower growing individuals, for 14 consecutive days.

Competition experiments

To investigate fungal growth under competition, we chose *C. angustispirale* as focal species competing against each of the five other species, as it showed moderate priming effects and could therefore be investigated under competition with species showing a higher and with species showing a lower primeability. That is, we use the term "pairwise" to refer to pairs that include *C. angustispirale* and did not investigate competition between the other species. Plugs of mycelium of *C. angustispirale* were inoculated pairwise with each of the five other species equally distant from each other and from the border of the Petri dish. As soon as the two colonies touched, the same treatments on priming and triggering as in the single species experiments were applied. In pairwise cultures, the individual colony shapes were not circular and colony area was determined using ImageJ (Schneider *et al.*, 2012). Scanning took place four times in total, until the Petri dish was filled. Eight replicates per competition setup and treatment were measured.

Determination of colony extension rates

We determined the species-specific growth rates by measuring the change of colony area over time for species in isolation in all four experimental treatments. Table 2 shows a quantification of the effect of the different treatments on growth (further illustrated in Figure B1).

For the C and P treatment, a linear fit was applied to the daily diameter values of the single species experiments resulting in colony growth measured as colony diameter change [mm/day]. For the T and PT treatments, we detected the occurrence of a stress-induced lag phase, i.e. a period of no growth, and determined the duration of the lag phase and the growth of the following phase, again with a linear fit. Further details are given in Appendix B.

Of the six species that were primed experimentally, four did not show priming costs concerning the growth rate (Table 2, P/C), while two showed a slight overall increase in

growth. To reduce the complexity of our model, we thus chose to exclude priming costs for further analyses. All fungi exhibited a lag phase without any growth under the T treatment, and four of the six species did not show a change in growth rate after the lag had ended compared to unstressed growth (Table 2, T/C). When being primed, the lag phase was shorter in five species and remained equal in *M. elongata*, and the growth rate after the end of the lag phase did not differ (Table 2, PT/T). Again, to avoid unnecessary model complexity, we assumed no difference in the growth rate after stress-induced lag phases for both T and PT treatments, as the changes were only marginal.

Table 2 Experimentally measured values of growth rates and their relative changes and lag phases after a stress stimulus. The significance levels between growth rates were assessed by a paired t-test * P ≤ 0.05, ** P ≤ 0.01, relative changes of 1 indicated a non-significant change in growth rate. The comparison between C and P treatment showed priming costs inflicted on a primed species, comparing C and T treatment revealed the effect of stress on growth, and comparing PT and T treatment quantified how much better a species grows if primed before stressed.
Abbreviations: C: control treatment without stress, P: primed treatment with a mild stress, T: triggered treatment with a strong stress, PT: primed and triggered treatment.

Species (competitor number)	Control growth rate g_{si} [mm/d]	Rel. change in growth T/C	Rel. change in growth PT/T	Rel. change in growth P/C	Non-primed lag phase $L_{si,T}$ [d]	Primed lag phase $L_{si,PT}$ [d]
C. angustispirale	9.38	1	1	1	3.17	1.9
F. sp. (1)	6.63	1	1	1	0.71	0.01
Amphisphaeriaceae strain *(2)*	7.13	1.15 (**)	1	1.10 (*)	1.577	1.03
P. sapidus (3)	3.58	1	1	1	1.93	1.56
F. oxysporum (4)	10.57	0.91 (**)	1	1.046 (*)	0.55	0.12
M. elongata (5)	14.74	1	1	1	2.82	2.82

Simulation Model

To simulate a fungal colony growing in a Petri dish, we developed a cellular automaton model and introduced the experimentally determined growth rates into this model. In the following section, we describe the model and how we converted the measured growth rates of the experiment, which are continuous over time and space, to the necessary discrete units of time and space of the cellular automaton.

Our model represents a Petri dish, i.e. a circular area, with an inner diameter $d = 86.5\,mm$ containing one or two fungal colonies. The area of the Petri dish is divided into square grid cells with a side length of $r_{spat} = 0.5\,mm$, leading to 173 grid cells along the diameter of the Petri dish. To mimic the laboratory experiments, the initial colony diameter of a fungus is set to $d = 6\,mm$. Colonies in isolation are placed into the centre of the Petri dish. Colonies in pairwise experiments are placed on the horizontal diameter equidistant from each other and the border of the Petri dish.

Simulation of cell division and colony growth follows the cellular automaton model of Gerlee and Anderson (2007). To realize radial extension of the initial colonies, each grid cell of the model is assigned one of two states: empty, or occupied by a fungal cell. Fungal cells conduct cell division, i.e. produced new daughter cells in an empty neighbouring grid cell, leading to an increase in colony area.

The temporal resolution r_{temp} is one hour. To match measured growth rates, it is necessary to determine the frequency of cell division, for which we introduce a linear increasing maturation value *m(t)* for each fungal cell. Cell division occurs when a cell reaches a maturation age of *m* ≥ 1. The increase in *m*, Δ*m*, is calculated based on the measured growth rate g_{si} relative to the temporal and spatial resolution:

$$\Delta m = \begin{cases} 0, & t_{treatment} < t < t + L_{si,treatment} \\ \frac{g_{si} \cdot r_{temp}}{2 \cdot r_{spat}}, & else \end{cases} \quad (1)$$

with *si* referring to the simulated species and *treatment* referring to the triggered only or primed and triggered treatment. Growth rate is corrected by factor ½, since the measured data of g_{si} include diameter growth into two directions, while simulated cell division for each side of the colony periphery is calculated separately. In case of heat stress (T or PT treatment), the maturation value remains constant for the duration of the post-stress lag phase, which starts after the application of a heat pulse (at t_T) or a priming stimulus followed by a heat pulse (at t_{PT}), and ends after the duration of the lag phase ($L_{si,T}$ or $L_{si,PT}$, respectively).

When a fungal cell reaches maturation age of $m \geq 1$, the cell divides and fills a random empty neighbouring grid cell (Moore neighbourhood with the eight surrounding cells) with a higher priority on the immediate four neighbouring grid cells, leading to an increase in area. The maturation value is then reduced by 1. The average division number corresponds to the measured radial colony extension. The daughter cell inherits its mother's new maturation age adjusted by a random variation term with a standard deviation of $\sigma = m/2$. If at the point of division none of the neighbouring grid cells is empty, the division failed and the maturation value of that fungal cell no longer increases.

Apart from competition for space, no other forms of interactions are included. The model setup leads to deadlock as only possible competitive outcome, which is a clear separation of space

between competing species and the most common competitive outcome of mycelial interactions (Stahl and Christensen, 1992; Schoeman *et al.*, 1996; Hiscox *et al.*, 2018).

The cellular automaton model was implemented in NetLogo 6.1.0 (Wilensky, 1999) and analysed using R (R., 2018) and the nlrx package (Salecker *et al.*, 2019).

Implementation of competition

Because fungi change their growth rates in dual cultures depending on their competitor (Stahl and Christensen 1992), we adjusted the growth rate $g_{si,ci}$ of species *si* under competition with competitor *ci*. For this, we applied a fit to non-stressed conditions (C), and only on data points before both fungal colonies touched to explicitly refer only to the effects that arise due to interactions at distance, as competition for space is already implemented in the model. The so-determined effect of competition was without further adjustment applied to the stress treatment (T) and primed-and-stressed treatment (PT) (shown as green line in the right panel of Figure 6). To validate the model, we simulated the growth of *C. angustispirale* as focal species in isolation and under competition with each one of the other five fungi under C, T and PT treatments.

Simulation experiments

As model output, we determined the colony area $A_{si} = N_{cells} \cdot {r_{spat}}^2$ [mm²], which serves as a measure of fitness. The relative benefit of priming b_{si} for *C. angustispirale* was then described as the colony area of a fungus under stress (T) compared to the area of a primed colony under stress (PT):

$$b_{si} = \frac{A_{si,PT}}{A_{si,T}} \quad (2)$$

A value of $b_{si} > 1$ thus refers to a situation where a species performs better with priming than without. To assess model performance, the colony area of *C. angustispirale* growing in competition with a competitor, as well as the simulated relative benefit from priming was compared to experimental data.

Subsequently, the sensitivity of this relative benefit of priming towards the following three response traits (summarized in Table 3) was evaluated in a full factorial experiment: i) The intrinsic colony extension rate in isolation g_{si} [mm/day], ii) the stress susceptibility defined as the length of the fungistatic lag phase under heat stress $sus_{si} = L_{si,T}$, and iii) the primeability of a species describing the reduction of the lag phase if primed before

stressed $prim_{si} = 1 - \frac{L_{si,PT}}{L_{si,T}}$. Both growth rate and stress susceptibility are absolute measures, while the primeability of a species is a relative value. By varying the response traits of the competing species, we could cover a broad range of possible competition scenarios that go far beyond the capacity of laboratory experiments.

Table 3 Model parameters and their description. A primeability value of $\boldsymbol{prim_{si}} = \mathbf{1}$ describes full primeability, i.e. a reduction of the lag phase to zero, while a primeability of $\boldsymbol{prim_{si}} = \mathbf{0}$ applies to non-primeable species that exhibit the same lag phase under T and PT treatment.

Parameter	Description	Source	Unit
g_{si}	intrinsic colony extension rate in isolation	measured	*mm/d*
$g_{si,ci}$	intrinsic colony extension rate under competition	measured	*mm/d*
$L_{si,T}$	duration of a phase of no growth after a 2 h heat stress	measured	*d*
$L_{si,PT}$	duration of a lag phase of no growth if primed before stressed	measured	*d*
sus_{si}	stress susceptibility	$sus_{si} = L_{si,T}$	*d*
$prim_{si}$	stress primeability (reduction of the stress-induced lag phase if primed)	$prim_{si} = 1 - \frac{L_{si,PT}}{L_{si,T}}$	-

To set a baseline, we first simulated the growth of *C. angustispirale* in competition with an identical species until the Petri dish was filled or up to a maximum of 15 days. We then systematically varied the three response traits of the competitor under T and PT conditions. Because the growth of *C. angustispirale* under competition proved to be variable depending on its competitor, we also varied the growth rate of *C. angustispirale*, while all other trait values of *C. angustispirale* remained fixed.

Secondly, to determine which competitor benefits more from priming, we measured the competitive shift $c_{C.a}$ of *C. angustispirale*

$$c_{C.a} = \ln\left(b_{C.a} \Big/ b_{competitor}\right) \tag{3}$$

which compares the benefits of both competitors and describes the influence of priming on competition, i.e. if the ratio of colony sizes of the two competing species is altered by a priming stimulus. Both the relative benefit of priming b_{si} and the competitive shift c_{si} depend on the performance of both competing species. However, while the relative benefit describes the potentially improved performance of a primed vs. a non-primed species under competition, the competitive shift describes which of the two competitors benefits more from priming. A value of $c_{c.a} = 0$ refers to no change in colony ratios between T and PT treatment,

i.e. both competitors benefit equally from priming in this competitive situation. For a value of $c_{C.a} > 0$, the colony size of *C. angustispirale* increases more than the one of its competitor.

Measuring both the relative benefit of *C. angustispirale* $b_{C.a}$ and the competitive shift $c_{C.a}$ allowed us to investigate whether certain parameter combinations affected these values differently, e.g. led to high priming benefit of *C. angustispirale* but still decreased its competitive strength because the competitor benefitted even more. We also compared the competitive shift that the model predicts for the five pairs with the experimental data to further validate our simulation model.

Results

After model fitting, we first validated the model by comparing the simulated output with experimental data of competition treatments not used for model parameterization. We then systematically varied different traits of an artificial species competing with *C. angustispirale* and assessed the benefit that *C. angustispirale* gained from priming. Additionally, we measured the effect of priming on competition strength under stress conditions.

Model Validation

With our model, we could well predict the growth in competition of four of five fungal pairs under stress with and without preceding priming cue (see Fig. 7 and Fig. B3; see Fig B4 for an observation vs prediction plot). In these successful cases, the effects of interactions and stress were additive. When competing with *M. elongata*, however, the model underestimated the performance of *C. angustispirale*: While the NRMSE (Normalized Root Mean Square Error, see Fig. B3), which quantifies the deviation of the model from the data, of the prediction ranged for most pairs between rather low values of 6 to 21, for this pair it reached a value of 45, indicating a relatively high deviance between modelled and observed data. Under control conditions, *M. elongata* overgrew *C. angustispirale* in the experiment and thus dominated strongly, but under stress, *M. elongata* changed its behaviour and could no longer overgrow *C. angustispirale*. For the sake of simplicity, however, our model does not yet take into account interactions between competition and stress nor alternative forms of fungal interactions such as overgrowth.

The priming benefit of *C. angustispirale* predicted by the simulation model was within the range of variation of the observed benefit for all five pairs (Fig. 8b). The model underestimation of growth when competing against *M. elongata* affected both T and PT treatment and thus cancelled out when calculating the relative benefit $b_{si} = \frac{A_{si,PT}}{A_{si,T}}$.

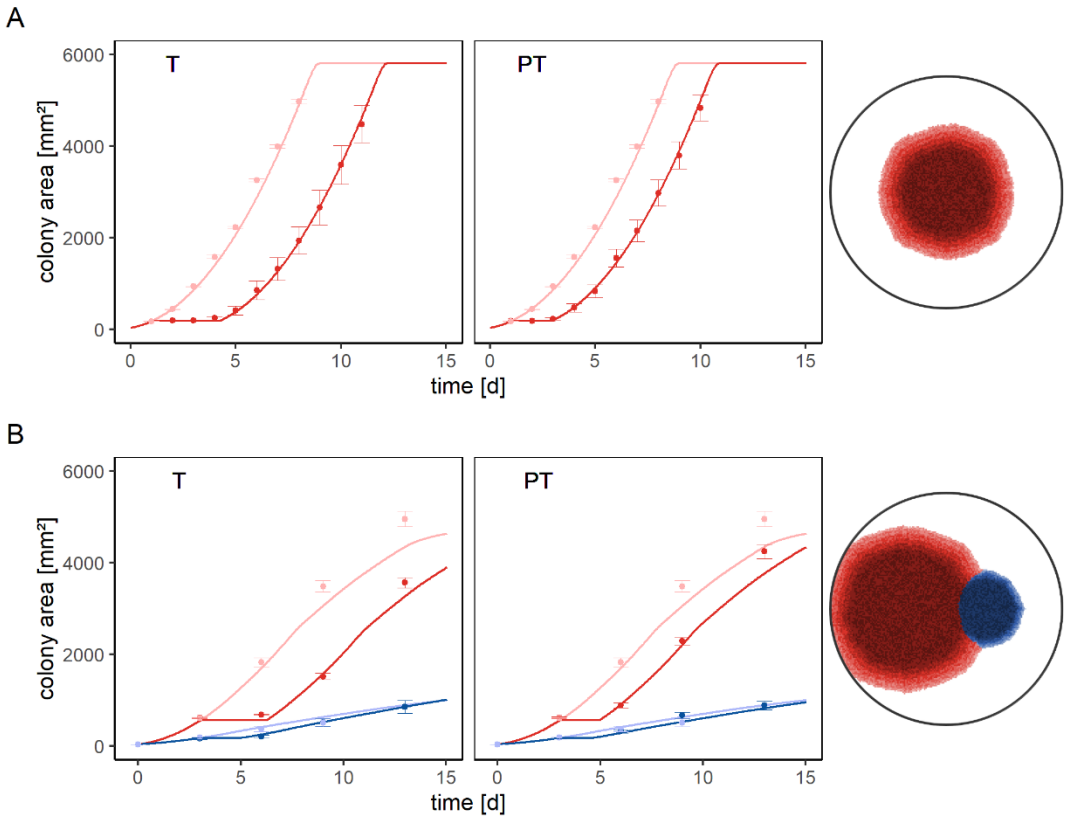


Figure 7 Measured and simulated growth dynamics of *C. angustispirale* (red) in **A** isolation and **B** competition with *P. sapidus* (blue). Points describe empirical measurements, and lines are the corresponding simulation model output. Light shades represent the control treatment, while darker shades represent the respective stress treatments (stressed, T, or primed and stressed, PT). Error bars show the standard error of the mean of the observed data. Examples at the right show the corresponding output of the cellular automaton model at day eight.

The relative benefit of priming

In four of the six investigated species, the post-lag growth phase was not significantly different from the control growth, and priming did not affect the growth of any of the species. Instead, the duration of the post-stress phase without growth was reduced in five species (see Table 2 and Figure B2).

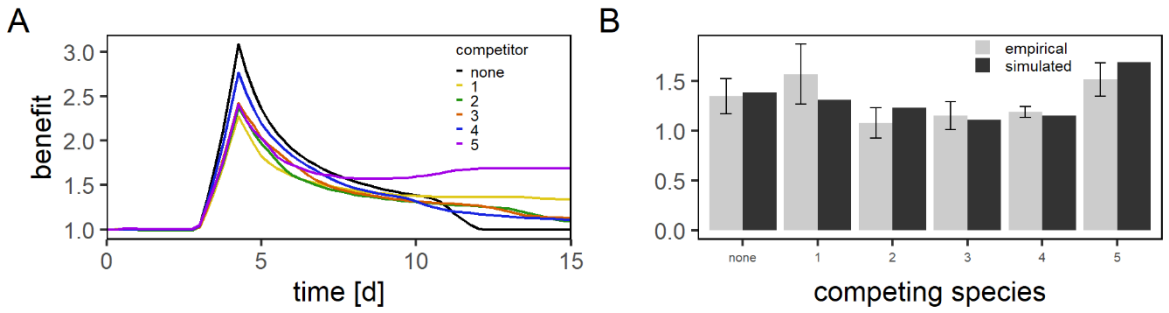


Figure 8 Priming benefit of *C. angustispirale.* **A** Simulation of the priming benefit of *C. angustispirale* over time in isolation or competition. **B** Comparison of the priming benefit of *C. angustispirale* in isolation or competition with one of five other soil fungi (represented by their competitor number listed in Table 1 and Table B1). Values represent the observed benefit at the last day of measurements and the simulated benefit for the same day.

When simulating the development of priming benefits over time, a consistent pattern emerged for both isolated and competitive growth (Fig. 8a): The relative benefit increased just after primed *C. angustispirale* restarted growth after the lag phase, and reached a maximum when the non-primed lag phase ended. The subsequent decrease in benefit results from the simultaneous increase of both primed and non-primed colony areas leading to a smaller relative difference between them.

For isolated growth, the immediate benefit was larger than under competition, because an isolated colony could expand without hindrance and benefit strongly from the shortened lag phase, while under competition, a competitor would have already claimed part of the space a species could grow on. The final relative benefit, however, was lowest in isolation, because without competitors there was no advantage in claiming space earlier, as eventually all available space would be overgrown. The final benefit was largest when *C. angustispirale* faced very competitive, i.e. fast growing, species (for example, *M. elongata)*.

When we systematically varied fungal response traits, for all combinations of traits within the investigated parameter space, priming was beneficial (i.e. relative benefit > 1, Fig. 9) eight days after inoculation, since priming involved no costs. However, under competition with a highly primeable and stress-susceptible competitor, priming was only marginally beneficial, especially when the competing species was fast-growing. Conversely, we observed the highest

benefit when *C. angustispirale* faced a stress-resistant and only moderately primeable competitor. Here, the negative effect of fast-growing competitors was reversed, and the relative benefit was highest for a fast-growing competitor (upper right vs. lower left panel of Fig. 9). A very susceptible competitor with high primeability strongly reduced its lag phase under priming. The faster that opponent grows, the more *C. angustispirale* will suffer from its gain in growing time. If, however, the opponent is less primeable, a priming cue will be of no great advantage to that species. In this case, *C. angustispirale* will benefit even more if the competitor is fast-growing.

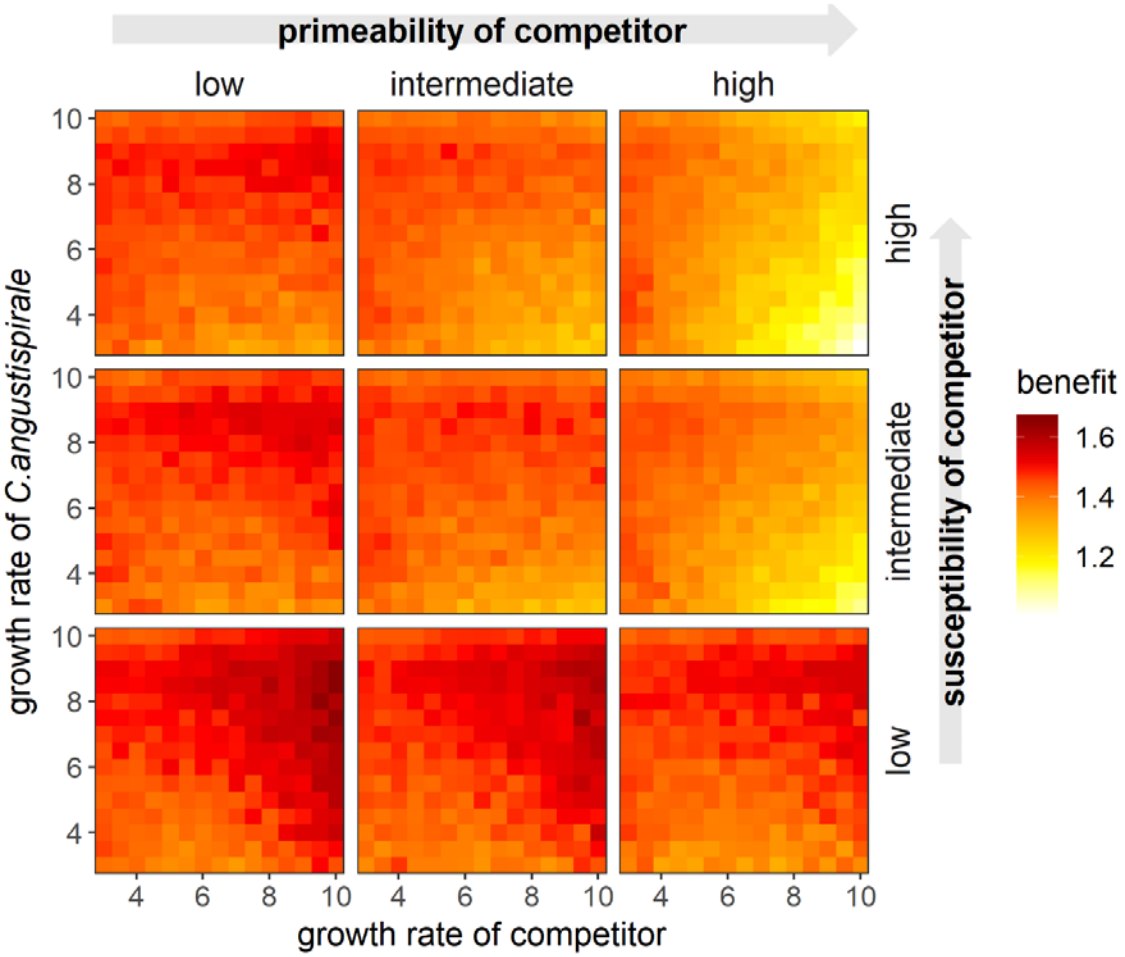


Figure 9 Priming benefit of *C. angustispirale* in competition with an artificial species. Benefits are shown for different trait combinations eight days after stress treatment. Levels of susceptibility correspond to different lengths of a stress-induced lag phase: low = 0.5 days, intermediate = 1.5 days, high = 2 days, and levels of primeability correspond to the reduction of this lag phase under priming conditions: low = 25%, intermediate = 50%, high= 100%.

Fifteen days after inoculation, when the Petri dish was filled and a steady state was reached, priming was not beneficial (i.e. $b_{C.a} < 1$) in case of a fast growing, highly primeable competitor with intermediate or high stress susceptibility (Fig. B5). During phases of growth, space that is lost to a more primeable competitor can still be compensated by colonizing empty

space elsewhere. If space is limited and the competitor is highly primeable, however, it can be more beneficial not to be primed at all.

The effect of priming on competition

Analogous to the investigation of priming benefits, we measured how the colony ratio between *C. angustispirale* and its competitor changed depending on different fungal traits (Fig. 10 and Fig. B6). A positive value indicates that priming favours *C. angustispirale* more than its competitor. Because of its intermediate primeability and high stress susceptibility stemming from a long lag phase in the non-priming treatment, for most investigated trait combinations, *C. angustispirale* benefited stronger than its competitor, as the long stress-induced lag phase of *C. angustispirale* was reduced substantially. If the dual cultures grown experimentally are positioned within Figure 10 according to the trait values of the competitors, it becomes evident that the effects of priming observed in the laboratory have been moderate. Our model shows that more extreme effects of priming on competition are possible: If competitors show a low

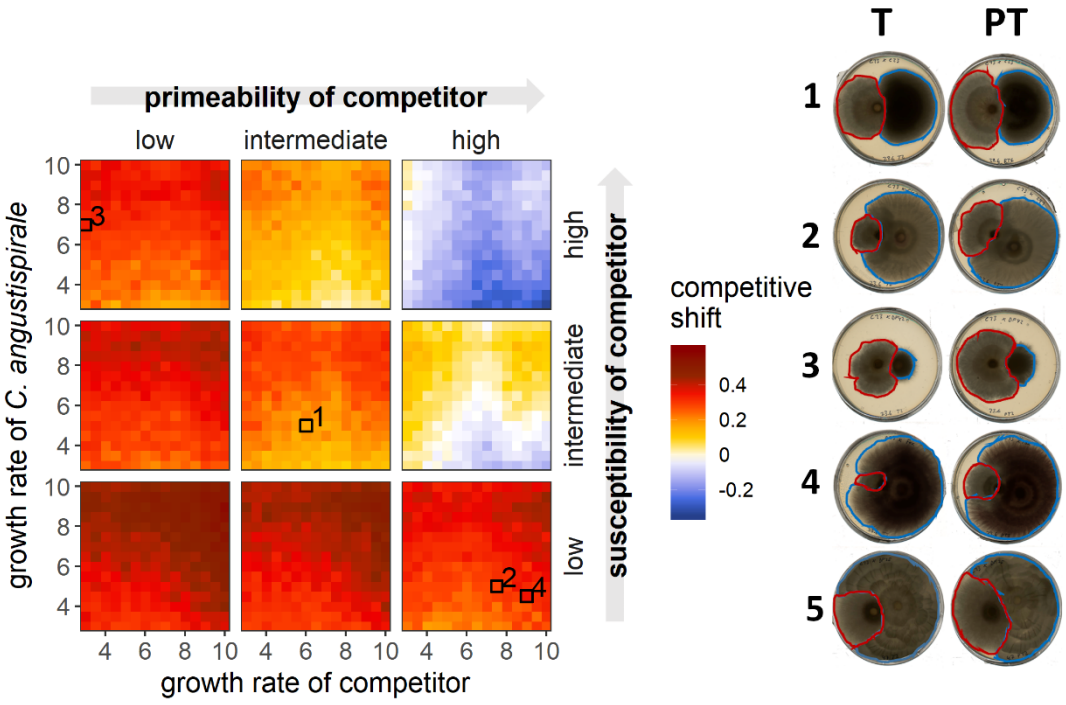


Figure 10 Competitive shift of *C. angustispirale* in competition with an artificial species. The shifts in competition are shown eight days after the stress treatment. Red shades indicate a shift in favour of *C. angustispirale*, and blue shades a shift favouring its competitor. Photos show exemplary pairwise cultures grown in the laboratory: Each pair is assigned the respective shift of competition predicted by the simulation model according to the parameter values of the competitor and growth of *C. angustispirale*. The pairs shown are *C. angustispirale* (red) competing against (blue) 1. *F. sp.*, 2. An *Amphisphaeriaceae* strain, 3. *P. sapidus* 4. *F. oxysporum,* 5. *M. elongata*. *M. elongata* is fast growing and not primeable, and is not represented in the visualized parameter space. Levels of susceptibility correspond to different lengths of a stress-induced lag phase: low = 0.5 days, intermediate = 1.5 days, high = 2 days, and levels of primeability correspond to the reduction of this lag phase under priming conditions: low = 25%, intermediate = 50%, high= 100%.

susceptibility to stress, the benefits of *C. angustispirale* can be much larger than observed in the experiments. If, in contrast, the susceptibility of the competitor is intermediate to high as well as its primeability, the benefits can be reversed leading to a shift towards the competitor.

When comparing Figure 9 and 10, the pattern in both plots seemed to be correlated: indeed, when *C. angustispirale* benefits from priming (i.e. $b_{c.a} > 1$), it will in many cases perform better than its competitor (i.e. $c_{c.a} > 0$), leading to similar patterns in both plots. Nevertheless, in some cases priming conferred a moderate relative benefit to *C. angustispirale*, while the corresponding competitive shift in these cases was variable: facing a primeable but stress susceptible and slow competitor, a competitive shift in favour of the competitor was found (i.e. $c_{c.a} < 0$). If on the other hand the competitor was less primeable or less stress susceptible, a competitive shift in favour of *C. angustispirale* occurred (i.e. $c_{c.a} > 0$), as the competitor benefited less from priming.

Discussion

We have performed laboratory experiments with competitors that cover different growth rates, degrees of primeability and stress susceptibilities. To increase the amount of trait combinations included in our analysis, we successfully developed a cellular automaton model that reproduces growth of competing fungi in a Petri dish under priming and heat stress conditions. With this model, we varied fungal traits such as stress susceptibility and primeability and assessed how these traits influence the species-specific benefit and competition outcomes.

The priming response of fungi

In our pre-experiments, we showed that while the chosen triggering stress pulse of 45 °C affects all six species negatively in their growth (i.e. pushes them away from their optimal growing temperature), it affects them to a different degree. This means that some species will perceive the stress as more severe than others. A stress cue will never affect all members of a community the same, and a priming cue can possibly induce priming in some species but not in others. Nevertheless, we use the term “community priming” to refer to a setting in which a whole community receives the same mild stress stimulus, which is known to prime at least some of the community members towards a second stronger stress stimulus. As not all species respond equally to both stress stimuli, competition can shift and the community might still change

differently than without a priming cue preceding a heat stress. Our approach does not allow for a direct comparison of physiological priming responses between species, but instead reflects the way stress priming affects communities in nature.

For all investigated species, a heat-induced no-growth phase was observed in the experimental data, and for four of six species, the post-lag growth phase was not significantly different from the control growth. Priming did not affect the growth of any of the species, but instead reduced the duration of the phase without growth. An analytical study by Wesener and Tietjen (2019) using coupled differential equations of microbial growth showed that stress of short duration is best met with an early defence and that a primed stress strategy that further shortens the time until the response is most successful. The current study confirms this pattern, as the fungi were treated with two-hour pulses of heat instead of prolonged periods of warming, and the primed colonies restarted growth earlier than those that had not been primed. Our model captured the dynamics for short stress durations and the effects of priming over several days before interaction types such as overgrowth dominate, while the community response to heat stress of longer duration remains to be investigated. Longer durations of heat stress should be applied as the fungal stress response types will likely differ for different types of heat stress.
Especially for species with a regeneration phase less than a day (*F. oxysporum* and *F. sp.*) the temporal resolution of measurements after stress should be increased to enable differentiation between an immediate but slow reversal to control level growth or a lag phase with no growth and "switch-like" change.
To parameterize the effect of inhibition at a distance, we used control measurements only and applied this parameterization to the other stress treatments. We could show that the inhibiting effect of growth due to a competitor is similar under all stress treatments. However, in some species combinations, heat stress could qualitatively alter the type of interaction between competitors, such as changing overgrowth to deadlock. This is in line with previous findings (Hiscox, Clarkson, *et al.*, 2016) and could be a valuable extension to our simulation model.

Priming costs

In this study, we aimed at accurately imitating growth dynamics of fungi under priming conditions. We did not implement any costs of priming, as there was no evidence under laboratory conditions that costs of priming are realized as reduced growth. Because priming usually involves the transient production of precursor molecules or transcription factors rather than the accumulation of resistance compounds, priming costs are generally expected to be low

(Heil, 2014) and might be hard to quantify. Especially under laboratory conditions, costs of induced resistance can be overseen, e.g. when they manifest as ecological costs (Heil, 2002). We investigated the effect of short-time stress pulses only and we expect the costs of priming to become apparent for longer duration of stress.

The distribution of resources between growth, resistance and reproduction is central to ecological theory, and any defence strategy must entail some costs (Harvell, 1990; Schulenburg *et al.*, 2009; Crowther *et al.*, 2014). A priming mechanism without costs would not bear any risks, and even in environments with low stress predictability (leading to organisms reacting to a priming cue, which is not followed by a triggering stress) priming would be of no disadvantage and would be ubiquitous in nature. To our knowledge, there is no study that investigated the costs of priming in microbes. Studies on priming costs in plants differed in their results for different species and priming cues, finding no direct costs of priming (Perazzolli *et al.*, 2011), costs realized as growth reduction (Hulten *et al.*, 2006), or reduced rhizome production (Yip *et al.*, 2019). Priming costs in fungi might thus also not be manifested in reduced growth, but rather in reduced spore production or competitive strength. Therefore, we want to stress the need of research on costs of induced resistance in microbes, which is necessary to fully comprehend the benefits and potential trade-offs of priming.

The benefit of priming

Because we did not implement any priming costs, during the growth phase priming is generally beneficial for *C. angustispirale* in all investigated scenarios. Therefore, we focus rather on the magnitude and not on the presence of this benefit.

Our results show that the relative benefit of priming under competition is highly dependent on fungal traits such as primeability, stress susceptibility and growth, as well as the time point during community build-up. We could show that depending on these factors, priming might not always be more beneficial under competition compared to the isolated benefit. Priming is least beneficial when a species faces a primeable, but stress susceptible and competitive (i.e. fast growing) species. Even when priming itself is relatively beneficial for a given species (i.e. it performs better than without priming), it might still be less competitive under priming conditions depending on the traits of its competitors. Priming can thus be beneficial when taking into account the change of the community structure and the resulting fitness of competing species, making it difficult to infer priming effects in a community from effects measured on species in isolation.

In future, more traits that influence the effect of priming should be analysed, such as the production of defence compounds. Scaling this production would allow a more dynamic response to the presence of a competitor and could result in different qualitative interactions such as inhibition at a distance or overgrowth – both interaction types are currently not implemented in the model.

Community priming

C. angustispirale exhibits moderate primeability and shows the longest stress-induced lag phase of the six investigated species. As a result, priming has the potential to strongly shorten its lag phase and thus to be highly beneficial in comparison to its competitors with lower susceptibility or lower primeability. Our model showed that when a steady state is reached and space is limited, priming is generally less advantageous, while during colonization of new territory it can be more beneficial, but also of greater disadvantage when facing primeable competitors. Because the competition for space in fungal communities is effectively competition for gaining access to nutrients (Boddy, 2000), it is of particular importance when colonizing new territory. We showed that a primed stress response that allows an organism to occupy empty space earlier than its competitors leads to the additional advantage of claiming space that would otherwise be colonized by another species. This result can be transferred to higher-order communities, where the order of species arrival in community assembly affects community structure and function (Fukami, 2015) and priority effects have been shown to be a common influence (Kennedy *et al.*, 2009). Because all modelled species compete for the same resources under severe space limitation, these priority effects constitute strong niche preemption (Fukami, 2015). However, our model does not take into account that priority effects can even be of increased importance when species further change environmental conditions or resource availability for later species via niche modification (Fukami, 2015). Environmental factors such as temperature have been shown to influence assembly of fungal community members (Hiscox *et al.*, 2015; Hiscox, Clarkson, *et al.*, 2016; Hiscox, Savoury, *et al.*, 2016). Therefore, heat priming can potentially influence the order of community assembly by letting certain species grow earlier than others.

Priming might not only affect community composition via community assembly, but also directly influence community structure: Sensitivity of microbial communities to disturbances is common, as they rarely return to pre-disturbance composition and reach alternative stable states (Schimel *et al.*, 2007; Allison and Martiny, 2008; Shade *et al.*, 2012). Environments with

fluctuating temperature show an increased species number in fungal communities (Toljander *et al.*, 2006), and post-stress communities can transiently consist of species that are generally more resistant to stress (Evans and Wallenstein, 2012; Jurburg, Nunes, Brejnrod, *et al.*, 2017). Priming, however, can influence community resistance, if less resistant but instead primeable species persist in a community. Stress responses at an individual level, such as priming, might therefore interact with legacy effects arising from pre-disturbance community composition (Meisner *et al.*, 2018), resulting in communities with different functions or stress resistance.

Our study advanced the understanding of ecological effects on priming in three ways. First, in our laboratory experiments we found that findings on priming benefits from microbial species in isolation cannot simply be transferred to species competing with other species. Second, the model showed that individual benefits of priming in a community context are highly dependent on the traits of both species and do not necessarily translate into a competitive advantage. And third, although the species chosen in our laboratory experiment showed a wide range of traits, the observed effects on competition were not at all representative for the full spectrum of potential effects as detected in our simulation study. This shows that inferring priming effects on communities from experiments on species in isolation can be highly misleading and that models are a valuable tool to complement laboratory experiments.

Acknowledgements

We are grateful to the German Research Foundation (DFG) for funding our Collaborative Research Centre 973 ‘Priming and Memory of Organismic Responses to Stress’ (www.sfb973.de).

We thank the high-performance computing service of the Freie Universität Berlin (ZEDAT) for providing the resources for running the simulation experiments.

References

Allison, S.D. and Martiny, J.B.H. (2008) resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A **105** Suppl: 11512–9.

Alvarez-Peral, F.J., Zaragoza, O., Pedreno, Y., and Argüelles, J.-C. (2002) Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in Candida albicans. Microbiology **148**: 2599–2606.

Andrade-Linares, Diana R, Lehmann, A., and Rillig, M.C. (2016) Microbial stress priming: a meta-analysis. Environ Microbiol **18**: 1277–1288.

Andrade-Linares, Diana R., Veresoglou, S.D., and Rillig, M.C. (2016) Temperature priming and memory in soil filamentous fungi. Fungal Ecol **21**: 10–15.

Berry, D.B. and Gasch, A.P. (2008) Stress-activated Genomic Expression Changes Serve a Preparative Role for Impending Stress in Yeast. Mol Biol Cell **19**: 4580–4587.

Boddy, L. (2001) Fungal Community Ecology and Wood Decomposition Processes in Angiosperms : From Standing Tree to Complete Decay of Coarse Woody Debris. Ecol Bull 43–56.

Boddy, L. (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol Ecol **31**: 185–194.

Boddy, L., Gibbon, O.M., and Grundy, M.A. (1985) Ecology of Daldinia concentrica: effect of abiotic variables on mycelial extension and interspecific interactions. Trans - Br Mycol Soc **85**: 201–211.

Cebrián, G., Sagarzazu, N., Pagán, R., Condón, S., and Mañas, P. (2010) Development of stress resistance in Staphylococcus aureus after exposure to sublethal environmental conditions. Int J Food Microbiol **140**: 26–33.

Crowther, T.W., Littleboy, A., Jones, T.H., and Boddy, L. (2012) Interactive effects of warming and invertebrate grazing on the outcomes of competitive fungal interactions. FEMS Microbiol Ecol **81**: 419–426.

Crowther, T.W., Maynard, D.S., Crowther, T.R., Peccia, J., Smith, J.R., and Bradford, M.A. (2014) Untangling the fungal niche: the trait-based approach. Front Microbiol **5**: 1–12.

Evans, S.E. and Wallenstein, M.D. (2012) Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? Biogeochemistry **109**: 101–116.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2008) Modelling interactions in fungi. J R Soc Interface **5**: 603–615.

Fukami, T. (2015) Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. Annu Rev Ecol Evol Syst **46**: 1–23.

Gerlee, P. and Anderson, A.R.A. (2007) Stability analysis of a hybrid cellular automaton model of cell colony growth. Phys Rev E - Stat Nonlinear, Soft Matter Phys **75**: 1–8.

Guhr, A., Horn, M.A., and Weig, A.R. (2017) Vitamin B 2 (Riboflavin) increases drought tolerance of Agaricus bisporus. Mycologia **109**: 860–873.

Harvell, C.D. (1990) The ecology and evolution of inducible defenses. Q Rev Biol **65**: 323–340.

Heil, M. (2002) Ecological costs of induced resistance. Curr Opin Plant Biol **5**: 345–350.

Heil, M. (2014) Trade-offs Associated with Induced Resistance. In, Induced Resistance for Plant Defense. Chichester, UK: John Wiley & Sons, Ltd, pp. 171–192.

Hernández, S.B., Cota, I., Ducret, A., Aussel, L., and Casadesús, J. (2012) Adaptation and Preadaptation of Salmonella enterica to Bile. PLoS Genet 8: e1002459.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., et al. (2016) Priming and memory of stress responses in organisms lacking a nervous system. Biol Rev **91**: 1118–1133.

Hiscox, J. and Boddy, L. (2017) Armed and dangerous – Chemical warfare in wood decay communities. Fungal Biol Rev **31**: 169–184.

Hiscox, J., Clarkson, G., Savoury, M., Powell, G., Savva, I., Lloyd, M., et al. (2016a) Effects of pre-colonisation and temperature on interspecific fungal interactions in wood. Fungal Ecol **21**: 32–42.

Hiscox, J., O'Leary, J., and Boddy, L. (2018) Fungus wars: basidiomycete battles in wood decay. Stud Mycol **89**: 117–124.

Hiscox, J., Savoury, M., Johnston, S.R., Parfitt, D., Müller, C.T., Rogers, H.J., and Boddy, L. (2016b) Location, location, location: priority effects in wood decay communities may vary between sites. Environ Microbiol **18**: 1954–1969.

Hiscox, J., Savoury, M., Müller, C.T., Lindahl, B.D., Rogers, H.J., and Boddy, L. (2015) Priority effects during fungal community establishment in beech wood. ISME J **9**: 2246–2260.

Hulten, M. van, Pelser, M., Loon, L.C. van, Pieterse, C.M.J., and Ton, J. (2006) Costs and benefits of priming for defense in Arabidopsis. Proc Natl Acad Sci **103**: 5602–5607.

Jurburg, S.D., Nunes, I., Brejnrod, A., Jacquiod, S., Priemé, A., Sørensen, S.J., et al. (2017) Legacy Effects on the Recovery of Soil Bacterial Communities from Extreme Temperature Perturbation. Front Microbiol **8**: 1832.

Kennedy, P.G., Peay, K.G., and Bruns, T.D. (2009) Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? Ecology **90**: 2098–2107.

Koutsoumanis, K.P. and Sofos, J.N. (2004) Comparative acid stress response of Listeria monocytogenes, Escherichia coli O157:H7 and Salmonella Typhimurium after habituation at different pH conditions. Lett Appl Microbiol **38**: 321–326.

Meisner, A., Jacquiod, S., Snoek, B.L., Ten Hooven, F.C., and van der Putten, W.H. (2018) Drought legacy effects on the composition of soil fungal and prokaryote communities. Front Microbiol **9**: 1–12.

Mitchell, A., Romano, G.H., Groisman, B., Yona, A., Dekel, E., Kupiec, M., et al. (2009) Adaptive prediction of environmental changes by microorganisms. Nature **460**: 220–224.

Perazzolli, M., Roatti, B., Bozza, E., and Pertot, I. (2011) Trichoderma harzianum T39 induces resistance against downy mildew by priming for defense without costs for grapevine. Biol Control **58**: 74–82.

R. (2018) R core team (2018). R A Lang Environ Stat Comput R Found Stat Comput Vienna, Austria URL http//www R-project org.

Rangel, D.E.N., Anderson, A.J., and Roberts, D.W. (2008) Evaluating physical and nutritional stress during mycelial growth as inducers of tolerance to heat and UV-B radiation in Metarhizium anisopliae conidia. Mycol Res **112**: 1362–1372.

Rillig, M.C., Rolff, J., Tietjen, B., Wehner, J., and Andrade-Linares, D.R. (2015) Community priming--effects of sequential stressors on microbial assemblages. FEMS Microbiol Ecol **91**: 1–7.

Salecker, J., Sciaini, M., Meyer, K.M., and Wiegand, K. (2019) The nlrx r package: A next-generation framework for reproducible NetLogo model analyses. Methods Ecol Evol **10**: 1854–1863.

Schimel, J., Balser, T.C., and Wallenstein, M. (2007) Microbial Stress-Response Physiology and Its Implications for Ecosystem Function. Ecology **88**: 1386–1394.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods **9**: 671–675.

Schoeman, M.W., Webber, J.F., and Dickinson, D.J. (1996) The effect of diffusible metabolites of Trichoderma harzianum on in vitro interactions between basidiomycete isolates at two different temperature regimes. Mycol Res **100**: 1454–1458.

Schulenburg, H., Kurtz, J., Moret, Y., and Siva-Jothy, M.T. (2009) Introduction. Ecological immunology. Philos Trans R Soc B Biol Sci **364**: 3–14.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., et al. (2012) Fundamentals of Microbial Community Resistance and Resilience. Front Microbiol **3**: 417.

Stahl, P.D. and Christensen, M. (1992) In vitro mycelial interactions among members of a soil microfungal community. Soil Biol Biochem **24**: 309–316.

Szymczak, A., Ryo, M., Roy, J., and Rillig, M.C. (2020) Diversity of Growth Responses of Soil Saprobic Fungi to Recurring Heat Events. **11**: 1–8.

Toljander, Y.K., Lindahl, B.D., Holmer, L., and Högberg, N.O.S. (2006) Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. Oecologia **148**: 625–631.

Trent, J.D. (1996) A review of acquired thermotolerance, heat-shock proteins, and molecular chaperones in archaea. In, FEMS Microbiology Reviews., pp. 249–258.

Wesener, F. and Tietjen, B. (2019) Primed to be strong, primed to be fast: modeling benefits of microbial stress responses. FEMS Microbiol Ecol **95**: 1–9.

Wilensky, U. (1999) NetLogo. http://ccl.northwestern.edu/netlogo/. Cent Connect Learn Comput Model Northwest Univ Evanst.

Yip, E.C., Tooker, J.F., Mescher, M.C., and De Moraes, C.M. (2019) Costs of plant defense priming: Exposure to volatile cues from a specialist herbivore increases short-term growth but reduces rhizome production in tall goldenrod (Solidago altissima). BMC Plant Biol **19**: 1–12.

CHAPTER IV

Revealing the causes of altered interactions between competing fungi under heat stress: insights from a modeling approach

Abstract

Fungal communities are shaped by competition for resources, which is characterized by various antagonistic interaction types. Both the competitive outcome and the qualitative type of interaction between fungi have been show to change under periods of heat stress. However, the underlying mechanisms of how fungal heat stress defense and competition interact remain unclear.

Here, we used a partial differential equation (PDE) model to simulate two fungal colonies competing in a two-dimensional space. With this model, we determined the growth, the production and secretion of antifungal compounds and the synthesis of heat shock proteins of both interacting colonies to understand the mechanisms leading to the observed change of fungal competition under heat stress. Our approach revealed that a heat stress-induced lag phase favored the accumulation of antifungal compounds and the build-up of an inhibitor field. This can lead to qualitatively changing type of interaction and to altered competition in favor of slower growing species, as these benefit stronger from the additional time during a stress-induced lag to build up a defense or block territory. This is an important step towards understanding drivers of fungal community dynamics and how they are affected by environmental changes.

Introduction

Fungi need to colonize territory to gain access to organic resources, and thus fungal competition for nutrients is synonymous with competition for space (Boddy, 2000; Hiscox and Boddy, 2017). This competition can be divided into two mechanisms: Primary resource capture describes the colonization of previously uncolonized territory, and is most effective for species that exhibit fast growth or high dispersal mechanisms (Klepzig and Wilkens, 1997; Boddy, 2000; Prospero *et al.*, 2003). Secondary resource capture, which is the capture of territory inhabited by another fungus, is characterized by various antagonistic interspecific interactions. In laboratory experiments with colonies extending on the horizontal plane, a number of qualitative interaction types defines fungal competition (Stahl and Christensen, 1992; Boddy, 2000; Falconer *et al.*, 2008): Inhibition at contact or at a distance lead to a local arrest of growth for both species. This is caused inhibiting compounds exuded by a competitor and usually results in a deadlock in which neither species invades territory inhabited by the other. Another interaction is overgrowth, which describes the invasion of inhabited territory and can ultimately lead to competitive exclusion of the inferior species. Intermediate interaction types can be partial overgrowth of one species as well as intermingling, when the hyphae of two species overlap and share certain regions. These fungal interaction types are mediated by an overwhelming amount of secondary metabolites, and their production varies not only between, but also within species (White and Boddy, 1992; Marx, 2004; Hiscox *et al.*, 2010; Knowles *et al.*, 2019). How exactly different interaction types emerge from the production of secondary metabolites, and how they affect competition between fungi, is yet to be fully understood.

Competitive outcomes in fungal pairs have been shown to change under heat stress (A'Bear *et al.*, 2013; Hiscox, Clarkson, *et al.*, 2016), as resistance and resilience towards stress can strongly differ across community members. Induced heat stress defenses can further influence the outcome of competition, if a species manages to build up a stress response earlier than its competitors (Wesener *et al.*, 2021). For example, a slow growing but stress-resistant species might be able to overgrow territory that has not yet been claimed by a faster competitor whose colony expansion is halted after a heat pulse. However, not only the distribution of territory between species might change under heat stress, but also the types of interactions have been observed to change (Schoeman *et al.*, 1996; Hiscox, Clarkson, *et al.*, 2016). Therefore, periods of heat might ultimately be a defining factor for fungal community assembly (Hiscox, Clarkson,

et al., 2016) and community composition (Allison and Martiny, 2008; Shade *et al.*, 2012). It is thus important to understand not only the effect of antifungal compounds on fungal combat, but also the interplay of heat stress and competition.

To shed light on the mechanisms underlying fungal growth and competition, several studies have mathematically simulated fungal colonies using Partial differential equations (PDE). Edelstein (Edelstein, 1982; Edelstein and Segel, 1983; Edelstein-Keshet and Ermentrout, 1989) was a pioneer for PDE models simulating mycelial growth in a two-dimensional space based on hyphal branching and merging, and her models have since inspired many other approaches. Some PDE models simulated macroscopic movement of biomass rather than hyphae (Davidson *et al.*, 1996, 1997), and follow-up studies have combined both approaches, simulating different types of biomass (Boswell *et al.* (2002, 2003), Falconer *et al.* (2005, 2007)). Some models have also included interactions between fungal competitors (Falconer *et al.*, 2008; Boswell, 2012), but so far no modeling study has included induced stress defenses in fungi. Therefore, a mechanistic explanation of changing fungal interactions under heat stress is still missing.

In this study, we extend the partial differential equation model of Falconer *et al.* (2008) by a dynamic heat stress defense mechanism. We use the model to simulate competitive growth between the two soil fungi under different scenarios to answer the following questions: Which competitive strategies are most successful with and without heat stress? Do optimal induced stress responses and competitive strategies differ for fungi with different growth rates? Under which conditions does a heat stress change the specific interaction type between competing fungi? Answering these questions will help disentangling the effects of induced defenses and secondary metabolite production and establish a link between processes at the species level and competition dynamics.

Methods

We used a model of partial differential equations (PDEs) based on Falconer *et al.* (2005, 2007, 2008) describing the growth of two fungal species and their interactions in space and time and extended it by the impacts of heat stress. To parameterize our model, we used growth data of two soil fungi from a laboratory experiment. We then used the model to assess the effects of

species' growth rates, their response to a heat shock as well as their production rate of antifungal compounds on fungal interactions.

Model Description

The PDE model simulates two fungal colonies growing and competing for space on a petri dish with substrate. It calculates the coupled dynamics of rigid (b_r), non-insulated (b_n), and mobile biomass (b_m), as well as substrate (s), inhibitors (i) and heat shock proteins (hsp) of both species (Fig. 11). In the following section, we describe these variables and how we simulated growth, the production of antifungal compounds as well as the heat shock defense.

Colony growth

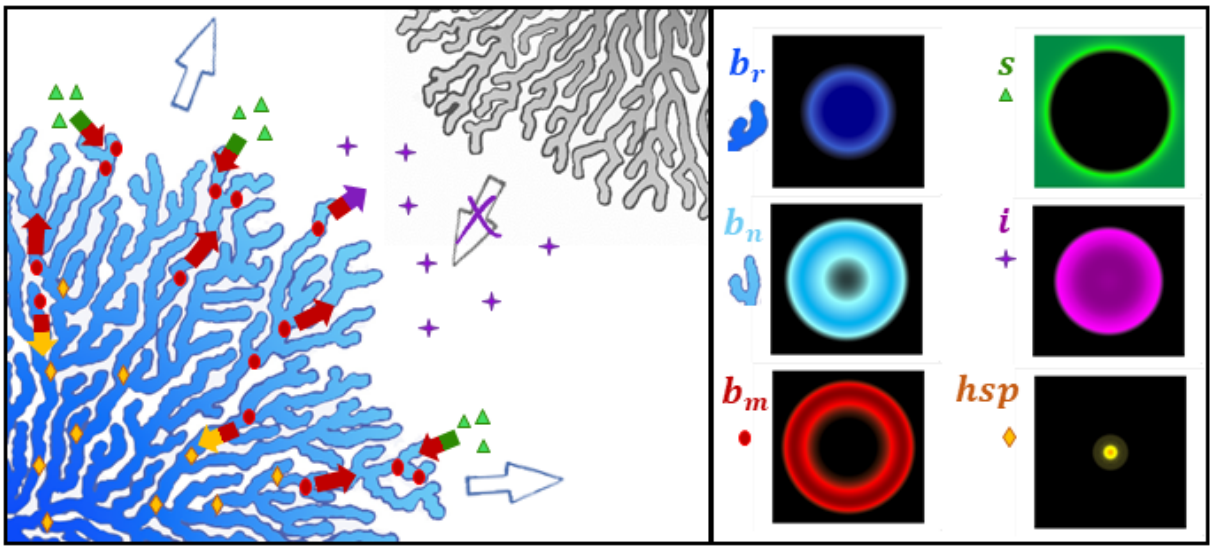


Figure 11 Schematic representation of the model dynamics. Non-insulated biomass (light blue, b_n) diffuses radially from the colony center (indicated by white outlined arrows) and is transformed into insulated, rigid biomass (dark blue, b_r) over time. Mobile biomass (red circles, b_m) is moved through the hyphae and immobilized (i.e. converted to non-insulated biomass). Red arrows show the movement of mobile biomass through the hyphae. New biomass is generated from the underlying substrate (green triangles, *s*), which is taken up and converted to mobile biomass. The inhibitors (purple stars, *i*), which are are produced by mobile biomass (red-purple arrows), can diffuse and stop the local growth of other fungal biomass (shown as grey colony). In case of a heat pulse event, heat shock proteins (yellow diamonds, *hsp*) are synthesized from mobile biomass (red-yellow arrows). The right box shows an exemplary distribution of the six state variables after some time.

For each of the two fungal species, the model assumes three different types of biomass growing on substrate s, which is replenished with a constant rate $\omega > 0$. With j referring to the simulated species, the non-insulated biomass b_{nj} represents the fungal hyphae and hyphal tips capable of high substrate uptake (rate λ_{nj}), that grow via a diffusion term scaled by the diffusion coefficient D_{nj}. The non-insulated biomass undergoes rigidifcation of the cell wall (rate c_j) and is converted to insulated, rigid biomass b_{rj} that describes hyphal sections that are significantly reduced in their uptake (rate λ_{rj}) of nutrients (Trinci, 1978).

The substrate is taken up by the hyphae and hyphal tips to then be converted to mobile biomass b_{mj}, which is transported via diffusion through the hyphae (diffusion coefficient D_{mj}). The diffusion coefficient D_{mj} of mobile biomass b_{mj} is nonlinearly dependent on the local amount of mobile biomass to account for limited transport pathways of the mycelial network. Similarly to Falconer *et al.* (2005) we assumed a simple nonlinear dependence:

$$D_{mj} = \begin{cases} D_{nj}, & b_{mj} \leq b_{m\_lim} \\ 10^{-7} D_{nj}, & b_{mj} > b_{m\_lim} \end{cases}$$

Where b_{m_lim} describes a local threshold of mobile biomass concentration.

Parts of the mobile biomass are immobilized (converted to non-insulated biomass b_{nj}) at rate α_j. Following Falconer *et al.* (2005), we assumed the immobilization of biomass to require elements of mobile biomass and implement the immobilization to be proportional to the ratio of mobile biomass to hyphae, i.e. $\alpha_j \pi_j$ with $\pi_j = \frac{b_{mj}}{b_{rj}+b_{nj}}$.

Interactions

To simulate fungal interactions, colonies can convert mobile biomass to inhibitor molecules i_j at a rate Ω_j, which can diffuse (via D_{ij}) and halt a competitor's colony growth. Given the general nature of the model, we did not use i to represent a certain antifungal compound, but instead aimed to capture the universal dynamics of antifungal compounds such as mycotoxins, chitinolytic enzymes or small antifungal proteins. For the sake of simplicity, we did not include the effect that volatile organic compounds (VOCs) can have on fungal interactions.

If a competitor's inhibitor concentration $i_{\neg j}$ is higher than a species' resistance ψ_j, the local diffusion coefficient of that species' non-insulated biomass is set to zero:

$$D_{nj} = \begin{cases} D_{nj}, & i_{\neg j} < \psi_j \\ 0, & i_{\neg j} \geq \psi_j \end{cases}$$

In this study, we did not consider possible effects of autophagy upon encounter of an antagonist proposed in some scenarios by Falconer et al. (2008) and thus did not include any other effects of i.

We assumed that a species is fully resistant to its own toxins and can grow unhindered, even though some fungi might locally disrupt their own mycelium when inhibiting other species (Hiscox and Boddy, 2017).

Heat shock response

We expanded the original model to include the effects of heat stress and stress defense mechanisms. In response to heat stress, fungi immediately stop growing, which can last up to several days, as cells are damaged, proteins are denatured and most cellular processes are halted. During this so-called lag phase (Wesener *et al.*, 2021), production of heat shock protein hsp_j is upregulated (Plesofsky-Vig and Brambl, 1995) in the colony, and heat shock defense molecules refold denatured proteins or protect nascent proteins (Tereshina, 2005; Liberek *et al.*, 2008). We assumed that low concentrations of biomass in the periphery of the fungal colony are degraded immediately and therefore set local biomass that is too small to zero. Similar to Boswell (2012), we defined the leading edge of the fungal colony as the line in space where the biomass ($b_{nj} + b_{rj} + b_{mj}$) surpasses 10^{-1}.

Even though we used the abbreviation hsp to refer to the state variable mediating the heat shock response, we again wanted to depict general stress defense dynamics. The molecules produced in our model could include different classes of heat shock proteins involved in unfolding and protecting proteins, as well as other substances such as the disaccharide Trehalose, which helps stabilizing proteins and membranes (Singer and Lindquist, 1998; Elbein *et al.*, 2003; Tereshina, 2005).

To simulate these processes, we introduced a binary variable z_j, which controls all affected cellular processes and set to $z_j = 1$ under non-stressed temperature conditions. By setting $z_j = 0$ upon transgression of a critical temperature, all modelled processes are immediately halted, except for the production of heat shock proteins, the diffusion of inhibitors, which are heat resistant (San-Lang *et al.*, 2002; Taechowisan *et al.*, 2003; Marx, 2004; Sena *et al.*, 2011), and the replenishment of substrate. At the same time, a part of the mobile biomass b_{mj} is converted to heat shock proteins hsp_j at a rate δ_j. When a certain threshold $hsp_j \geq hsp_{lim}$ is reached, the local cellular processes revert to pre-disturbance levels, i.e. $z_j = 1$, representing the protection of nascent proteins as well as the unfolding or disaggregation of denatured proteins. Assuming self-regulation of heat shock protein production (Tereshina, 2005), the production is set back to $\delta_j = 0$ when $hsp_j \geq hsp_{lim}$.

Therefore,

$$z_j = \begin{cases} 1, & t < t_T \\ 0, & t \geq t_T \; and \; hsp_j < hsp_{lim} \\ 1, & t > t_T \; and \; hsp_j > hsp_{lim} \end{cases}$$

and

$$\delta_j = \begin{cases} 0, & t < t_T \\ \delta_j, & t \geq t_T \; and \; hsp_j < hsp_{lim} \\ 0, & t > t_T \; and \; hsp_j \geq hsp_{lim} \end{cases}$$

Where t_T is the time of the heat pulse treatment.

The partial differential equation system

The processes described above resulted in the following set of equations for a species:

$$\frac{\partial \mathrm{b_r}}{\partial \mathrm{t}} = \overbrace{\mathrm{c} \cdot \mathrm{b_n} \cdot \mathrm{z}}^{\text{rigidifcation}}$$

$$\frac{\partial \mathrm{b_n}}{\partial \mathrm{t}} = \overbrace{\mathrm{D_n}\left(\frac{\partial^2 \mathrm{b_n}}{\partial \mathrm{x}^2} + \frac{\partial^2 \mathrm{b_n}}{\partial \mathrm{y}^2}\right) \cdot \mathrm{z}}^{\text{tip movement}} + \overbrace{\gamma\alpha\pi\mathrm{b_n} \cdot \mathrm{z}}^{\text{immobilization}} - \overbrace{\mathrm{c} \cdot \mathrm{b_n} \cdot \mathrm{z}}^{\text{rigidifcation}}$$

$$\frac{\partial \mathrm{b_m}}{\partial \mathrm{t}} = \overbrace{\mathrm{D_m}\left(\frac{\partial^2 \mathrm{b_m}}{\partial \mathrm{x}^2} + \frac{\partial^2 \mathrm{b_m}}{\partial \mathrm{y}^2}\right) \cdot \mathrm{z}}^{\text{transport of biomass}} - \overbrace{\alpha\pi\mathrm{b_n} \cdot \mathrm{z}}^{\text{immobilization}} + \overbrace{(\lambda_\mathrm{n}\mathrm{b_n} + \lambda_\mathrm{r}\mathrm{b_r})\mathrm{s} \cdot \mathrm{z}}^{\text{substrate uptake}} - \overbrace{\Omega\mathrm{b_m}\mathrm{b_n} \cdot \mathrm{z}}^{\text{inhibitor production}} - \overbrace{\delta\mathrm{b_m}\mathrm{b_n}}^{\text{hsp production}}$$

$$\frac{\partial \mathrm{s}}{\partial \mathrm{t}} = \overbrace{\omega(\mathrm{s_m} - \mathrm{s})}^{\text{replenishment}} - \overbrace{(\lambda_\mathrm{n}\mathrm{b_n} + \lambda_\mathrm{r}\mathrm{b_r})\mathrm{s} \cdot \mathrm{z}}^{\text{substrate uptake}}$$

$$\frac{\partial \mathrm{i}}{\partial \mathrm{t}} = \overbrace{\Omega\chi\mathrm{b_m}\mathrm{b_n} \cdot \mathrm{z}}^{\text{production}} + \overbrace{\mathrm{D_i}\left(\frac{\partial^2 \mathrm{i}}{\partial \mathrm{x}^2} + \frac{\partial^2 \mathrm{i}}{\partial \mathrm{y}^2}\right)}^{\text{inhibitor diffusion}}$$

$$\frac{\partial \mathrm{hsp}}{\partial \mathrm{t}} = \overbrace{\delta\mathrm{b_m}\mathrm{b_n}}^{\text{production}}$$

where

$$\pi = \frac{b_m}{b_n + b_r}$$

We discretized the system of PDEs over a 172 mm by 172 mm grid with one grid cell representing an area of 0.5 mm by 0.5 mm to approximate fungal growth on a petri dish of 86 mm diameter. To calibrate growth and heat shock responses, we simulated species in isolation with initial colonies of 6 mm diameter placed in the center of the dish. For dual cultures, two initial colonies of 6 mm diameter were placed on the horizontal diameter equidistant from each other and the border of the Petri dish. The initial conditions of the state variables describing the

biomass b_{nj}, b_{rj}, b_{mj} were therefore set to b_{n0}, b_{r0}, b_{m0} within a radius of 3mm around the center of the initial colonies, and 0 everywhere else. The substrate s was distributed homogeneously over the grid, and initially no inhibitors i and heat shock proteins hsp were present.

The system was solved numerically with the Finite Element Method using the Package FiPy (Guyer *et al.*, 2009) in Python (Van Rossum and Drake Jr, 1995).

Parameterization

We parameterized our model for two species with experimental data from Wesener *et al.* (2021) of the Mucoromycete *Morteriella elongata*, which is a fast-growing soil fungus with a high stress susceptibility (i.e. a long lag phase after stress), as well as the Basidiomycete *Pleurotus sapidus*, which grows much slower, but has a shorter post-stress lag phase. To do so, we used a trait set from Falconer *et al.* (2008) ($\alpha = 0.87, \lambda_n = 0.97, \lambda_r = 0.1$), but adapted the growth parameter D_n to simulate growth observed in laboratory experiments for the two species. By changing D_n but keeping the biomass conversion rate α fixed, we assumed a growth rate / biomass density trade-off. The parameter regulating the heat shock protein production δ was adapted in a way that the lag phase after the heat pulse matched the lag phases observed in the laboratory. To define the rate of cell wall rigidification c, we took a conversion rate of 0.5 day^{-1} from active to inactive mycelium used by Boswell *et al.* (2002).

Table 4 Model parameters and their description and derivation. Parameters with several values were varied to obtain different scenarios. Numbers 1-4 in brackets refer to the different simulated species

Parameter	Value	Description	Units	How is it derived
λ_n	0.97	Substrate intake of non-insulated biomass	$mm\ day^{-1}$	Falconer et al. (2008)
λ_r	0.1	Substrate intake of rigid biomass	$mm\ day^{-1}$	Falconer et al. (2008)
c	0.5	Conversion of non-insulated to insulated, rigid biomass beyond the extension zone	day^{-1}	Boswell et al. (2002)
D_n	22.6 (1)(3) 1.005 (2) (4)	Diffusion of non-insulated biomass	$mm^2\ day^{-1}$	Calibrated
D_m	$10^{-7} \cdot D_n$	Diffusion of mobile biomass	$mm^2\ day^{-1}$	Falconer et al. (2005)
β	0	Mobilization of biomass	$mol\ mm^{-1} day^{-1}$	Falconer et al. (2008)
α	0.87	Immobilization of biomass	$mol\ mm^{-1} day^{-1}$	Falconer et al. (2008)
γ	1	Efficiency of conversion from mobile to immobile biomass	$mm\ mol^{-1}$	assumed
ω	0.01	Replenishment of external substrate	day^{-1}	Falconer et al. (2005)
s_m	10	Maximum substrate per cell	$mol\ cm^{-2}$	assumed
Competition				
Ω	$0.1/10^{-6}$	Inhibitor production	$mm\ day^{-1}$	Varied
D_i	10/0	Inhibitor diffusion rate	$mm^2\ day^{-1}$	Varied
ψ	0.000001	Resistance to competitor's inhibitor	$mol\ mm^{-2}$	assumed
Heat stress response				
δ	0.119 (1)/0.18 (3) 0.047 (2)/0.028 (4)	Max. production rate of hsp	$mm\ day^{-1}$	calibrated
hsp_{lim}	0.001	Minimal concentration of hsp needed to allow normal process rates	$mol\ mm^{-2}$	calibrated
Initial conditions				
b_{n0}	1	Initial amount of non-insulated biomass	mm^{-1}	assumed
b_{r0}	0	Initial amount of rigid biomass	mm^{-1}	assumed
b_{m0}	1	Initial amount of mobile biomass	$mol\ mm^{-2}$	assumed
s_0	10	Initial amount of substrate	$mol\ mm^{-2}$	assumed
r	6	Radius of initial mycelial plug	mm	assumed

Simulation experiments

To investigate a balanced pair of competitors, we simulated competition between the fast growing but stress susceptible fungus *M. elongata* (diameter expansion of 14.75 mm/day and a stress-induced lag phase of 2.8 days), and the slower but more stress resistant *P. sapidus* (diameter expansion of 3.5 mm/day and a lag phase of 1.93 days). We investigated low and

high inhibitor production ($\Omega = 10^{-6}$ and $\Omega = 0.1$) for both species, as well as the production of diffusible and nondiffusible inhibitors ($D_i = 0$ and $D_i = 10$), leading to four possible response trait combinations per species and therefore 16 possible scenarios on two competing species. To gain a more complete picture, we repeated these 16 scenarios with a competitively rather imbalanced pair, namely a superior fast growing and stress resistant or an inferior slow and stress susceptible species. For this, we exchanged the stress responses of *M. elongata* and *P. sapidus*.

As model output, we determined the qualitative outcome of interaction ten days after inoculation. We differentiated between four types of interaction, namely inhibition at contact, inhibition at a distance, intermingling, as well as partial overgrowth and full overgrowth. Moreover, we determined the ratio of biomass under control conditions compared to biomass under a stress treatment for each species. Here, we differentiated between biomass of a species in areas covered by a single species and total biomass of both species in shared areas. Shared areas occur for the interaction types overgrowth and intermingling. These metrics allowed us to assess the effect of heat stress on the type of interspecific interactions and on competition for space.

Results

After parameterizing and calibrating the PDE model, we systematically varied different response traits of two fungal species exhibiting different growth rates competing for space with and without heat stress. We qualitatively determined interaction types for each scenario 10 days after inoculation. In addition, we calculated the change in biomass under stress conditions for each species in each scenario.

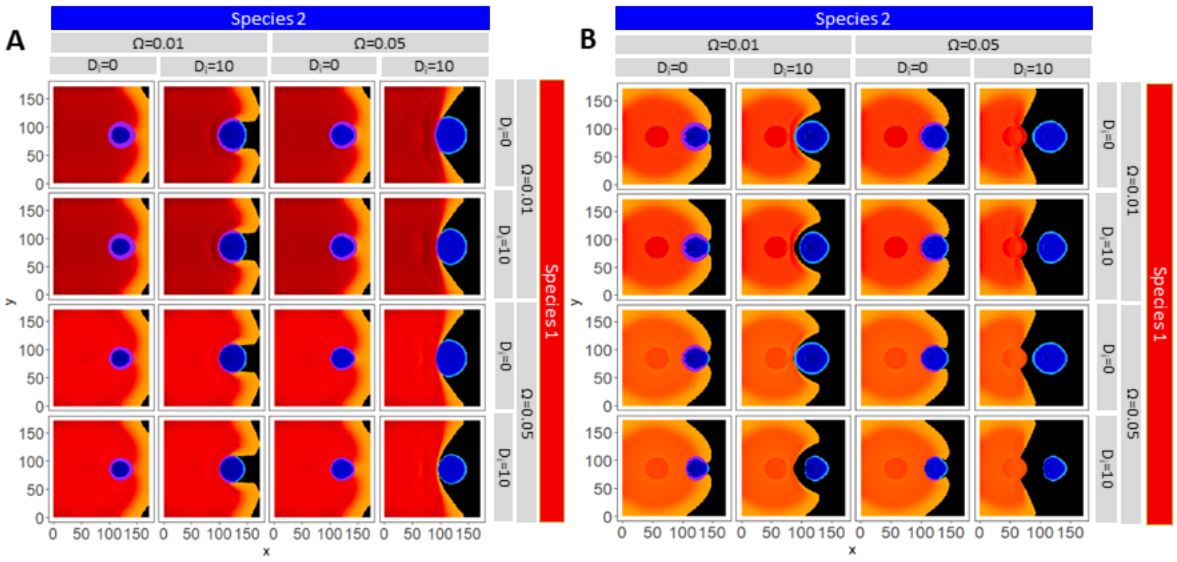


Figure 12 Biomass distribution of two competing fungi for different values of inhibitor production Ω and inhibitor diffusivity Di. Competitive outcomes are shown ten days after inoculation A under control conditions B after experiencing a 2h-heat pulse at day 1. Red/orange tones represent species 1 (M. elongata), blue tones represent species 2 (P. sapidus), purple represents territory that is overgrown, i.e. colonized by both species, and black shows territory that has not been claimed by either species. The biomass of each species is normalized to values between 0 and 1.

Generally, independent of the inhibitor production (Ω) and whether the inhibitors are diffusible (D_i), the fast-growing Species 1 occupies more space than Species 2 because of its higher growth rate (Fig. 12A). At the same time, it suffers more from a heat stress than the slower Species 2, as its colony size is relatively smaller (Fig. 12B and Fig. 13A). Also, for several response trait combinations, the type of interaction between the two fungal colonies changes (Table 2). As we observed different effects of heat stress for inhibitors that diffuse and those that stay in the colony (non-diffusible), we will first describe the effect of diffusible inhibitors vs. non-diffusible inhibitors on competition without heat stress. We will then explain how heat stress affects these findings, and finally show the results for two other species, namely a fast growing and stress resistant Species 3 and the slow growing and stress susceptible Species 4.

Table 5 Changes of interaction types between control and heat treatment for all combinations of Inhibitor production and diffusion. Yes/No refers to whether there was a qualitative change in interaction type.

Qualitative change of interaction type			Slow growing species (Species 2)			
			Low production Ω of inhibitors		High production Ω of inhibitors	
			No diffusion D_i of inhibitors	Diffusion D_i of inhibitors	No diffusion D_i of inhibitors	diffusion D_i of inhibitors
Fast growing species (Species 1)	Low production Ω of inhibitors	Diffusion D_i of inhibitors	No, slight reduction of overgrowth	Yes, partial overgrowth -> contact inhibition	No, reduction of overgrowth	Yes, intermingling -> distance inhibition
		No diffusion D_i of	No, slight reduction of overgrowth	Yes, partial overgrowth -> distance inhibition	No, reduction of overgrowth	Yes, intermingling -> distance inhibition
	High production Ω of inhibitors	Diffusion D_i of inhibitors	No, slight reduction of overgrowth	Yes, partial overgrowth -> contact inhibition	Yes, partial overgrowth -> contact inhibition /intermingling	Yes, intermingling -> distance inhibition
		No diffusion D_i of	No, slight reduction of overgrowth	Yes, partial overgrowth -> distance inhibition	Yes, partial overgrowth -> contact inhibition /intermingling	Yes, contact inhibition -> distance inhibition

Diffusible vs. non-diffusible inhibitors

Under control conditions, diffusion of inhibitors seems to be useful especially for slower growing species. These can use diffusive inhibitors offensively to claim territory that the species has not yet grown on, as shown for the slow-growing Species 2, for which the colony size is bigger and the overgrowth by the other species is smaller in cases with diffusion of inhibitors than without their diffusion (columns $D_{i2} = 0$ and $D_{i2} = 10$ of Fig. 12A). In contrast, the fast-growing Species 1 does not gain additional territory when producing diffusible compared to non-diffusible inhibitors (Fig. 12A, $D_{i1} = 10$ vs. $D_{i1} = 0$), because it follows a strategy of fast biomass expansion. The production and secretion of diffusible inhibitors does not grant much territory relative to the colony size.

Without diffusion, inhibitors take on a defensive role and, especially for slower growing species, reduce the effect of overgrowth depending on the amount of inhibitors produced (Fig.

12A, $D_{i2} = 0$, columns $\Omega_2 = 10^{-6}$ vs. $\Omega_2 = 0.1$). For the fast-growing Species 1, a higher amount of inhibitor production does not change the qualitative outcome, as long as the low amount can reach inhibitory concentrations. However, the overall biomass of Species 1 is lower for higher production rates, because more resources are divested from biomass production (Fig. 12A, rows $\Omega_1 = 10^{-6}$ vs. $\Omega_1 = 0.1$)).

Effect of heat pulse

Heat stress can induce the change from contact inhibition to inhibition at distance if the production of diffusible inhibitors is high enough (Table 2). Under stress, the inhibitors of the slow Species 2 can diffuse further while both species are in the post-stress lag phase, especially when produced in a high amount (Fig. 12B, columns $D_{i2} = 10$ and $\Omega_2 = 0.1$).

However, the response of the faster Species 1 modulates the interaction: When both species produce diffusible inhibitors, they "block" each other from claiming free territory (See $D_{i1} = 10$ and $D_{i2} = 10$ in Fig. 12B). When Species 1 does not produce diffusible inhibitors, the slower Species 2 benefits and can even produce more biomass under a stress treatment compared to the control (See $D_{i1} = 0$ and $D_{i2} = 10$ in Fig. 13). This happens only if production is not too high, as high costs manifested as resource divestment outweigh the benefits (Appendix C, Fig. C2, $D_{i2} = 10$, $\Omega_2 = 10^{-6}$ vs. $\Omega_2 = 0.1$).

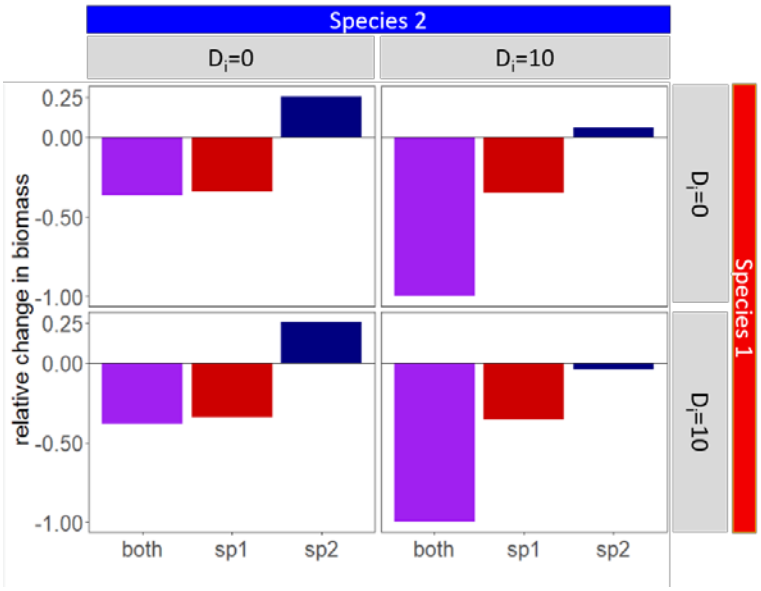


Figure 13 Effect of heat stress on the biomass of the two competitors. Relative change of total biomass under stress treatment compared to control conditions ten days after inoculation for low inhibitor production $\Omega=10^{-6}$ and a lag phase of 2.8 days (fast species 1), or 1.9 days (slow species 2). Positive values indicate a gain in biomass after stress, and a value of -1 indicates a reduction to zero. Red and blue bars refer to the cumulative biomass of species 1 and 2 (sp1 and sp2), respectively, and purple bars refer to biomass that is on shared territory, i.e. where one species has overgrown the other.

The defensive effect of non-diffusible inhibitors for the slow Species 2 is increased under heat treatment as inhibitors have more time to accumulate and thus decrease overgrowth (Fig. 13) which can even be reduced to a deadlock (Table 2).

Sensitivity towards heat stress resistance

In the prior experiment, a fast growing but stress-susceptible (Species 1) and a slow-growing but stress-resistant species (Species 2) were grown in direct competition. In a second step, we exchanged the stress resistance of both investigated species, leading to a superior fast growing and stress-resistant (Species 3) and an inferior slow-growing and stress-susceptible species (Species 4), to find out whether the heat resistance determined the benefit of inhibitor production. Surprisingly, in all cases, the respective interaction type did not change qualitatively (Fig. C1). This shows that the inhibitors that had been produced before the species start interacting influenced the resulting interaction type rather than the stress resistance of a species. That is, if a colony has already produced enough inhibitors to suppress the other species, these are also effective during its phase of no growth.

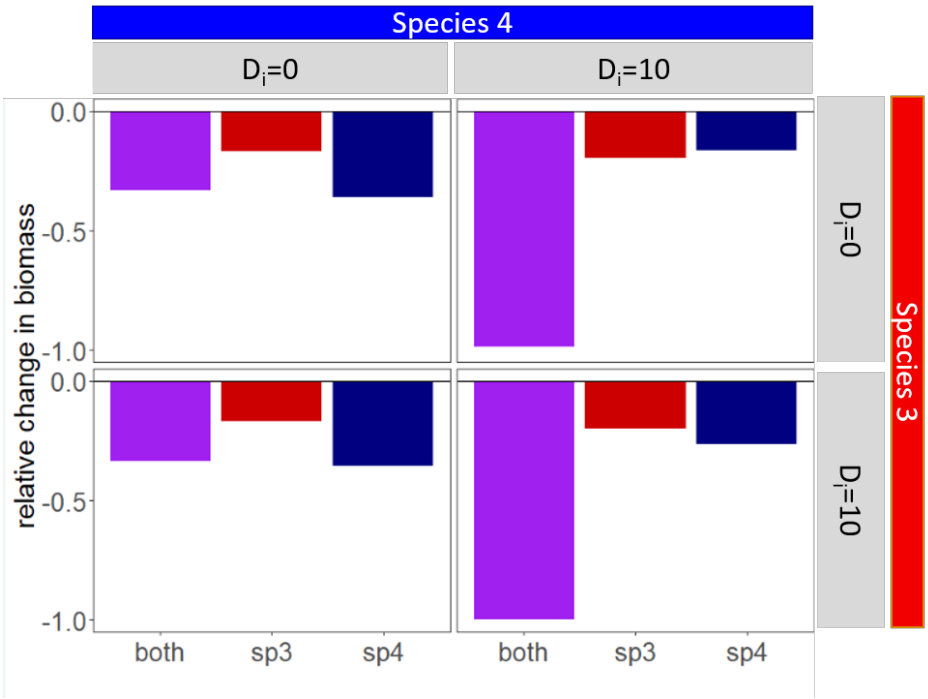


Figure 14 Effect of heat stress on the biomass of the two competitors. Relative change of total biomass under stress treatment compared to control conditions ten days after inoculation for low inhibitor production $\Omega=10^{-6}$ and a lag phase of 1.9 days (fast species 3), or 2.8 days (slow species 3). Positive values indicate a gain in biomass after stress, and a value of -1 indicates a reduction to zero. Red and blue bars refer to the cumulative biomass of species 3 and 4 (sp3 and sp4), respectively, and purple bars refer to biomass that is on shared territory, i.e. where one species has overgrown the other.

Quantitatively, the fast growing and more stress resistant Species 3 suffered less from the impacts of the heat pulse than if it was stress-susceptible, as it builds up more biomass (Fig.

14). Still, unlike the slow growing and stress resistant Species 2, it never gains any biomass when stress resistant compared to control conditions. Because Species 1 and 3 focus on rapid colony extension, a lag phase of no growth is very disadvantageous and the loss in biomass cannot compensated for by a high stress resistance and earlier inhibitor production. The slow and stress susceptible Species 4 builds up less biomass under stress for all scenarios. Because the colony cannot produce defensive compounds when the cellular processes are halted during heat stress, the time lag has a large impact. However, even though it is slower and more stress susceptible, Species 4 may still suffer less from stress than the fast and more stress resistant Species 3: When Species 4 produces low amounts of diffusible compounds and Species 3 does not (Fig. 14), the diffusible inhibitors acting as heat-stable agents are especially beneficial and can stop fast growing and stress resistant species locally from claiming territory.

Discussion

The PDE model could reproduce the typical response of a lag phase after a heat pulse as well as changing fungal interaction types under heat stress. We varied the production of antifungal compounds and heat stress proteins and could show that the change in interaction type as well as the gain in biomass under heat stress conditions differed greatly for different fungal response trait combinations.

The effect of heat stress on competition for space

Our result show that under control conditions, the production of antifungal compounds is most beneficial for slower growing species, which can claim territory they cannot quickly overgrow, or build up a defense against overgrowth by faster species. Conversely, ruderal species focusing on fast colony expansion and primary resource capture gain less territory relative to their colony size than slower species if they invest into the production of inhibitors. However, even ruderal species can produce diffusible inhibitors to counter opponents that also exude inhibitors.

Under heat stress, these benefits have increased. We found that the increase in benefit does not necessarily depend on an effective stress response, i.e. how fast heat shock proteins can be produced, but mostly on a species' general strategy of resource capture, i.e. whether a species is a fast or slow growing species.

Combative mechanisms function aggressively and/or defensively (Crowther *et al.*, 2014; Hiscox and Boddy, 2017) and are sometimes divided into a competitive effect and a competitive response (Boddy, 2000). Our model includes these two mechanisms. If slow growing species follow a defensive strategy and produce inhibitors locally, they benefit from heat stress if they can gain time to build up resistance and stop competitors from overgrowing them. This is in line with a study on fungal secondary metabolite production, in which a mutant exhibiting reduced secondary metabolism was overgrown by its competitor, while the wild type formed a deadlock (Knowles *et al.*, 2019). Especially for sessile organisms which form spatially structured communities, competitive exclusion mostly happens via interference competition (in this case, overgrowth) (Maynard *et al.*, 2017) and a build-up of defensive compounds within the fungal colony is of high benefit.

A slow-growing species that exudes diffusive inhibitors and thus follows a more offensive strategy can still profit from a heat stress even when its stress resistance is lower than that of their fast-growing opponents. If they have produced enough antifungal compounds before a stress-induced lag phase, these compounds still diffuse and block territory that a fast competitor might have claimed if not stress-lagged. This dependence on an early claim of territory is thus subject to priority effects.

In earlier studies, we have shown the importance of priority effects in competition if heat stress was applied to microbial communities (Wesener and Tietjen, 2019; Wesener *et al.*, 2021). Here we can show that these results are also applicable to the production of antifungal compounds. We show that not only fast growing and stress resistant species that can quickly claim territory after as short lag phase benefit from priority effects. Slower species might benefit as well if they are able to produce and exude sufficient amounts of inhibiting compounds, even when they exhibit a longer post-stress lag phase. By giving species different lag-induced temporal advantages, priority effects play a role for community assembly as well as in an established community after short, non-lethal disturbances. This is in accordance with studies showing that temperature changes might counteract priority effects that would occur under constant conditions (Tucker and Fukami, 2014).

However, priority effects might be promoted by small habitat sizes or other factors that favor fast community dynamics (Fukami, 2004), some of which apply under laboratory conditions and might bias our model results. Still, many studies confirm the importance of priority effects in controlled natural experiments (Mergeay *et al.*, 2011; Hiscox *et al.*, 2015).

Faster species benefit less from the stress-induced lag phase that grants time for inhibitor diffusion and the build-up of an inhibitor field. In our model, this is not caused by a colonization-competition trade-off (Kennedy *et al.*, 2011; Maynard *et al.*, 2019) mediated by a divestment of resources, or stress-induced gene silencing (Gasch, 2007), but rather by the fact that a ruderal species focusing on fast colonization gains less relative territory when additionally exuding inhibitory compounds. In this study, we investigate the interplay of heat defense and competition at the ecological level and thus do not see this as a contradicting result, but rather as an additional factor that should be considered when investigating colonization-competition trade-offs.

Changing Interaction Types

In our model, the dynamics of the inhibitor field produced by a fungus determined the type of interaction between competitors. We could show that heat stress impacts the production and distribution of inhibitor fields and thus in many cases can lead to altered interaction types. A post stress phase of reduced or no growth can lead to bigger inhibitor fields or higher inhibitor concentration in faster recovering colonies and thus reduces the overall amount of overgrown territory, favoring contact and distance inhibition. Hiscox *et al.* (2016) found that for most pairs the effect of overgrowth increased under elevated temperatures, which could mean that intracellular and diffusible antifungal compounds are only one factor determining interactions between fungi. However, they did not investigate the effect of heat pulses that induce fungistatic lag phases, but rather the effect of continuous temperature of up to 30°C. Also, they evaluated interaction types several months after inoculation. Interactions have been shown to change weeks after a competitive outcome has been determined (White and Boddy, 1992). Active degradation of antifungal compounds or the effect of nutrient depletion might play a role and could be integrated in the existing PDE model.

Strengths & limitations of the model

With the PDE model presented here, we were able to produce different interaction types, such as overgrowth and inhibition, and thus include secondary resource capture in our analyses of heat effects on fungal communities, which is an important aspect of fungal competition describing a way to deprive opponents of resources in their own territory. Moreover, the extension rate of a fungal colony is separate from its biomass production, as it has been shown to be independent of each other (Heilmann-Clausen and Boddy, 2005; Maynard *et al.*, 2017). This is particular important as some combat strategies depend on local biomass production,

such as the formation of barriers to keep competitors from overgrowing (White and Boddy, 1992; Hiscox and Boddy, 2017). This could be implemented in the model, and it would likely influence the results. This defensive strategy might benefit a species that can fend off inhibitors of antagonists, and would thus decrease the efficacy of inhibitor production. Combativeness of a fungus is a trait complex consisting of many response traits (Crowther *et al.*, 2014), and entangling the contributions of these single traits will help understanding fungal competition.

In this study, we did not aim for reproducing the behavior of certain species, but understanding the effect of different traits on competitive dynamics. Fungal traits are an important metric to obtain conceptual outlines of fungal community development and functioning, as they provide generality and predictability (Crowther *et al.*, 2014; Aguilar-Trigueros *et al.*, 2015). Especially because traits within functional groups are plastic (Naeem and Wright, 2003), and many species show different traits and also different life strategies (ruderal, combative, or stress-tolerant) at different development stages and in different environments (Pugh and Boddy, 1988), a focus on traits is more informative than predictions based on taxonomic classification. By not parameterizing our model for several species but instead varying response trait combinations in a full-factorial manner, we aim to add to this generalist trait-based approach.

We present the first PDE model to include induced stress defenses into a simulation of fungal growth. The model could show how the interplay of inhibitor dynamics, heat stress response and fungal growth strategy influenced fungal combative ability. We observed shifts of interaction types under heat stress and showed that this change was independent of the heat stress defenses of the competing fungi. With our study, we provide an important step towards a precise understanding of fungal secondary metabolism as well as stress defense mechanisms as the base of fungal interactions at the ecological level.

Acknowledgements

We are grateful to the German Research Foundation (DFG) for funding our Collaborative Research Centre 973 ‘Priming and Memory of Organismic Responses to Stress’ (www.sfb973.de).

We thank the high-performance computing service of the Freie Universität Berlin (ZEDAT) for providing the resources for running the simulation experiments.

References

A'Bear, A.D., Crowther, T.W., Ashfield, R., Chadwick, D.D.A., Dempsey, J., Meletiou, L., et al. (2013) Localised invertebrate grazing moderates the effect of warming on competitive fungal interactions. Fungal Ecol 6: 137–140.

Aguilar-Trigueros, C.A., Hempel, S., Powell, J.R., Anderson, I.C., Antonovics, J., Bergmann, J., et al. (2015) Branching out: Towards a trait-based understanding of fungal ecology. Fungal Biol Rev 29: 34–41.

Allison, S.D. and Martiny, J.B.H. (2008) resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A 105 Suppl: 11512–9.

Boddy, L. (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol Ecol 31: 185–194.

Boswell, G.P. (2012) Modelling combat strategies in fungal mycelia. J Theor Biol 304: 226–234.

Boswell, G.P., Jacobs, H., Davidson, F.A., Gadd, G.M., and Ritz, K. (2003) A positive numerical scheme for a mixed-type partial differential equation model for fungal growth. Appl Math Comput 138: 321–340.

Boswell, G.P., Jacobs, H., Davidson, F.A., Gadd, G.M., and Ritz, K. (2002) Functional consequences of nutrient translocation in mycelial fungi. J Theor Biol 217: 459–477.

Crowther, T.W., Maynard, D.S., Crowther, T.R., Peccia, J., Smith, J.R., and Bradford, M.A. (2014) Untangling the fungal niche: the trait-based approach. Front Microbiol 5: 1–12.

Davidson, F.A., Sleeman, B.D., Rayner, A.D.M., Crawford, J.W., and Ritz, K. (1996) Context-dependent macroscopic patterns in growing and interacting mycelial networks. Proc R Soc London Ser B Biol Sci 263: 873–880.

Davidson, F.A., Sleeman, B.D., Rayner, A.D.M., Crawford, J.W., and Ritz, K. (1997) Travelling waves and pattern formation in a model for fungal development. J Math Biol 35: 589–608.

Edelstein-Keshet, L. and Ermentrout, B. (1989) Models for Branching Networks in Two Dimensions. SIAM J Appl Math 49: 1136–1157.

Edelstein, L. (1982) The propagation of fungal colonies: a model for tissue growth. J Theor Biol.

Edelstein, L. and Segel, L.A. (1983) Growth and metabolism in mycelial fungi. J Theor Biol.

Elbein, A.D., Pan, Y.T., Pastuszak, I., and Carroll, D. (2003) New insights on trehalose: A multifunctional molecule. Glycobiology 13: 17–27.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2007) Biomass recycling: a key to efficient foraging by fungal colonies. Oikos 116: 1558–1568.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2005) Biomass recycling and the origin of phenotype in fungal mycelia. Proc R Soc B Biol Sci 272: 1727–1734.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2008) Modelling interactions in fungi. J R Soc Interface 5: 603–15.

Fukami, T. (2004) Assembly history interacts with ecosystem size to influence species diversity. Ecology 85: 3234–3242.

Gasch, A.P. (2007) Comparative genomics of the environmental stress response in ascomycete fungi. Yeast 24: 961–976.

Guyer, J.E., Wheeler, D., and Warren, J.A. (2009) FiPy: Partial Differential Equations with Python. Comput Sci Eng 11: 6–15.

Heilmann-Clausen, J. and Boddy, L. (2005) Inhibition and stimulation effects in communities of wood decay fungi: Exudates from colonized wood influence growth by other species. Microb Ecol 49: 399–406.

Hiscox, J., Baldrian, P., Rogers, H.J., and Boddy, L. (2010) Changes in oxidative enzyme activity during interspecific mycelial interactions involving the white-rot fungus Trametes versicolor. Fungal Genet Biol 47: 562–571.

Hiscox, J. and Boddy, L. (2017) Armed and dangerous – Chemical warfare in wood decay communities. Fungal Biol Rev 31: 169–184.

Hiscox, J., Clarkson, G., Savoury, M., Powell, G., Savva, I., Lloyd, M., et al. (2016) Effects of pre-colonisation and temperature on interspecific fungal interactions in wood. Fungal Ecol 21: 32–42.

Hiscox, J., Savoury, M., Müller, C.T., Lindahl, B.D., Rogers, H.J., and Boddy, L. (2015) Priority effects during fungal community establishment in beech wood. ISME J 9: 2246–2260.

Kennedy, P.G., Higgins, L.M., Rogers, R.H., and Weber, M.G. (2011) Colonization-Competition Tradeoffs as a Mechanism Driving Successional Dynamics in Ectomycorrhizal Fungal Communities. PLoS One 6: e25126.

Klepzig, K.D. and Wilkens, R.T. (1997) Competitive interactions among symbiotic fungi of the southern pine beetle. Appl Environ Microbiol 63: 621–627.

Knowles, S.L., Raja, H.A., Wright, A.J., Lee, A.M.L., Caesar, L.K., Cech, N.B., et al. (2019) Mapping the fungal battlefield: Using in situ chemistry and deletion mutants to monitor interspecific chemical interactions between fungi. Front Microbiol 10: 1–15.

Liberek, K., Lewandowska, A., and Ziętkiewicz, S. (2008) Chaperones in control of protein disaggregation. EMBO J 27: 328–335.

Marx, F. (2004) Small, basic antifungal proteins secreted from filamentous ascomycetes: A comparative study regarding, expression, structure, function and potential application. Appl Microbiol Biotechnol 65: 133–142.

Maynard, D.S., Bradford, M.A., Covey, K.R., Lindner, D., Glaeser, J., Talbert, D.A., et al. (2019) Consistent trade-offs in fungal trait expression across broad spatial scales. Nat Microbiol 4: 846–853.

Maynard, D.S., Bradford, M.A., Lindner, D.L., van Diepen, L.T.A., Frey, S.D., Glaeser, J.A., and Crowther, T.W. (2017) Diversity begets diversity in competition for space. Nat Ecol Evol 1: 156.

Mergeay, J., De Meester, L., Eggermont, H., and Verschuren, D. (2011) Priority effects and species sorting in a long paleoecological record of repeated community assembly through time. Ecology 92: 2267–2275.

Naeem, S. and Wright, J.P. (2003) Disentangling biodiversity effects on ecosystem functioning: Deriving solutions to a seemingly insurmountable problem. Ecol Lett 6: 567–579.

Plesofsky-Vig, N. and Brambl, R. (1995) Disruption of the gene for hsp30, an alpha-crystallin-related heat shock protein of Neurospora crassa, causes defects in thermotolerance. Proc Natl Acad Sci 92: 5032–5036.

Prospero, S., Holdenrieder, O., and Rigling, D. (2003) Primary resource capture in two sympatric Armillaria species in managed Norway spruce forests. Mycol Res 107: 329–338.

Pugh, G.J.F. and Boddy, L. (1988) A view of disturbance and life strategies in fungi. Proc R Soc Edinburgh Sect B Biol Sci 94: 3–11.

Van Rossum, G. and Drake Jr, F.L. (1995) Python tutorial. Tech Rep CS-R9526, Cent voor Wiskd en Inform.

San-Lang, W., Shih, I.L., Wang, C.H., Tseng, K.C., Chang, W.T., Twu, Y.K., et al. (2002) Production of antifungal compounds from chitin by Bacillus subtilis. Enzyme Microb Technol 31: 321–328.

Schoeman, M.W., Webber, J.F., and Dickinson, D.J. (1996) The effect of diffusible metabolites of Trichoderma harzianum on in vitro interactions between basidiomycete isolates at two different temperature regimes. Mycol Res 100: 1454–1458.

Sena, A.R., Júnior, G.L.V., Neto, A.G., Taranto, A.G., Pirovani, C.P., Cascardo, J.C.M., et al. (2011) Production, purification and characterization of a thermostable β-1,3-glucanase (laminarinase) produced by moniliophthora perniciosa. An Acad Bras Cienc 83: 599–609.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., et al. (2012) Fundamentals of Microbial Community Resistance and Resilience. Front Microbiol 3: 417.

Singer, M.A. and Lindquist, S. (1998) Thermotolerance in Saccharomyces cerevisiae: The Yin and Yang of trehalose. Trends Biotechnol 16: 460–468.

Stahl, P.D. and Christensen, M. (1992) In vitro mycelial interactions among members of a soil microfungal community. Soil Biol Biochem 24: 309–316.

Taechowisan, T., Peberdy, J.F., and Lumyong, S. (2003) Chitinase production by endophytic Streptomyces aureofaciens CMUAc130 and its antagonism against phytopathogenic fungi. Ann Microbiol 53: 447–461.

Tereshina, V.M. (2005) Thermotolerance in fungi: The role of heat shock proteins and trehalose. Microbiology 74: 247–257.

Trinci, A. (1978) Wall and hyphal growth. Bull Br Mycol Soc 12: 116–122.

Tucker, C.M. and Fukami, T. (2014) Environmental variability counteracts priority effects to facilitate species coexistence: Evidence from nectar microbes. Proc R Soc B Biol Sci 281:.

Wesener, F., Szymczak, A., Rillig, M.C., and Tietjen, B. (2021) Stress priming affects fungal competition - evidence from a combined experimental and modelling study. Environ Microbiol 00: 1–12.

Wesener, F. and Tietjen, B. (2019) Primed to be strong, primed to be fast: modeling benefits of microbial stress responses. FEMS Microbiol Ecol 95: 1–9.

White, N.A. and Boddy, L. (1992) Extracellular enzyme localization during interspecific fungal interactions. FEMS Microbiol Lett 98: 75–79.

CHAPTER V

General discussion

In this thesis, I took several steps to investigate induced stress defenses in fungal communities. I started with a conceptual approach evaluating the benefit of different primed response strategies (Chapter II). I transferred these findings from the population to the community level and showed that the benefits of these strategies changed under competition. Then, I assessed the effect of priming in a spatially explicit model of a fungal pair competing for space for different trait combinations (Chapter III). This assessment revealed that priming was not necessarily more beneficial in the community context compared to isolation, but that the benefit depended on the traits of the community members. Finally, I considered the production of antifungal compounds and heat shock proteins (Chapter IV). I could show that heat stress affected fungal competition and interaction types by influencing the build-up and spread of inhibitors, but that the type of interactions (e.g. overgrowth or inhibition) was mostly influenced by growth, not the stress defense.

In the following, I will discuss several insights from my studies that I deem important for the understanding of an induced defense at the level of the community. First, I will discuss the costs and benefits of an induced defense, and how they may change under competition compared to isolation. I will then move on to talk about the interplay of an induced stress defense and the community, covering several aspects that influence the dynamics of a community under heat stress. Finally, I will give an overview of potential future research directions for which my models may be useful tools.

Costs and benefits of induced stress defenses

Induced defenses are a cost saving strategy, as investments are only made on an encounter with a stress. Priming is further enhancing this mechanism (Mitchell and Pilpel, 2011; Heil, 2014; Hilker *et al.*, 2016). During the development of my simulation models, I could not find any literature that quantified the costs of induced defenses in microbes, and the question arose how to implement and scale the costs of induced stress defenses. It is a principle of ecological theory that any strategy that entails a benefit must come with some costs or risk, otherwise an inducible

defense could just be expressed constitutively (Harvell, 1990). Therefore, the investments that come with any stress defense are an important factor in assessing the benefit of a strategy.

Costs and benefits in isolation

Because of the lack in literature on the scale and realization of priming costs, or microbial defense costs in general, I varied allocation costs (i.e. the divestment of resources) realized as growth rate in my analysis of Chapter II. My results revealed that costs did not affect which of the different stress responses were most beneficial. However, priming costs controlled whether an organism benefitted from priming at all. The costs paid off for longer or more detrimental stresses, because then the priming benefit was highest.

I could not find evidence for reduced growth of fungi that had experienced a priming cue in the data that I had received (Chapter III). This led to the hypothesis that costs of priming and other induced defenses might not be realized as reduced growth rate but possibly as another reduced fitness component, such as spore production, biomass density, or production of secondary metabolites. This assumption was affirmed in Chapter IV: When implementing the processes underlying a stress defense, I did not have to explicitly impose any costs. The production of heat shock proteins led to a divestment of the resources that would otherwise have been invested in the production of biomass or defense compounds. As predicted, the costs barely manifested as significantly reduced growth, but rather in lower colony density (i.e. less dense hyphae) and lower inhibitor production. This is in line with the claim that costs of induced defenses take on many forms and are hence difficult to separate and quantify (Harvell, 1990).

Costs and benefits in the community

In Chapter II, I showed that the negative effect of allocation costs on fitness was much smaller in the community, especially for an early stress response. Reacting earlier to a stress than an opponent granted a high benefit that would in many cases compensate even higher priming costs (causing a priority effect). However, I found that in some cases, allocation costs of a defense could be the factor differentiating between a benefit and a disadvantage. For example, a slow growing species that invested too much in building up a defense would benefit less than a fungus that only produced moderate amounts of defense compounds but could produce more biomass instead (Chapter IV). In addition, the costs of a primed response were dependent on how likely a stress was to occur after a priming stimulus (Chapter II). Under community conditions, it is likely that the allocation costs paid for a primed response are combined with

opportunity costs (temporary reduction of fitness that cannot be made up for, Heil, 2002) when an organism had prepared for a stress that never occurred, while other competitors had not prepared. I thus conclude that costs in a community pay off more easily, but also come with a higher risk due to the possibility of opportunity costs.

In contrast to the model used in Chapter II, I did not implement any allocation costs of stress priming into the Cellular Automaton (Chapter III), and only compared the relative effects of traits on the benefit. However, I found that in some cases priming was not beneficial: If a community member benefitted from priming, but its competitor benefitted even more, the overall effect of priming could be disadvantageous to that species. For example, a fungus that benefits from priming in isolation could perform worse with than without priming stimulus if it faced a more stress-susceptible and primeable species that benefitted more from priming. Here, it is important to differentiate between stress defense of the individual, which would not be disadvantageous without allocation costs, and of the community, which depends on the diverse responses of all community members that experience a priming stimulus. Again, this shows that while at the individual level priming can be beneficial, it entails a risk at the community level.

Simulation models proved to be very useful for investigating defense costs, as they allow to dissect different types of costs by "turning off" certain types, e.g. allocation costs. I could show that even though allocation costs are more easily compensated for under competition, they might control whether a stress response is beneficial or not. Additional costs not observed in isolation, like opportunity costs, as well as the benefit of other community members, can negatively affect benefits under competition. This shows that an induced defense in the community context is a strategy that is of higher risk, but also of a higher possible reward, than in isolation.

The interplay of induced stress responses and the community

In this thesis, I have been able to show that the benefit of priming in isolation does not directly transfer to the community context. In the following, I will discuss several aspects of community ecology that interact with induced defenses: Competition between community members, community assembly (the immigration of species into a community), and community structure (the species composition in a community). For greater clarity, I will discuss these aspects separately, even though they cannot be completely disentangled. Community assembly, for

example, affects community structure (Fukami *et al.*, 2010), and is in turn affected by competition between species (Hiscox *et al.*, 2015).

Competition

In their review, Crowther *et al.* (2014) suggest that fungal combativeness is controlled by traits such as growth rate and toxic secondary metabolite production. The results of my research corroborate this proposition, as they showed that a high growth rate granted dominance that could partly be countered by production of antifungal compounds. However, environmental conditions are never static and I propose to include the ability to respond to environmental fluctuations in the set of traits influencing fungal competition. My claim is based on the following two findings: First, in Chapter III, I showed that a primed response can shift the competition for space between two species towards one or the other competitor. The shift occurred for different combinations of growth rates, depending on the primeability of the competitors. This shows a high degree of control that an induced stress defense can exert over competitive outcomes. Second, my findings in Chapter IV indicate that a heat pulse does not only favor a high stress resistance, but might also function as an environmental filter promoting the production of antifungal compounds. Slower growing species under the effect of heat could build up a better defense against overgrowing activity if they had a short lag phase, and diffusible compounds could block territory more effectively when the competitors were lagged due to a heat pulse. This reveals the interplay of stress defense effects and competition.

Induced stress defenses thus do not only affect competition by favoring stress-resistant species, but directly interact with combative traits such as growth and inhibitor production. This is an important aspect to take into account when assessing the competitive strength of community members.

Community assembly

Especially during community assembly, priority effects are important, when space and nutrients are still abundant and an early claim grants a high benefit (Fukami, 2015). My analysis of primed response types in Chapter II was in line with this statement and showed that in a well-mixed and resource limited community, the early response strategy was very beneficial. This conceptual response type was found in soil fungi (Chapter III), where priming grants fungi an earlier return to growth after a disturbance. Taking into account the spatially structured competition of soil fungi, I could show that induced defenses had a large impact on priority

effects in the community. A primed response that grants an earlier response was very beneficial under competition for space. This benefit was highest when a primed species with an earlier response faced a fast growing, less primeable species, because then a head start induced by priming would make a large difference for the slower species. For filamentous fungi, being early is important: competitive outcomes have been shown to correlate with timing (Kennedy *et al.*, 2009), because as soon as a territory is occupied by a competitor, a fungus has to invest in secondary resource capture (Heilmann-Clausen and Boddy, 2005; Hiscox *et al.*, 2015), which is costly. When including the production of antifungal compounds and spatially discrete interaction types (Chapter IV), the priority effect played an important role in the production of secondary metabolites as well. Even when stress-lagged, a species could claim territory that would be overgrown by faster competitors, as long as its antifungal compounds reached a territory before its competitor. This strategy again was most beneficial for slower species that can enhance their range using diffusible compounds.

My findings are in line with other studies that have found an effect of temperature fluctuations on community assembly via priority effects (Tucker and Fukami, 2014; Hiscox, Clarkson, *et al.*, 2016) and showed that variable temperature altered or even weakened the effect of the timing of species arrival. I could specify the effects of heat stress on community assembly observed in other studies and identified induced direct defenses as an important factor for fungal community assembly via priority effects, which could be further enhanced by strategies such as priming. This influence is applicable to the expansion of biomass as well as the production of secondary metabolites.

Community structure

Several studies showed that heat disturbances can have a positive effect on biodiversity in microbial communities (Heilmann-Clausen and Boddy, 2005; Toljander *et al.*, 2006). This is in agreement with the results of my thesis, which suggested that induced defenses granting a head start favor less competitive, slower growing species. They can thus have an equalizing effect promoting coexistence and increasing the diversity of a community. Additionally, in Chapter IV, I showed that a deadlock between competitors was a more common outcome after a heat stress than under control conditions, because a species could gain additional time to accumulate antifungal compounds and stop competitors from overgrowing them. As competitive exclusion in fungal communities is often realized via overgrowth (Maynard *et al.*,

2017), this result also promotes the idea that a heat stress can favor biodiversity by decreasing the exclusion of community members.

Another way heat stress can influence community composition is via legacy effects, which describe the changes of community composition that persist after a disturbance (Meisner *et al.*, 2018; Ballauff *et al.*, 2020). For example, a heat stress event can impact community composition by excluding susceptible community members, leading to a community composed of mostly resistant species (Jurburg *et al.*, 2017a). I want to include the effect of priming into this framework, as priming can benefit otherwise stress-susceptible species (Chapter III) and protect them from exclusion. Thus, a post-stress community that would be dominated by stress-resistant species might result in very different composition if subjected to a priming stimulus before. Other findings support this, connecting plastic responses with higher compositional stability of a community (Shade *et al.*, 2012).

My results revealed that induced defenses are an important driver of community structure under heat stress. It is unclear whether changes in community structure caused by disturbances will have long-term effects (Schimel *et al.*, 2007), or whether communities are likely to return to pre-stress composition (Jurburg *et al.*, 2017b). I thus want to stress the need of more long-term studies including priming and other induced defenses to assess lasting effects that defense mechanisms at the species level can have on the community.

Outlook

Additional factors influencing fungal interactions

Even though competition for space is influenced by heat stress defenses, I could not determine an effect of these defenses on the qualitative interaction between fungi (such as overgrowth or deadlock). While the results of my thesis support the findings that raised temperatures can change fungal interaction types (Schoeman *et al.*, 1996; A'Bear *et al.*, 2012; Hiscox, Clarkson, *et al.*, 2016), this change depended mostly on growth rates and the production and type of secondary metabolites. However, to determine the qualitative outcome of fungal interactions, additional factors might play a role, such as volatile organic compounds (Guo *et al.*, 2021). Other modes of combat between fungi might reduce the impact of diffusing antifungal compounds on competition. For example, the active degradation or inhibition of secondary metabolites has been shown to change patterns of inhibitor distribution (Hiscox *et al.*, 2010), or change their effect over time (Hiscox and Boddy, 2017). Mycelial barriers (Rayner *et al.*,

1994) may shield a species from toxins and thus reduce their efficacy. Finally, the translocation of nutrients to an interaction site can constitute a local supply of resources and affect competition (Lindahl *et al.*, 2001). This could especially play a role in nutrient-poor environments (Hiscox *et al.*, 2010) and would favor bigger colonies that have access to a larger pool of resources.

This study is an important contribution to the understanding of fungal combative ability. Identifying more traits that form this trait complex (Crowther *et al.*, 2014) will help to further comprehend the competitive dynamics in fungal communities. Because one could stepwise implement the different traits described above, simulation models can provide a useful means to systematically assess their impact.

Interpolation to higher order communities

This study focused on pairwise interactions between fungal competitors to assess the mechanisms underlying fungal competition and interaction types at a low level of complexity. While some studies found that competitive outcomes for multispecies communities were similar to those of pairwise cultures (Robinson *et al.*, 1993), most others reported difficulties in interpolating from pairs to three-species communities (Boddy, 2000; Hiscox *et al.*, 2017). In communities of higher order, a third species can modify the interaction of two other species, and new competitive dynamics can shape the community (Levine *et al.*, 2017; Maynard *et al.*, 2017). For example, many studies observe intransitive relationships, i.e. rock-paper-scissor like dynamics, that can result in the survival of weaker individuals and promote coexistence (Hiscox *et al.*, 2017), but can also have destabilizing effects (Gallien *et al.*, 2017).

To which extent one can transfer the results from dual cultures to more complex fungal communities remains unclear, and efforts should be undertaken to shed light on this matter. Extending the simulation models of this thesis by a third species and comparing the effect of induced defenses to the results of this study could, with higher computational demand, be an important step towards a better comprehension of complex fungal communities.

Interpolation to three-dimensional growth

While planar growth of fungi occurs in nature, e.g. on mineral surfaces (Gorbushina, 2007), in the soil, fungal colonies can develop unconstrained in all directions. Soil fungal communities therefore usually develop in three-dimensional space and might exhibit dynamics that are very different from growth on flat agar medium. A study on wood decaying fungi compared growth

in wood blocks arranged in a line, a plane or a cube (O'Leary *et al.*, 2018). This revealed that dimensionality influences competitive outcomes and especially affects transitive relationships in three-species communities. Several simulation models replicated fungal growth in three dimensions for one (Meškauskas *et al.*, 2004; Vidal-Diez de Ulzurrun *et al.*, 2017) or two species (Falconer *et al.*, 2008), but a systematic assessment of the effect of three dimensional space on fungal competition is still lacking.

To reliably reproduce and predict soil fungal community dynamics, it is essential to know to which degree one can transfer results from simulation models and laboratory experiments to natural observations. The PDE model presented in Chapter IV could be extended with an additional dimension to investigate how the interactions of induced stress defenses and fungal competition would change in three-dimensional space.

Conclusion

I diversified the approaches of previous studies on induced defenses in microbial communities by taking into account different priming strategies and costs, different stress durations, and different fungal traits such as stress resistance, growth rate and production rate of secondary metabolites. This enabled me to perform an assessment of the costs and benefits of induced defenses in microbes, and to show that not only the community context changes the benefit of defense strategies of a species, but also that induced defenses can influence the community. With this thesis, I have provided a link between processes at the species level and at the community level. By elucidating the mechanisms behind this link, the studies presented here take an important step towards a better understanding of the drivers of fungal community characteristics, such as community assembly or composition. Fungal communities fulfill fundamental roles in the soil ecosystem, and the findings of my study promote a better understanding of soil fungal community functioning and, ultimately, of the soil ecosystem response to the effects of environmental changes.

References

A'Bear, A.D., Boddy, L., and Hefin Jones, T. (2012) Impacts of elevated temperature on the growth and functioning of decomposer fungi are influenced by grazing collembola. Glob Chang Biol **18**: 1823–1832.

Ballauff, J., Zemp, D.C., Schneider, D., Irawan, B., Daniel, R., and Polle, A. (2020) Legacy effects overshadow tree diversity effects on soil fungal communities in oil palm-enrichment plantations. Microorganisms **8**: 1–18.

Boddy, L. (2000) Interspecific combative interactions between wood-decaying basidiomycetes. FEMS Microbiol Ecol **31**: 185–194.

Crowther, T.W., Maynard, D.S., Crowther, T.R., Peccia, J., Smith, J.R., and Bradford, M.A. (2014) Untangling the fungal niche: the trait-based approach. Front Microbiol **5**: 1–12.

Falconer, R.E., Bown, J.L., White, N.A., and Crawford, J.W. (2008) Modelling interactions in fungi. J R Soc Interface **5**: 603–15.

Fukami, T. (2015) Historical Contingency in Community Assembly: Integrating Niches, Species Pools, and Priority Effects. Annu Rev Ecol Evol Syst **46**: 1–23.

Fukami, T., Dickie, I.A., Paula Wilkie, J., Paulus, B.C., Park, D., Roberts, A., et al. (2010) Assembly history dictates ecosystem functioning: Evidence from wood decomposer communities. Ecol Lett **13**: 675–684.

Gallien, L., Zimmermann, N.E., Levine, J.M., and Adler, P.B. (2017) The effects of intransitive competition on coexistence. Ecol Lett **20**: 791–800.

Gorbushina, A.A. (2007) Life on the rocks. Environ Microbiol **9**: 1613–1631.

Guo, Y., Jud, W., Weikl, F., Ghirardo, A., Junker, R.R., Polle, A., et al. (2021) Volatile organic compound patterns predict fungal trophic mode and lifestyle. Commun Biol **4**: 1–12.

Harvell, C.D. (1990) The ecology and evolution of inducible defenses. Q Rev Biol **65**: 323–340.

Heil, M. (2002) Ecological costs of induced resistance. Curr Opin Plant Biol **5**: 345–350.

Heil, M. (2014) Trade-offs Associated with Induced Resistance. In, Induced Resistance for Plant Defense. Chichester, UK: John Wiley & Sons, Ltd, pp. 171–192.

Heilmann-Clausen, J. and Boddy, L. (2005) Inhibition and stimulation effects in communities of wood decay fungi: Exudates from colonized wood influence growth by other species. Microb Ecol **49**: 399–406.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., et al. (2016) Priming and memory of stress responses in organisms lacking a nervous system. Biol Rev **91**: 1118–1133.

Hiscox, J., Baldrian, P., Rogers, H.J., and Boddy, L. (2010) Changes in oxidative enzyme activity during interspecific mycelial interactions involving the white-rot fungus Trametes versicolor. Fungal Genet Biol **47**: 562–571.

Hiscox, J. and Boddy, L. (2017) Armed and dangerous – Chemical warfare in wood decay communities. Fungal Biol Rev **31**: 169–184.

Hiscox, J., Clarkson, G., Savoury, M., Powell, G., Savva, I., Lloyd, M., et al. (2016) Effects of pre-colonisation and temperature on interspecific fungal interactions in wood. Fungal Ecol **21**: 32–42.

Hiscox, J., Savoury, M., Müller, C.T., Lindahl, B.D., Rogers, H.J., and Boddy, L. (2015) Priority effects during fungal community establishment in beech wood. ISME J **9**: 2246–2260.

Hiscox, J., Savoury, M., Toledo, S., Kingscott-Edmunds, J., Bettridge, A., Waili, N. Al, and Boddy, L. (2017) Threesomes destabilise certain relationships: multispecies interactions between wood decay fungi in natural resources. FEMS Microbiol Ecol **93**: 1–11.

Jurburg, S., Nunes, I., Brejnrod, A., Jacquiod, S., Priemé, A., Sørensen, S.J., et al. (2017) Legacy Effects on the Recovery of Soil Bacterial Communities from Extreme Temperature Perturbation. Front Microbiol **8**: 1832.

Jurburg, S., Nunes, I., Stegen, J.C., Le Roux, X., Priemé, A., Sørensen, S.J., and Salles, J.F. (2017) Autogenic succession and deterministic recovery following disturbance in soil bacterial communities. Sci Rep **7**: 45691.

Kennedy, P.G., Peay, K.G., and Bruns, T.D. (2009) Root tip competition among ectomycorrhizal fungi: Are priority effects a rule or an exception? Ecology **90**: 2098–2107.

Levine, J.M., Bascompte, J., Adler, P.B., and Allesina, S. (2017) Beyond pairwise mechanisms of species coexistence in complex communities. Nature **546**: 56–64.

Lindahl, B., Stenlid, J., and Finlay, R. (2001) Effects of resource availability on mycelial interactions and 32P transfer between a saprotrophic and an ectomycorrhizal fungus in soil microcosms. FEMS Microbiol Ecol **38**: 43–52.

Maynard, D.S., Bradford, M.A., Lindner, D.L., van Diepen, L.T.A., Frey, S.D., Glaeser, J.A., and Crowther, T.W. (2017) Diversity begets diversity in competition for space. Nat Ecol Evol **1**: 156.

Meisner, A., Jacquiod, S., Snoek, B.L., Ten Hooven, F.C., and van der Putten, W.H. (2018) Drought legacy effects on the composition of soil fungal and prokaryote communities. Front Microbiol **9**: 1–12.

Meškauskas, A., Fricker, M.D., and Moore, D. (2004) Simulating colonial growth of fungi with the neighbour-sensing model of hyphal growth. Mycol Res **108**: 1241–1256.

Mitchell, A. and Pilpel, Y. (2011) A mathematical model for adaptive prediction of environmental changes by microorganisms. Proc Natl Acad Sci U S A **108**: 7271–6.

O'Leary, J., Eastwood, D., Müller, C., and Boddy, L. (2018) Emergent properties arising from spatial heterogeneity influence fungal community dynamics. Fungal Ecol **33**: 32–39.

Rayner, A.D.M., Griffith, G.S., and Wildman, H.G. (1994) Induction of metabolic and morphogenetic changes during mycelial interactions among species of higher fungi. Biochem Soc Trans **22**: 389–394.

Robinson, C.H., Dighton, J., and Frankland, J.C. (1993) Resource capture by interacting fungal colonizers of straw. Mycol Res **97**: 547–558.

Schimel, J., Balser, T.C., and Wallenstein, M. (2007) Microbial Stress-Response Physiology and Its Implications for Ecosystem Function. Ecology **88**: 1386–1394.

Schoeman, M.W., Webber, J.F., and Dickinson, D.J. (1996) The effect of diffusible metabolites of Trichoderma harzianum on in vitro interactions between basidiomycete isolates at two different temperature regimes. Mycol Res **100**: 1454–1458.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Bürgmann, H., et al. (2012) Fundamentals of Microbial Community Resistance and Resilience. Front Microbiol **3**: 417.

Toljander, Y.K., Lindahl, B.D., Holmer, L., and Högberg, N.O.S. (2006) Environmental fluctuations facilitate species co-existence and increase decomposition in communities of wood decay fungi. Oecologia **148**: 625–631.

Tucker, C.M. and Fukami, T. (2014) Environmental variability counteracts priority effects to facilitate species coexistence: Evidence from nectar microbes. Proc R Soc B Biol Sci **281**:.

Vidal-Diez de Ulzurrun, G., Baetens, J.M., Van den Bulcke, J., and De Baets, B. (2017) Modelling three-dimensional fungal growth in response to environmental stimuli. J Theor Biol **414**: 35–49.

APPENDICES

Appendix A: Supplementary Information for Chapter II

1. Parameter analysis

To determine the effect of the parameter choice for our baseline scenario, we analytically investigated the benefit of priming under different parameter sets. To do so, we solved the differential equation described by Equation 1 and assessed the effect of parameter choice on the three different response parameters. Because the mortality $m(t)$ is defined by a step function depending on the stress phase that the organism is currently experiencing, we obtained three different solutions:

$$S(t) = \begin{cases} S_0 \cdot e^{(g-m_I)\cdot t}, & t < t_L \\ S_0 \cdot e^{(g-m_I)\cdot t + \frac{(L^2+t^2)(m_I s_R)}{2} - tLm_I s_R}, & t_L \le t < t_R \\ S_0 \cdot e^{\frac{m_I s_R t_R^2}{2} - Lm_I s_R t_R + m_R t_R - m_I t_R + \frac{L^2 m_I s_R}{2} - tm_R + gt}, & t \ge t_R \end{cases} \quad \text{(A1)}$$

with $t_R = L + \frac{m_I - m_R}{m_I s_R}$: (A2)

$$S(t) = \begin{cases} S_0 \cdot e^{(g-m_I)\cdot t}, & t < t_L \\ S_0 \cdot e^{(g-m_I)\cdot t + \frac{(L^2+t^2)(m_I s_R)}{2} - tLm_I s_R}, & t_L \le t < t_R \\ S_0 \cdot e^{(g-m_R)\cdot t + (m_R - m_I)\cdot L + \frac{2m_I m_R - m_I^2 - m_R^2}{2m_I s_R}}, & t \ge t_R \end{cases} \quad \text{(A3)}$$

For the baseline scenario, we define the primed and nonprimed population as being of equal size at time point t_B, i.e. $S_p(t = t_B) = S_{np}(t = t_B)$. The parameters determining the improved primed response thus compensate the priming costs that initially lead to a higher population size of the nonprimed population at the beginning of the stress $S_p(t = t_{TS}) < S_{np}(t = t_{TS})$. These improved response parameters are either a shorter length of the primed lag phase L_p (earlier response), the higher speed of the primed response s_{Rp} (faster response), or the improved final primed response level m_{Rp} (stronger response).

In case of $t_L \le t < t_R$, the stress response is building up, and the parameters affect the system dynamics differently than after the response buildup. For this case, only the primed response speed s_{Rp} and the primed lag phase L_p are investigated, because the final response level m_R

has not yet been reached and the primed stronger response cannot show any positive effect. We analytically investigate, which response brings a higher benefit during response buildup and whether the parameter choice influences our results. To do so, we can assess the population size during the response buildup ($t_L \leq t < t_R$) for the earlier and faster results with:

$$S_{p\_early} = S_p \cdot e^{(g-m_I)\cdot t + \frac{((L-\Delta L)^2+t^2)(m_I s_R)}{2} - t(L-\Delta L)m_I s_R} \quad \text{(A4)}$$

$$S_{p\_fast} = S_p \cdot e^{(g-m_I)\cdot t + \frac{(L^2+t^2)(m_I s_{R\cdot\Delta s_R})}{2} - tLm_I s_R\cdot\Delta s_R} \quad \text{(A5)}$$

In the following, we will refer to the initial vale of a primed or naïve population as S_p or S_{np}, respectively. Figure 3 shows that if we define our baseline scenario (i.e. that time point when all three responses confer a benefit as high as the nonprimed response) at any point in time $t > t_R$, the earlier and faster response will be of equal benefit at $t \geq t_R$, because these responses do not accumulate any additional benefit after the response buildup, unlike the stronger response. We can thus assume $S_{p\_early}(t = t_R) = S_{p\_fast}(t = t_R) = S_{np}(t = t_R)$ and use our second solution (Eq. A3) to solve for the necessary reduction in the lag phase ΔL and the increase in speed Δs_R to compensate for priming costs:

$$\Delta L = L - L_p = L - t_R + \sqrt{\frac{2\ln\left(\frac{S_{np}}{S_p}\right)}{m_I s_R} + (L-t_R)^2} \quad \text{(A6)}$$

$$\Delta s_R = \frac{s_{Rp}}{s_R} = 1 + \frac{\ln\left(\frac{S_{np}}{S_p}\right)}{(L-t_R)^2 m_I s_R} \quad \text{(A7)}$$

By inserting Equations A6 and A7 into A4 and A5, respectively, we can assess the difference between the relative population sizes $\frac{S_{p\_early}-S_{p\_fast}}{S_{np}}$:

$$S_{p\_early} - S_{p\_fast} = S_p \cdot e^{(g-m_I)\cdot t + \frac{((L-\Delta L)^2+t^2)(m_I s_R)}{2} - t(L-\Delta L)m_I s_R} - S_p \cdot e^{(g-m_I)\cdot t + \frac{(L^2+t^2)(m_I s_{R\cdot\Delta s_R})}{2} - tLm_I s_R\cdot\Delta s_R}$$

To find out whether this equation is negative or positive, it is sufficient to investigate only the exponential terms that are different between S_{p_early} and S_{p_fast}:

$$\frac{({L_p}^2 + t^2)(m_I s_R)}{2} - tL_p m_I s_R - (\frac{(L^2 + t^2)(m_I s_{Rp})}{2} - tLm_I s_{Rp})$$

$$= \frac{m_I}{2}(\Big(\big(t^2 - 2L_p t + L_p^2\big)s_R - (L^2 - 2tL + t^2)s_{Rp}\Big)$$

$$= \frac{m_I}{2}(\big(t - L_p\big)^2 s_R - (t - L)^2 s_{Rp})$$

Here, we can focus on two terms. If the left term is larger than the right, then the early population will be larger than the faster.

$$\big(t - L_p\big)^2 s_R > (t - L)^2 s_{Rp}$$

We can now substitute L_p and s_{Rp} from Equations A6 and A7, respectively:

$$\left(t - t_R + \sqrt{\frac{2\ln\left(\frac{S_{np}}{S_p}\right)}{m_I s_R} + (L - t_R)^2}\right)^2 s_R > (t - L)^2 s_R + \frac{\ln\left(\frac{S_{np}}{S_p}\right)(t - L)^2}{(L - t_R)^2 m_I}$$

We can show that for no priming costs (i.e. $S_{np} = S_p$) , both terms are equal:

$$(t - L)^2 s_R = (t - L)^2 s_R$$

And, ignoring the additive terms, we can show that costs higher than zero lead to a benefit of the early response:

$$\left(\sqrt{\frac{2\ln\left(\frac{S_{np}}{S_p}\right)}{m_I s_R}}\right)^2 s_R > \frac{\ln\left(\frac{S_{np}}{S_p}\right)(t - L)^2}{(L - t_R)^2 m_I}$$

$$2\ln\left(\frac{S_{np}}{S_p}\right) > \frac{(t - L)^2}{(t_R - L\,)^2}\ln\left(\frac{S_{np}}{S_p}\right)$$

$$2 > \frac{(t - L)^2}{(t_R - L\,)^2}$$

$S_{p\_early} > S_{p\_fast}$ for all $t < t_R$ and any value of m_I, s_R, L, m_R, or g. Thus, at any point during the response buildup, the earlier response is more beneficial than the faster response due to the earlier setoff. This benefit is compensated for by the faster response only later in time when the buildup has been finished. We can now assess the impacts parameter choice on our results: A

faster response buildup (higher s_R), a shorter lag phase L or a higher final response level m_R will decrease the value of t_R (Eq. A2 and Fig. 3) and the response buildup is finished earlier. An increase in stress intensity m_I reduces the strength of the primed responses ΔL and Δs_R (Eq. A6 and A7, respectively), because under more intense stress the same priming costs can be compensated for with less investments. The maximum difference between the benefit of early and fast response during the response buildup is higher for higher m_I due to the higher benefit gain of the early response during the response lag $t < t_L$.

In case of $t \geq t_R$, the final response level has been reached, and the third solution of Equation A3 describes the population dynamics. Again, we use the time point t_B as reference point at which nonprimed and primed population are of equal size $S_p(t = t_B) = S_{np}(t = t_B)$, with $t_R < t_B$ (Fig. 3).

$$S_{p\_early} = S_p \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot(L-\Delta L)+\frac{2m_Im_R-m_I^2-m_R^2}{2m_Is_R}} \quad \text{(A8)}$$

$$S_{p\_fast} = S_p \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot L+\frac{2m_Im_R-m_I^2-m_R^2}{2m_Is_R\Delta s_R}} \quad \text{(A9)}$$

$$S_{p\_strong} = S_p \cdot e^{(g-m_R\Delta m_R)\cdot t+(m_R\Delta m_R-m_I)\cdot L+\frac{2m_Im_R\Delta m_R-m_I^2-(m_R\Delta m_R)^2}{2m_Is_R}} \quad \text{(A10)}$$

In this phase, the three response parameters are defined as:

$$\Delta L = L - L_p = \frac{\ln\left(\frac{S_{np}}{S_p}\right)}{m_I-m_R} \quad \text{(A11)}$$

$$\Delta s_R = s_{Rp}/s_R = \frac{(m_R-m_I)^2}{-2\ln\left(\frac{S_{np}}{S_p}\right)m_Is_R+(m_R-m_I)^2} \quad \text{(A12)}$$

$$\Delta m_R = m_R - m_{Rp} = -\frac{ln\left(\frac{S_{np}}{S_p}\right)}{t_R-t_B} \quad \text{(A13)}$$

We can now compare the population size for any $t \geq t_R$ with equations A8-A10, and the response parameter values derived in Equations A11-A13. We can show that $S_{p\_early} = S_{p\_fast}$ for all $t > t_R$, independently of any other parameters:

$$S_{p_{early}} = S_{p_{fast}}$$

$$S_p \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot L_p+\frac{2m_Im_R-m_I^2-m_R^2}{2m_Is_R}} = S_p \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot L+\frac{2m_Im_R-m_I^2-m_R^2}{2m_Is_{Rp}}}$$

$$S_p \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot L+\ln\left(\frac{S_{np}}{S_p}\right)-\frac{(m_R-m_I)^2}{2m_Is_R}} = S_p \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot L+\ln\left(\frac{S_{np}}{S_p}\right)-\frac{(m_R-m_I)^2}{2m_Is_R}}$$

$$= S_{np} \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot L-\frac{(m_R-m_I)^2}{2m_Is_R}}$$

S_{p_strong} is lower than the populations following the other strategies up to t_B, when all responses are of equal benefit. Afterwards, the stronger strategy further accumulates benefit and is therefore superior to the other strategies. This can be shown by looking at the third solution of Equation A1:

$$S_{p\_strong} = S_p \cdot e^{\frac{m_Is_Rt_R^2}{2}-Lm_Is_Rt_R+m_R\Delta m_Rt_R-m_It_R+\frac{L^2m_Is_R}{2}-tm_R\Delta m_R+gt}$$

And inserting Δm_R as defined in Equation A13.

$$S_p \cdot e^{\frac{m_Is_Rt_R^2}{2}-Lm_Is_Rt_R+m_Rt_R+\frac{ln\left(\frac{S_{np}}{S_p}\right)t_R}{t_R-t_B}-m_It_R+\frac{L^2m_Is_R}{2}-tm_R-\frac{ln\left(\frac{S_{np}}{S_p}\right)t}{t_R-t_B}+gt}$$

$$= S_p \cdot e^{\frac{m_Is_Rt_R^2}{2}-Lm_Is_Rt_R+m_Rt_R-m_It_R+\frac{L^2m_Is_R}{2}-tm_R+\frac{ln\left(\frac{S_{np}}{S_p}\right)(t_R-t)}{t_R-t_B}+gt}$$

$$= S_p \cdot e^{\frac{m_Is_Rt_R^2}{2}-Lm_Is_Rt_R+m_Rt_R-m_It_R+\frac{L^2m_Is_R}{2}-tm_R+gt} \cdot e^{\frac{ln\left(\frac{S_{np}}{S_p}\right)(t_R-t)}{t_R-t_B}}$$

For $t = t_B$, we obtain

$$S_{p\_strong} = S_p \cdot e^{\frac{m_Is_Rt_R^2}{2}-Lm_Is_Rt_R+m_Rt_R-m_It_R+\frac{L^2m_Is_R}{2}-tm_R+gt} \cdot e^{ln\left(\frac{S_{np}}{S_p}\right)}$$

$$= S_{np} \cdot e^{\frac{m_Is_Rt_R^2}{2}-Lm_Is_Rt_R+m_Rt_R-m_It_R+\frac{L^2m_Is_R}{2}-tm_R+gt} = S_{nonprimed}$$

For all values $t > t_B$, the value of S_{p_strong} will be larger than $S_{nonprimed}$, because $e^{\frac{ln\left(\frac{S_{np}}{S_p}\right)(t_R-t)}{t_R-t_B}}$ is growing with $t > t_R$.

2. Effect of resource limitation

We numerically analyze the differential equation model described by Equation 1 under the assumption of resource limitation, i.e. carrying capacity $K = 10{,}000$. The system is parametrized as described in the Methods section of the main manuscript. We systematically assess the effect of different priming costs and stress durations on the benefit of the three primed stress responses and can show an increased benefit of the faster response for intermediate durations of stress compared to the solution without K (Fig. A1a).

3. Combined Responses

Just as the analysis shown above, we can analyze the benefit of two response types combined into one defense strategy, namely a response that starts earlier and builds up faster than a nonprimed response, or a response with a higher defense level that either starts earlier or is realized faster. To realize a combined response and facilitate comparison, we directly transfer the change of response parameters $\Delta L, \Delta s_R, \Delta m_R$ that provide the same benefit at $t = t_B$ in the single response case to the combined response case. That is, we do not seek for parameters to make the combined response equal to the nonprimed case or to the single response at $t = t_B$, since this would add an additional degree of freedom to the parameter choice. Instead, we compare the three combined responses among each other, focusing on the stress defense phase after the response buildup $t > t_R$:

$$S_{p\_early\_fast} = S_p \cdot e^{(g-m_R)\cdot t+(m_R-m_I)\cdot(L-\Delta L)+\frac{2m_I m_R - m_I^2 - m_R^2}{2m_I s_R \Delta s_R}} \quad \text{(A14)}$$

$$S_{p\_fast\_strong} = S_p \cdot e^{(g-m_R\Delta m_R)\cdot t+(m_R\Delta m_R-m_I)\cdot L+\frac{2m_I m_R\Delta m_R - m_I^2 - (m_R\Delta m_R)^2}{2m_I s_R \Delta s_R}} \quad \text{(A15)}$$

$$S_{p\_early\_strong} = S_p \cdot e^{(g-m_R\Delta m_R)\cdot t+(m_R\Delta m_R-m_I)\cdot(L-\Delta L)+\frac{2m_I m_R\Delta m_R - m_I^2 - (m_R\Delta m_R)^2}{2m_I s_R}} \quad \text{(A16)}$$

Here, $S_{p\_early\_fast} > S_{p\_fast\_strong} > S_{p\_early\_strong}$ holds during shorter stress durations after t_R, while for longer stress durations the combination of a faster buildup and a stronger response is most beneficial ($S_{p\_fast\_strong} > S_{p\_early\_strong} > S_{p\_early\_fast}$).

We numerically implemented the combined responses with the same parameter set as the isolated response scenario and a carrying capacity $K = 10{,}000$ and could reproduce the analytical results (see Fig. A1b). The parameter space favoring priming is much larger, because the combined responses yield a higher benefit, compensating the priming costs. However, these more complex responses will most likely come with higher costs.

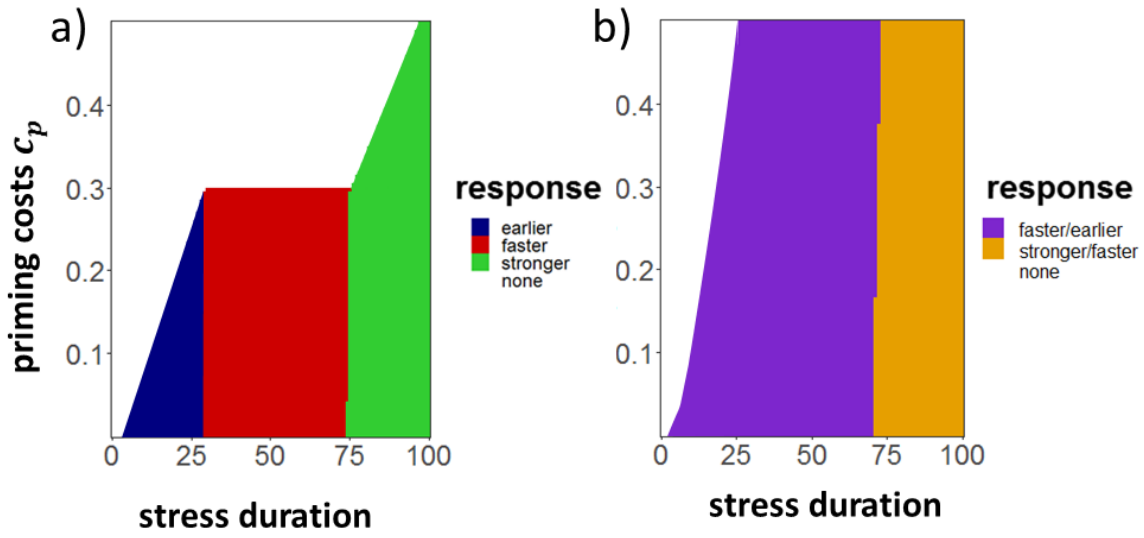


Figure A1 Parameter space favoring the different primed stress responses under resource limitation depending on stress duration and priming costs; a) single response strategies and b) combined response strategies.

4. Predictability of stress

To approximate the costs of poor predictability of stress, we differentiate two alternative options: If a priming cue is followed by a triggering stress (denoted as $T = True$), it can lead to a fitness gain ΔF_{PT} that we define as difference between primeable $S_p(t)$ and nonprimeable population $S_{np}(t)$.

$$\Delta F_{PT} = S_p(T = True, t = t_{TE}) - S_{np}(T = True, t = t_{TE}) \quad \text{(A17)}$$

Note that in our model, ΔF_{PT} can be negative if the priming costs are high or the stress duration is short. t_{TE} is the time point of measurement just after the triggering stress has ended.

If no triggering stress occurs after an organism has been primed (denoted as $T = False$), the costs of establishing a preliminary priming response have still been paid, but the possible

benefit of reacting earlier/faster/stronger to a stress will not be gained and the difference ΔF_P will be calculated as

$$\Delta F_P = S_p(T = False, t = t_{TE}) - S_{np}(T = False, t = t_{TE}) \quad \text{(A18)}$$

ΔF_P will always be negative (or zero), because without triggering stress, no benefit is gained by committing to a priming strategy and the nonprimed population S_{np} will perform better.

We can now use these values for a given stress duration and given costs of priming to assess the effect of predictability, i.e. the probability $p(T = True)$ that a priming cue is followed by a triggering stress. We calculate the overall fitness by

$$\Delta F = p(T = True) \cdot \Delta F_{PT} + (1 - p(T = True)) \cdot \Delta F_P \quad \text{(A19)}$$

For a poorly predictable stress, $(1 - p(T = True))$ will be higher and the overall Fitness gain ΔF will be lower, as more priming events are followed by no stress and thus the overall costs invested per successfully predicted triggering stress increase. ΔF_P is independent of the priming responses, because in the case of no triggering stress, no primed stress response occurs. The benefits of the responses occurring in the case of ΔF_{PT} will thus all be equally reduced by $(1 - p(T = True)) \cdot \Delta F_P$.

5. Community dynamics

To better understand the dynamics in the community model and the time point of changes in dominance, a visualization of the population dynamics in a community is given in Figure A2. The early population is dominant because of the earlier reduction of mortality that takes place while the other responses are still delayed in their response. The faster population cannot compensate for this delay, because it shares the same capacity and is limited by the larger population size of the early population. Only for longer stress duration, the stronger population dominates the community because of its smaller mortality, leading to higher overall growth.

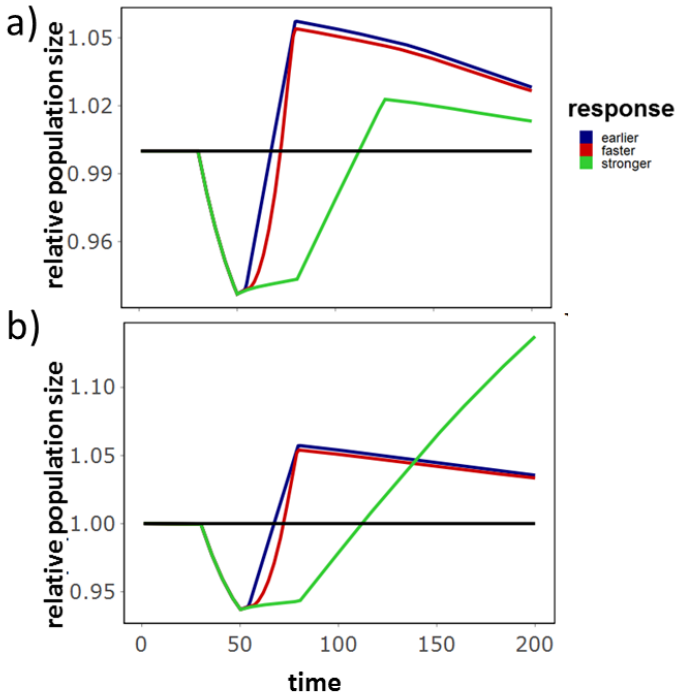


Figure A2 Population sizes of the three primed response strategies under a) short stress ($\boldsymbol{TD} = \mathbf{75}$) and b) long stress ($\boldsymbol{TD} = \mathbf{150}$) and intermediate competition ($\boldsymbol{\alpha} = \mathbf{0.5}$). Population size is relative to the nonprimed strategy (shown as black line), i.e. a value of 1 signifies a population size equal to the naïve population. The Priming stimulus takes place at $\boldsymbol{t} = \mathbf{30}$, and the triggering stress begins at $\boldsymbol{t} = \mathbf{50}$.

Appendix B: Supplementary Information for Chapter III

1. Taxonomic identification of the fungal species

The six isolates were identified as ascomycetes *Chaetomium angustispirale*, *Fusarium oxysporum*, *Fusarium sp.* and a strain of the *Amphisphaeriaceae* family, the basidiomycete *Pleurotus sapidus* and the mucoromycete *Morteriella elongata* (see Table S1 for details). Not all isolates could be identified to the species level. For information on how the species were identified, see Lehmann *et al.*, 2019. The soil saprotrophic fungi were originally taken from a semi-arid grassland site in Mallnow (Mallnow Lebus, Brandenburg, Germany, 52°27.778' N, 14°29.349' E). All six species were cultured from the top 10 cm of soil and thus occur in close proximity in nature where they compete for space and/or nutrients. The investigated combinations of competitors are therefore likely to be observed in nature.

Table B1 . Taxonomic identification of the soil filamentous fungi used in this study, and accession numbers. The order of species in the table is by phylogeny. The neighbor-joining tree was based on the ITS (intergenic transcribed spacer) and a part of the large rRNA subunit (LSU). Phylogenetic annotations were based on bootstrap analysis, and assumed valid when supported in 80% of the bootstraps.

Strain ID	Competitor No.	Genus species	Order	Phylum	NCBI	DSMZ	Partial 18s-, full ITS-, partial LSU sequence accession number
RLCS06	-	*Chaetomium angustispirale*	Sordariales	Ascomycota	KT582096	DSM 100400	MT453288
RLCS05	1	*Fusarium sp.*	Hypocreales	Ascomycota	KT582097	DSM 100403	MT453291
RLCS07	2	*Amphisphaeriaceae* strain 1	Xylariales	Ascomycota	KT582088	DSM 100284	MT453269
RLCS16	3	*Pleurotus sapidus*	Agaricales	Basidiomycota	KT582080	DSM 100408	MT453295
RLCS32	4	*Fusarium oxysporum*	Hypocreales	Ascomycota	KT582095	DSM 100409	MT453296
RLCS02	5	*Mortierella elongata*	Mortierellales	Mucoromycota	KT582072	DSM 100407	MT453294

2. Determination of treatment temperatures

The durations and intensities of priming stimulus and strong heat stress (triggering stimulus) were determined in a pre-experiment (Figure S1): For each species we measured the colony size at ambient temperature (22 °C) as well as 4 days after treating them with two-hour temperature pulses of 35, 40, 42, 45, 48 or 50 °C. Because some species exhibited a lag phase without any growth for up to three days after higher temperature stress, the chosen time point is well after this possible lag phase and thus combines the effects of a post-stress lag phase and reduced growth.

To obtain the temperature pulse curves in the pre experiment, species were grown under control temperature of 22 °C and treated with 2h heat pulses of varying temperatures (shown on the x-axis of Figure S1). For a wide range of low temperatures, they do not show significantly reduced growth, either because they do not suffer from short pulses of moderate heat, or because they can recover from any damage by day 4. Therefore, they differ from classical temperature response curves in that they do not show an optimum growing temperature (T_{opt}) for each species, but rather indicate under which heat pulse they start suffering from temperature stress.

We selected 35 °C as two-hour priming stimulus, as this temperature did not significantly change the growth in any of the chosen species and can therefore be defined as mild stimulus that does not induce a phenomenological stress response. A two-hour heat pulse of 45 °C was selected as triggering stimulus, because it proved to be growth reducing but not lethal for all species, albeit to a different degree.

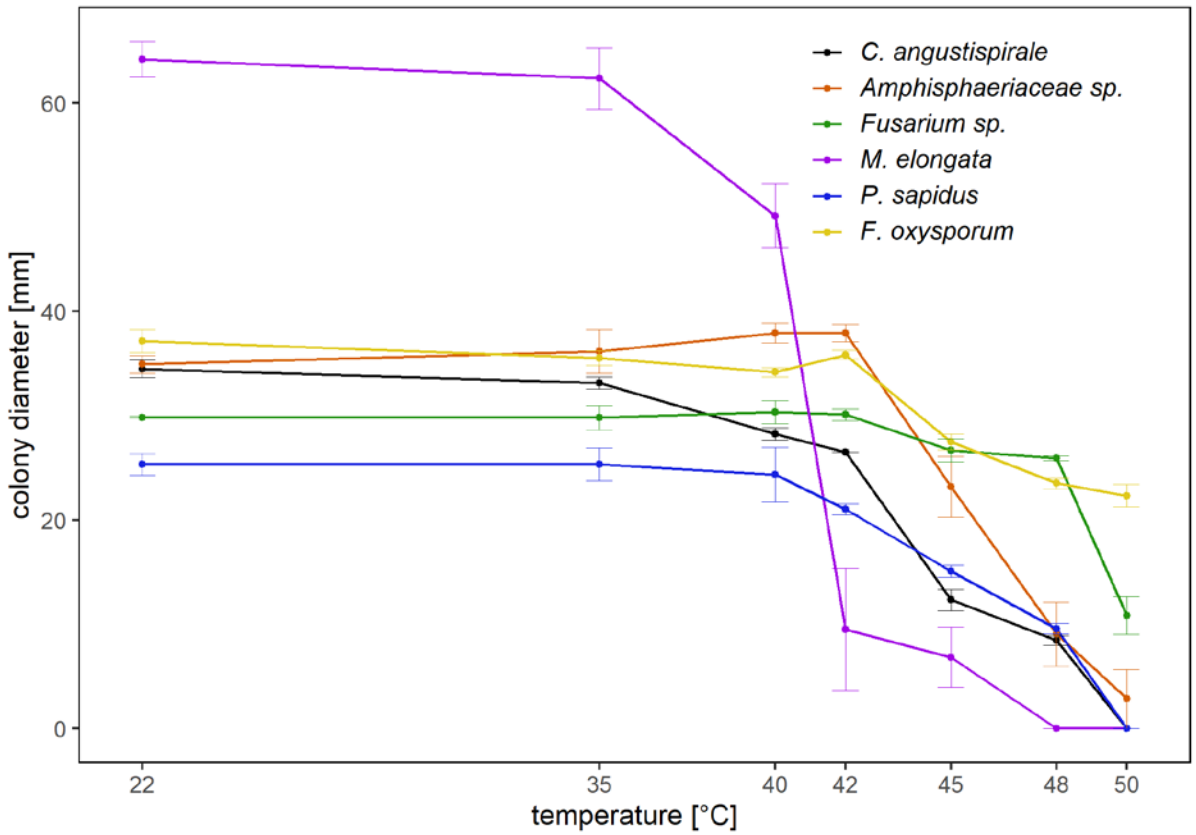


Figure B1 Mean response of the six investigated species in isolation towards a two-hour temperature treatment. The heat treatment was followed by a four-day growth period at 22 °C after which colony diameters were measured. Error bars show the standard error of the mean

3. Single species stress responses

Figure S2 as well as Table 1 illustrate that the investigated species exhibit different degrees of stress susceptibility and primeability and thus cover a range of possible competitor responses. This way, when validating our model we can make sure that very different competitor responses can be represented by our simulations.

To determine the length of the post stress lag phase and the growth of the following phase for each species, we compared the colony diameter distribution of the single species replicates on day 1 (day of treatment) with each respective following day using a paired t-test after testing for normality and homoscedasticity. We measured the duration of the lag phase $L_{si,T}$ and $L_{si,PT}$ as the time period following a T and PT treatment, in which the colony diameter does not differ significantly from day 1. Growth rates after the lag were determined with a linear fit. The exact time point of transition from lag phase to growth was the intersection of the lag and growth fit lines.

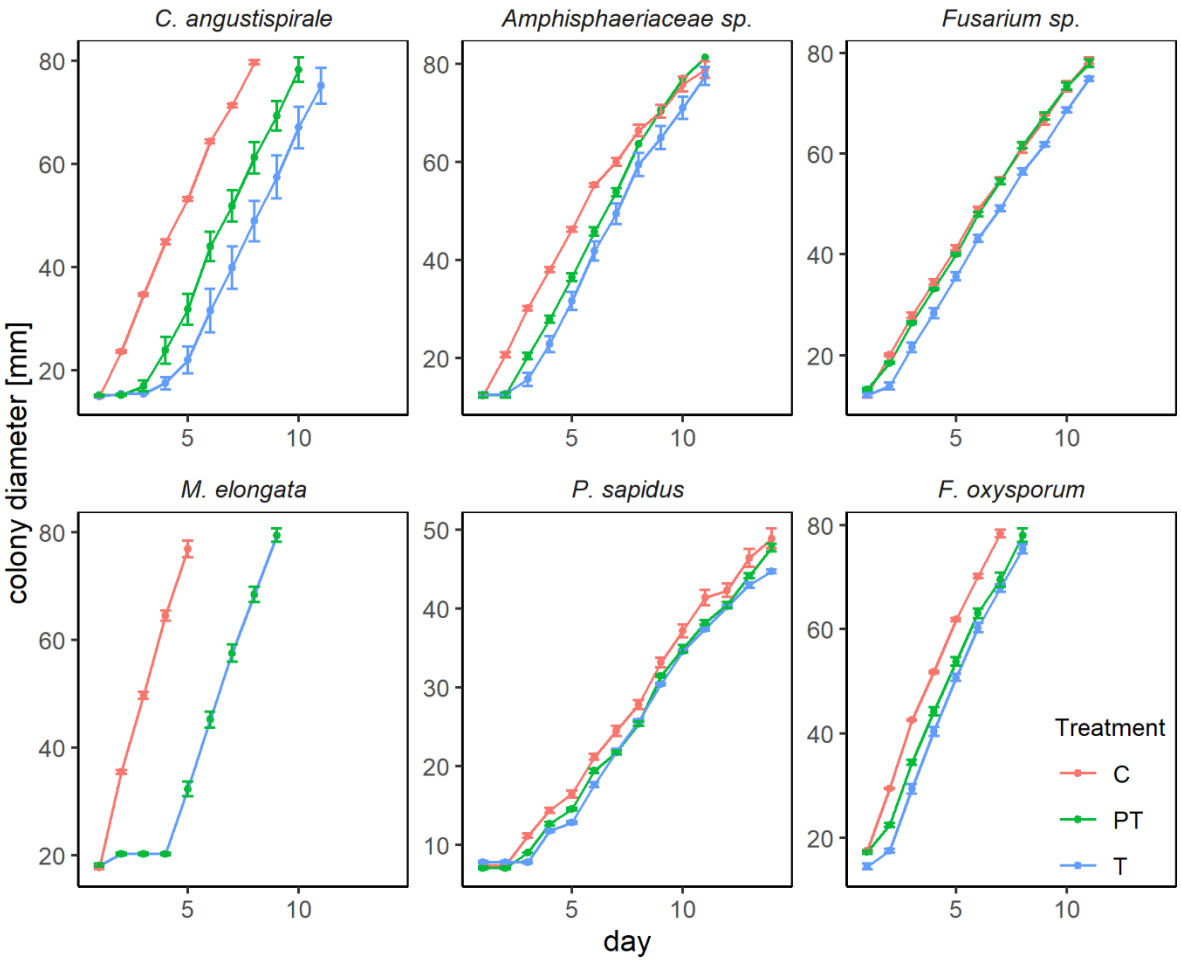


Figure B2 Experimentally measured colony diameters of the six species grown in isolation. For all species, T and PT treatment were applied on day 1.

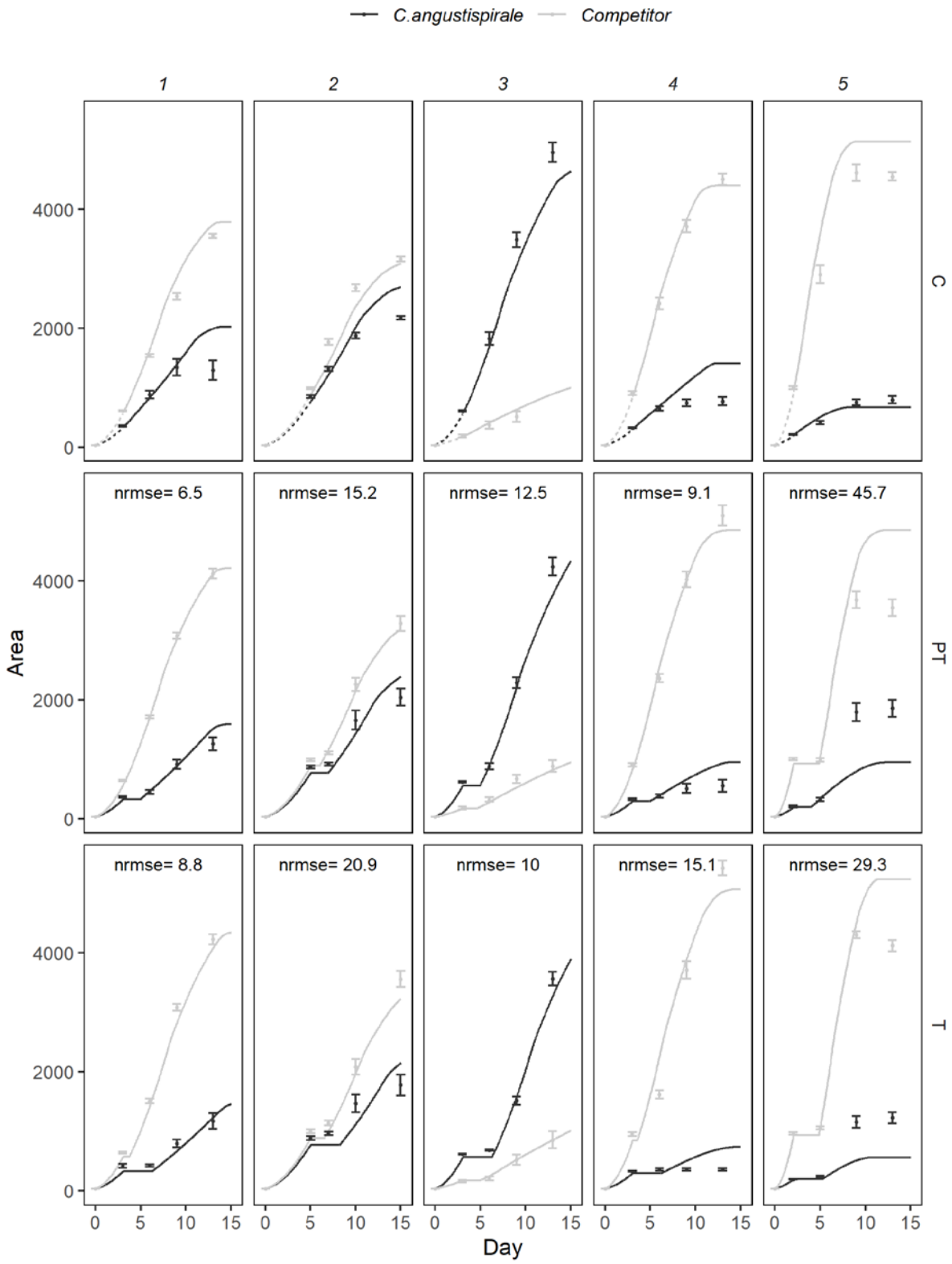


Figure B3 Growth dynamics of *C. angustispirale* competing with five other soil fungi (represented by their competitor number listed in table S1). Points describe empirical measurements, and lines are the corresponding simulation model output. C = control treatment, T = stressed treatment, PT = primed and stressed treatment. Error bars show the standard error of the mean of the observed data. NRMSE is the normalized root mean square error of the simulation model for the respective pair.

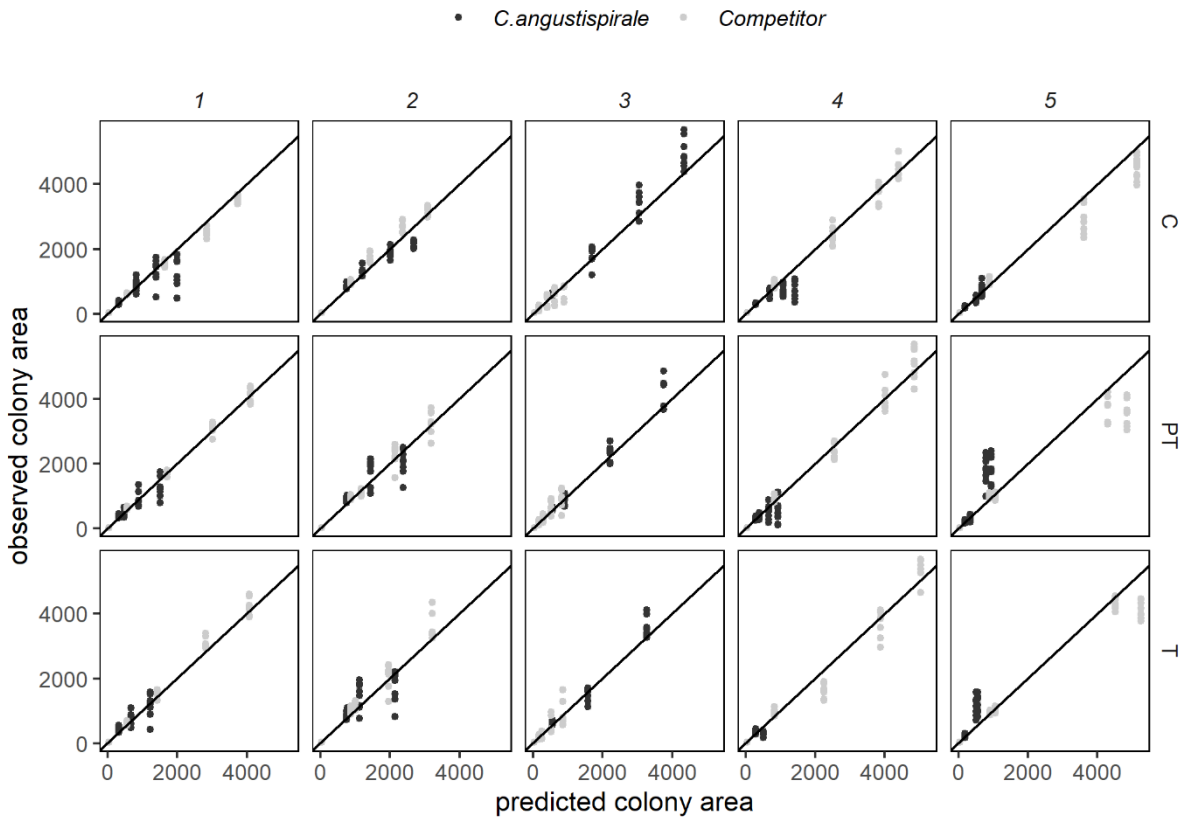


Figure B4 Laboratory observation vs. model prediction of colony area of *C. angustispirale* competing with five other soil fungi (represented by their competitor number listed in table S1). One point represents one measurement. C = control treatment, T = stressed treatment, PT = primed and stressed treatment. The lines show the 1:1 line for observed versus predicted colony areas.

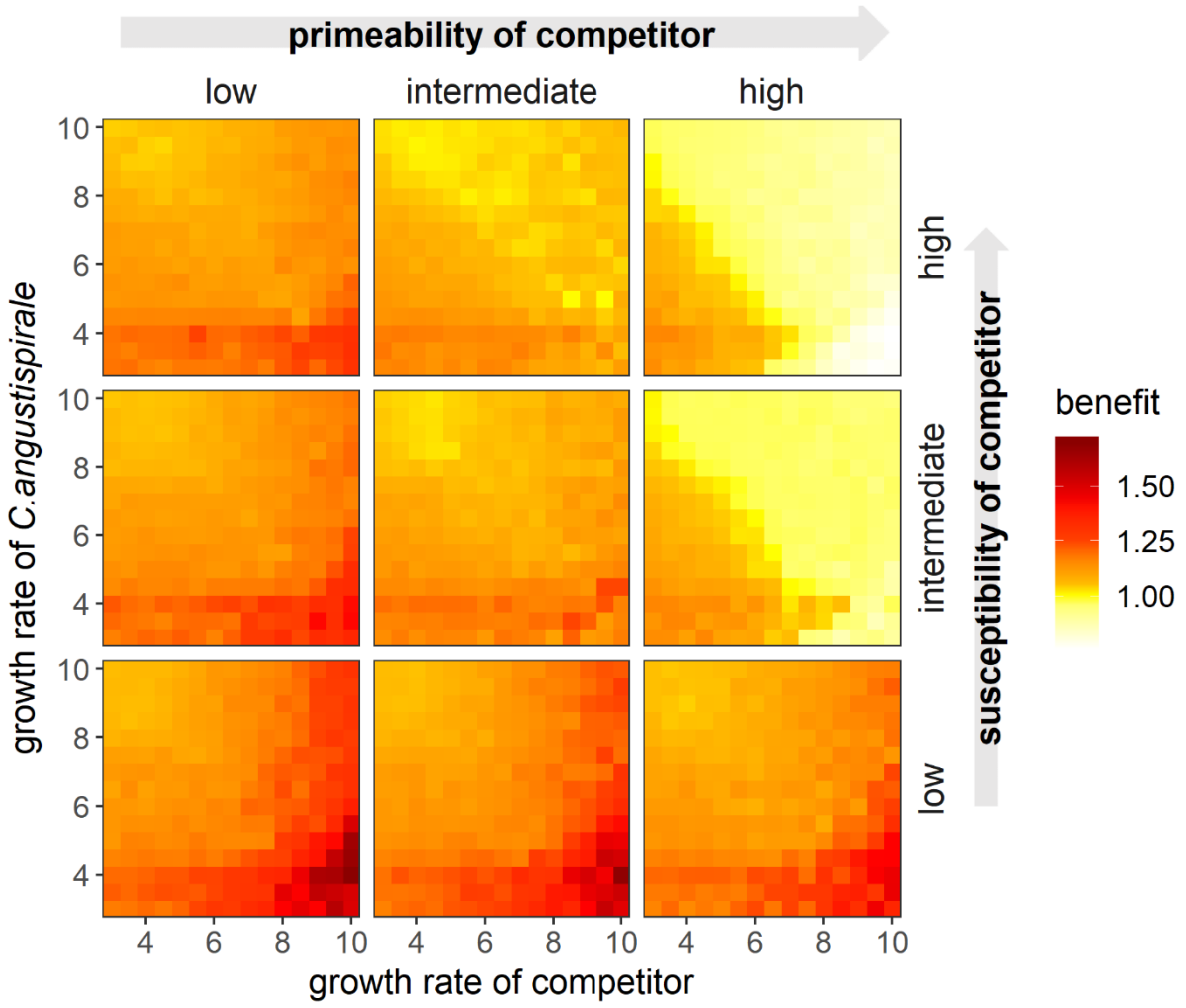


Figure B5 Priming benefit of *C. angustispirale* in competition with an artificial species. Benefits are shown for different trait combinations fifteen days after stress treatment at the end of the simulation. Levels of susceptibility correspond to different lengths of a stress-induced lag phase: low = 0.5 days, intermediate = 1.5 days, high = 2 days, and levels of primeability correspond to the reduction of this lag phase under priming conditions: low = 25%, intermediate = 50%, high= 100%.

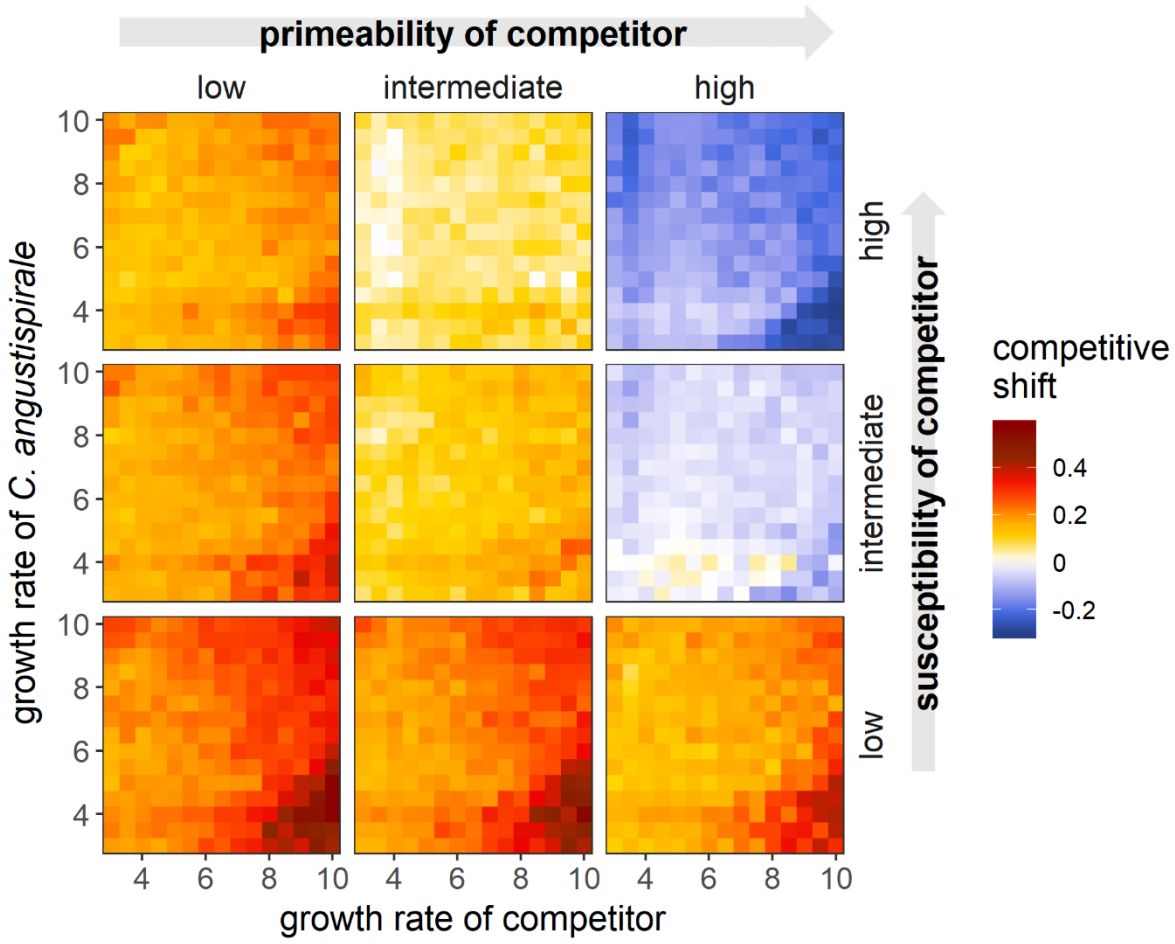


Figure B6 Competitive shift of *C. angustispirale* in competition with an artificial species. The shifts in competition are shown fifteen days after the stress treatment. Red shades indicate a shift in favor of *C. angustispirale*, and blue shades a shift favoring its competitor. Levels of susceptibility correspond to different lengths of a stress-induced lag phase: low = 0.5 days, intermediate = 1.5 days, high = 2 days, and levels of primeability correspond to the reduction of this lag phase under priming conditions: low = 25%, intermediate = 50%, high= 100%.

References

1. Lehmann, A. et al. Tradeoffs in hyphal traits determine mycelium architecture in saprobic fungi. Sci. Rep. 9, 1–9 (2019).

Appendix C: Supplementary Information for Chapter IV

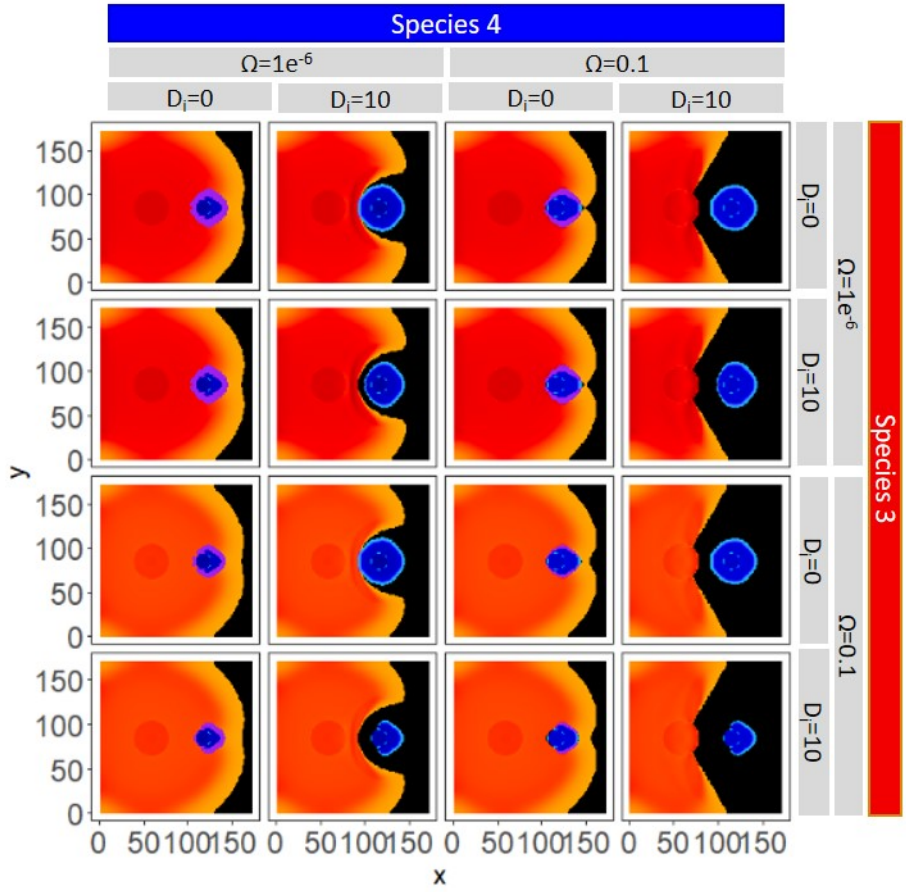


Figure C1 Biomass distribution of two competing fungi for different values of inhibitor production Ω and inhibitor diffusivity Di. Competitive outcomes are shown ten days after inoculation after experiencing a 2h-heat pulse at day 1. Red/orange tones represent species 3, blue tones represent species 4, purple represents territory that is overgrown, i.e. colonized by both species, and black shows territory that has not been claimed by either species. The biomass of each species is normalized to values between 0 and 1.

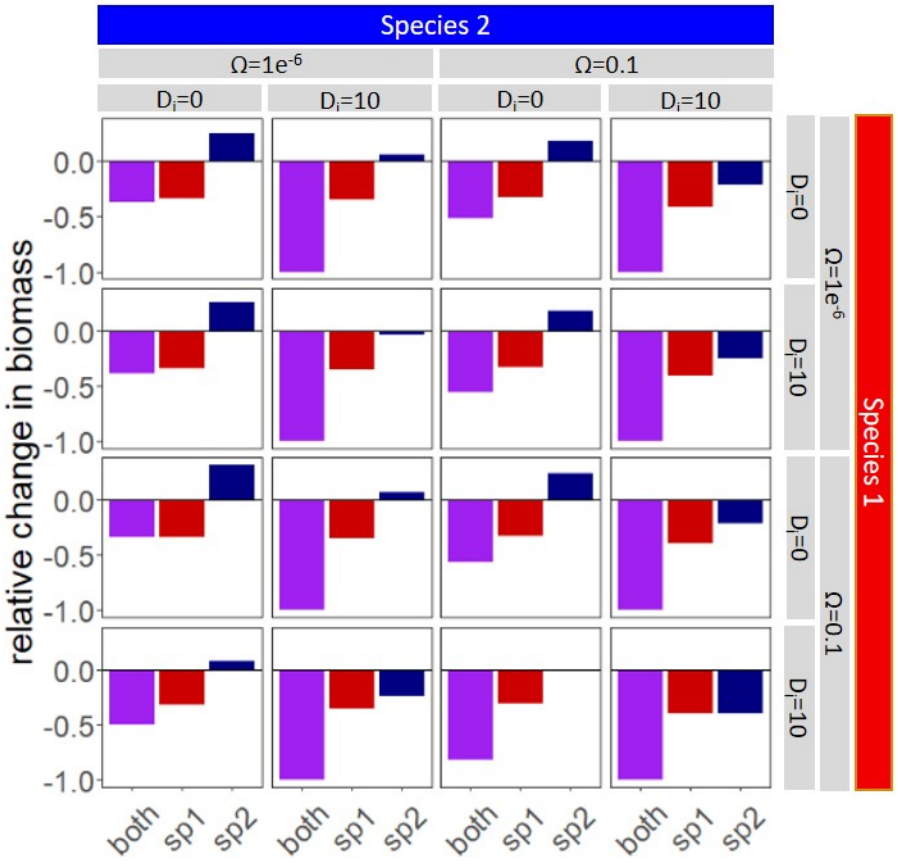


Figure C2 Effect of heat stress on the biomass of the two competitors. Relative change of total biomass under stress treatment compared to control conditions ten days after inoculation for different values of inhibitor production Ω and inhibitor diffusivity Di and for a lag phase of 2.8 days (fast species 1), or 1.9 days (slow species 2). Positive values indicate a gain in biomass after stress, and a value of -1 indicates a reduction to zero. Red and blue bars refer to the cumulative biomass of species 1 and 2 (sp1 and sp2), respectively, and purple bars refer to biomass that is on shared territory, i.e. where one species has overgrown the other.

Selbstständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Doktorarbeit eigenständig verfasst und keine anderen als die angegebenen Hilfsmittel und Quellen verwendet habe.

Berlin, den 30.06.2021

Felix Wesener