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Soil fungi invest into asexual sporulation under resource scarcity, but trait spaces of individual isolates are unique

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Summary

During the last few decades, a plethora of sequencing studies provided insight into fungal community composition under various environmental conditions. Still, the mechanisms of species assembly and fungal spread in soil remain largely unknown. While mycelial growth patterns are studied extensively, the abundant formation of asexual spores is often overlooked, though representing a substantial part of the fungal life cycle relevant for survival and dispersal. Here, we explore asexual sporulation (spore abundance, size and shape) in 32 co-occurring soil fungal isolates under varying resource conditions, to answer the question whether resource limitation triggers or inhibits fungal investment into reproduction. We further hypothesized that trade-offs exist in fungal investment towards growth, spore production and size. The results revealed overall increased fungal investment into spore production under resource limitations; however, effect sizes and response types varied strongly among fungal isolates. Such isolate-specific effects were apparent in all measured traits, resulting in unique trait spaces of individual isolates. This comprehensive dataset also elucidated variability in sporulation strategies and trade-offs with fungal growth and reproduction under resource scarcity, as only predicted by theoretical models before. The observed isolate-specific strategies likely underpin mechanisms of co-existence in this diverse group of saprobic soil fungi.

Introduction

Saprobic fungi are highly relevant for soil nutrient dynamics, and their mycelia efficiently explore and use heterogeneous resource patches in soil (Moore *et al*., 2021a). Many experimental studies and fungal growth models focus on these flexible mycelia, which enable fungi to adapt colonization intensity and occupation of different patches for the optimal use of spatially heterogeneous nutrients (Watkinson *et al*., 2006; Fricker *et al*., 2008; Vidal-Diez de Ulzurrun *et al*., 2017). Still, one important part of this fungal life-history strategy is often overlooked, despite being an important strategy for colonization and survival (Gilchrist *et al*., 2006): the production of asexual spores.

As dispersal agents, asexual spores are highly relevant for the colonization of new resource patches, securing survival especially in suboptimal and disturbed environments (Wyatt *et al*., 2013; Boddy and Hiscox, 2016). An enormous diversity of asexual spore types and sporogenesis modes exists throughout the Eumycota, with spores being relevant mainly for dispersal in space but also persistence over time (e.g. thick-walled chlamydospores; Walther *et al*., 2005; Domsch *et al*., 2007; Wyatt *et al*., 2013). The ecological significance of asexual spore production is further demonstrated by the evolutionary persistence of these structures since early eukaryotic lineages, despite concurrent development of more sophisticated sexual reproduction organs in higher fungi. Within fungi, there was a shift in asexual reproductive structures from motile zoospores found in chytrids and other early lineages inhabiting aquatic environments to non-motile spores found in more derived lineages including the Mucoromycota (Moore *et al*., 2021a). The significance of asexual spores for dispersal in soil compared with sexual asco- and basidiospores is currently unknown, but high proportions of asexual spores in airborne samples suggest their likely relevance (Després *et al*., 2012; Pashley

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et al., 2012). While shape and size of asexual spores are often described as taxonomic characters for many soil fungi (Domsch *et al*., 2007), assessments of numbers and variability in spore formation in the diverse group of soil saprobic fungi remain scarce.

Many authors have emphasized the relevance of analysing spore production in fungi as an ecological trait and indicator of fungal fitness (Crowther *et al*., 2014; Aguilar-Trigueros *et al*., 2015; Dawson *et al*., 2019). However, most studies so far have only resolved detailed sporulation patterns in model fungi (Park and Yu, 2012; Ruger-Herreros and Corrochano, 2020) or economically relevant species including pathogenic and biocontrol strains (Steyaert *et al*., 2010; Su *et al*., 2012). Some interesting insights for saprobic fungi are derived from theoretical models of fungal growth strategies. Since resource colonization by spores requires optimal starting conditions due to low resource carry-over, the amount of spores and their size is crucial for successful establishment and fungal reproduction (Fricker *et al*., 2008). Consequently, fungal growth strategies must be seen as a trade-off of energy investment into growth, resource use and reproduction (Heaton *et al*., 2016). Gilchrist *et al*. (2006) calculated that the optimal fitness can be reached by a 'bang-bang resource allocation strategy', with investment into fungal growth until resource uptake of about 50% of available resources, followed by a sudden switch to spore production. Their model also suggests that the exact timing depends on resource quality and spatial (patch) limitations, and that this strategy may be less optimal in more disturbed environments. In line with this, Boddy and Hiscox (2016) propose variations in timing and spore numbers along the spectrum of C-S-R strategists, with more 'rapid and substantial' investment to spores in ruderals compared to competitive and stress-tolerant fungi. Similarly, a model by Heaton *et al*. (2016) differentiates efficient growth strategies for fast-growing fungi on rich but transiently available resource patches with short reproduction cycles, compared with mycelia with slow growth on complex resources (e.g. wood decomposers).

Such species-specific differences also apply to the environmental factors that trigger or shift sporulation. In the model fungi *Aspergillus nidulans*, *Neurospora crassa* and *Trichoderma* spp., the genetic mechanisms and cellular differentiation during spore formation have been studied in detail (Steyaert *et al*., 2010; Park and Yu, 2012). The main environmental signals for asexual sporulation involve light, hyphal exposure to air and resource quality, but also many other factors have been reported including temperature, humidity, pH, calcium and physical injury (Dahlberg and Etten, 1982; Steyaert *et al*., 2010; Su *et al*., 2012). Especially the factor resource quality is highly complex in this context: sufficient nitrogen (N) supply is relevant for spore production in *Trichoderma*, contrary to N limitation inducing spores in *Aspergillus*, while in *Neurospora* C starvation initiates sporulation (Steyaert *et al*., 2010; Ruger-Herreros and Corrochano, 2020; Moore *et al*., 2021a). In general, theoretical models predict that resource quality determines the energy available for reproduction (Heaton *et al*., 2016); however, resource limitation can also trigger dispersal to new patches (Dahlberg and Etten, 1982; Money, 2016). In fact, existing studies provide contradictory results, with nutrient availability and also type having different effects (Morton and Brian, 1961; Olutiola, 1976; Larmour and Marchant, 1977). These differential effects may relate to species-specific strategies, since optimal spore formation on different media was found to vary interspecifically (Ritz, 1995; Gao *et al*., 2007; Su *et al*., 2012).

Most ecological (and economic) studies focus on spore numbers, but spore size is a trait that also determines the reproductive strategy of fungal species. Larger spores increase germination and colonization success but also elicit a trade-off with the numbers of spores that can be produced (Kauserud *et al*., 2008; Aguilar-Trigueros *et al*., 2019). Spore size and shape may also be related to dispersal ability: smaller spores can be dispersed further by wind and also an elongated form may be advantageous (Kauserud *et al*., 2008; Norros *et al*., 2014), though the relevance of different dispersal agents for asexual spores in soil is not well understood (Golan and Pringle, 2017). While spore size and shape can also be an indicator of nutritional mode of species (Kauserud *et al*., 2008; Pringle *et al*., 2015), studies in the sexual spores of basidiomycetes revealed that both spore size and shape in fungal communities shift along a gradient of resource supply (Halbwachs *et al*., 2017). For asexual spores, Williams (1959) demonstrated that also within species size responds to differences in C and N availability in media, indicating that this trait may even vary intraspecifically and respond to resource supply.

Here, we aimed to develop a comprehensive picture of the patterns of asexual spore formation in co-occurring soil saprobic fungi, focusing on the shifts in investment to reproduction under varying resource supply and intra- and interspecific variability that may contribute to niche differentiation mechanisms. We addressed the hypotheses that (i) low nutrient supply will increase fungal investment into spores, but decrease spore size; (ii) fungal isolates will differ in their sporulation strategies, regarding responses to nutrient supply and also nutrient type; (iii) sporulation strategies over time within isolates (e.g. bang-bang resource allocation strategy) will be fixed but shift in time and extent with resource supply; and (iv) when comparing traits among isolates, trade-offs will be apparent in the fungal investment of resources towards growth, spore production and spore size. We

measured the size and number of asexual spores as a trait for 32 fungal isolates within the Mucoromycota, Ascomycota and Basidiomycota isolated from the same grassland soil (Fig. 1) and explored the variability of these traits in response to resource availability, the timing of measurements and the respective nutrient type. Fungi were grown in resource-rich [100% potato dextrose agar (full-strength PDA)] or –poor (1% full-strength PDA) conditions with C as the main limiting element. Fungal growth, spore production and spore size and shape were analysed as response variables. For a subset of five isolates, detailed measurements over time in 1% and 100% PDA were included, to analyse fungal sporulation strategies and its shift with resource supply in more detail. Additionally, to explore fungal niche spaces in respect to different nutrient types, fungal spore production was also measured on media varying only in nitrogen (N) or phosphorus (P) supply (Methods and Results are presented in Supporting Information S2).

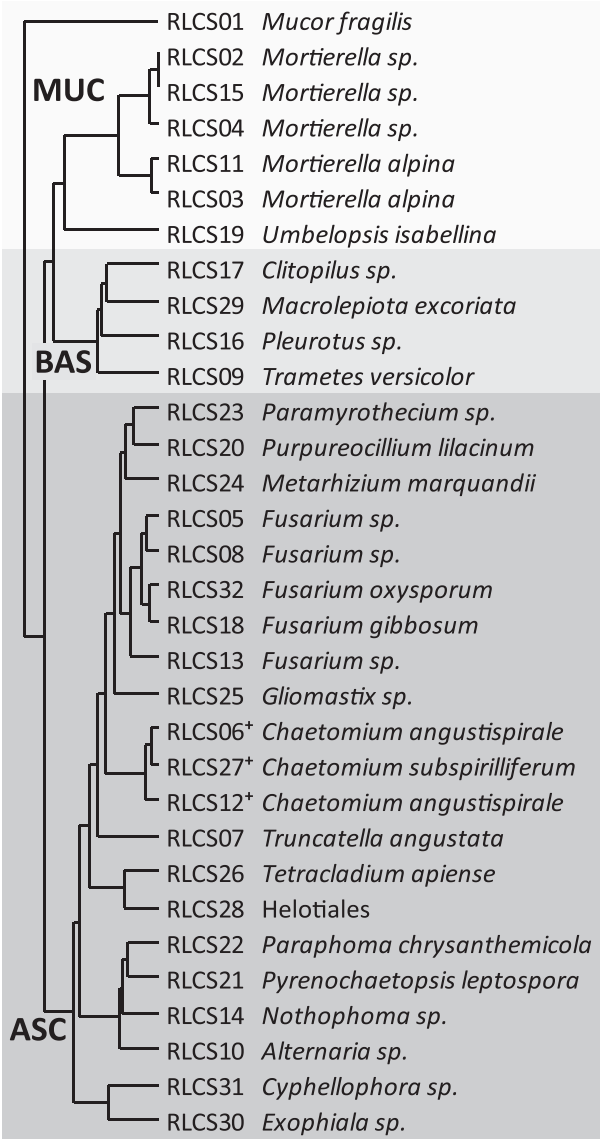

Fig. 1. Phylogenetic classification of fungal isolates and the related phylogenetic tree. Taxon names are given based on taxonomic classification of ITS and LSU marker regions according to the UNITE database and the RDP LSU dataset. Phylum names follow Spatafora *et al*. (2017), in case of synonymous names the 'current name' according to *Species Fungorum* was chosen (www.speciesfungorum.org). Plus signs identify isolates of the genus *Chaetomium*, which only formed sexual ascospores on growth media. Grey shadings differentiate the fungal isolates according to phylum affiliations. MUC: Mucoromycota, BAS: Basidiomycota, ASC: Ascomycota.

Results

After 6 weeks of growth most fungal isolates had produced spores, though the quantity varied widely among isolates (Fig. 2). Only three isolates did not form any spores in all the tested media [RLCS21 (*Pyrenochaetopsis leptospora*), RLCS26 (*Tetracladium apiense*) and RLCS29 (*Macrolepiota excoriate*); one isolate (RLCS22, *Paraphoma chrysanthemicola*) produced spore numbers below our method's detection limit (see Fig. S2)]. Further, six isolates did not sporulate in 100% PDA while eight isolates did not sporulate in 1% PDA. Within some isolates large variability was observed, driven by one or two repetitions with no apparent spore formation (Fig. 2). Looking in detail, in these cases actual spore counts in the Neubauer chamber for the other repetitions were also extremely low, in all cases <6 spores. In isolates with substantial sporulation observed in all three repetitions, variability in spore abundance was in fact mostly lower within isolates than among isolates (Fig. 2, S4).

The spores formed by the three isolates of the genus *Chaetomium* were identified as ascospores formed within perithecia, which were clearly visible on the mycelia (Berkson, 1966). These isolates were excluded from statistical analyses, since the initiation of sexual spore production may differ ecologically from asexual spore production. All other isolates formed asexual spores only. Some of the species included may form different spore types, e.g. *Fusarium* produces micro- and macroconidia as well as chlamydospores. However, the spore types observed within individual isolates were uniform, only varying in size and potentially also age (Fig. 3, spore types were identified following descriptions by Domsch *et al*. (2007)).

Fungal growth and sporulation in 1% and 100% PDA

The total amount of spores extracted from fungal cultures increased in all isolates on 100% compared to 1% PDA (except for two isolates only sporulating on 1% PDA

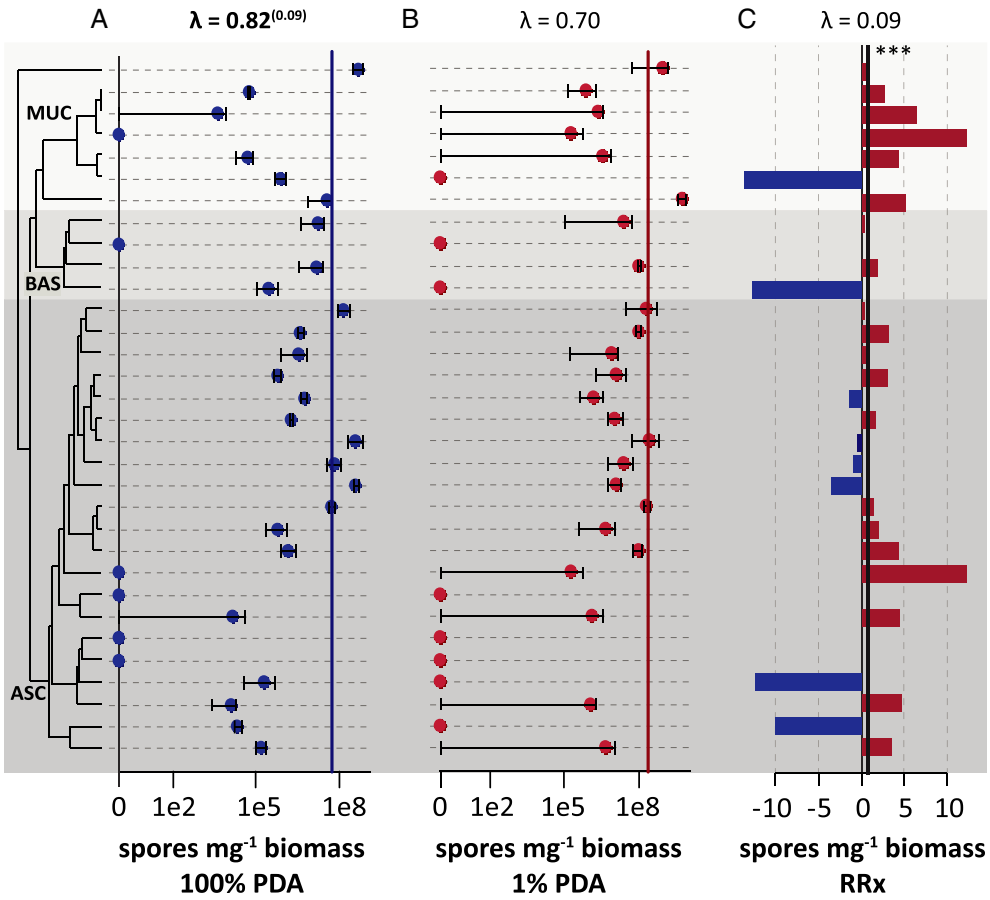

Fig. 2. Relative spore production (mg^{-1} biomass; log-scale) of individual isolates in 100% (A) and 1% potato dextrose agar (PDA; B), and the average response ratios RRx (C).
A and B. Dots indicate mean values of each isolate and growth medium, respectively ($n = 3$), bars the respective complete data range (non-log transformed data were used for the calculation of average values). Coloured vertical lines indicate the average values of all isolates.
C. Bars represent the response ratio RRx [natural logarithm (spores mg^{-1} biomass in 1% PDA/spores mg^{-1} biomass in 100% PDA)], with blue bars indicating higher relative spore production in 100%, red bars higher numbers in 1% PDA medium. The black line indicates the average value of all isolates, asterisks the significant overall medium effects (***$P < 0.001$). The phylogenetic signal calculated as Pagel's λ (lambda) is shown for each trait, with P values in brackets indicating significant model deviations from models assuming $\gamma = 0$. Grey shadings differentiate fungal phyla – MUC: Mucoromycota, BAS: Basidiomycota, ASC: Ascomycota. Plus signs identify isolates of the genus *Chaetomium*, which only formed sexual ascospores and were excluded from statistical analyses.

medium; Fig. S4), with an average total spore number of 3.2e9 and 3.6e7 in 100% and 1% PDA medium, respectively (Table 1, Fig. S4). However, since fungal biomass was increased 53-fold in 100% PDA, the relative spore production (spores mg^{-1} biomass) was in fact higher on 1% PDA medium ($P < 0.001$) showing higher relative investment into spores under low resource conditions (Table 1, Fig. 2). Despite this overall positive response of relative spore production (spores mg^{-1} biomass) to low resources supply, we also observed substantial variation in the magnitude of this response across isolates (Fig. 2).

The larger biomass values of fungal mycelia in 100% PDA were driven by a much denser mycelium (defined as biomass cm^{-2} mycelium), whereas mycelial extension rate was on average lower in 100% PDA (Table 1, Fig. S5). Similarly to relative spore production, the direction of responses to medium type in mycelial extension depended on the fungal isolate (Fig. S5), indicating species-specific strategies in response to resource limitations. The variability in magnitude and direction of responses in fungal traits was also supported by the interaction term in two-way models of medium type and isolate identity, as observed for most response variables (Table 1).

When analysing these differential directions and magnitudes in responses of fungal traits to medium type, no phylogenetic signal was detected (Fig. 2, Fig. S4), meaning variations in fungal trait responses (strategies) were not conserved within certain fungal clades. Only individual fungal traits showed phylogenetic signals, especially extension rate, mycelial density (in 100% PDA) and to a smaller extent the number of spores produced (Table 1, Fig. 2 and S4).

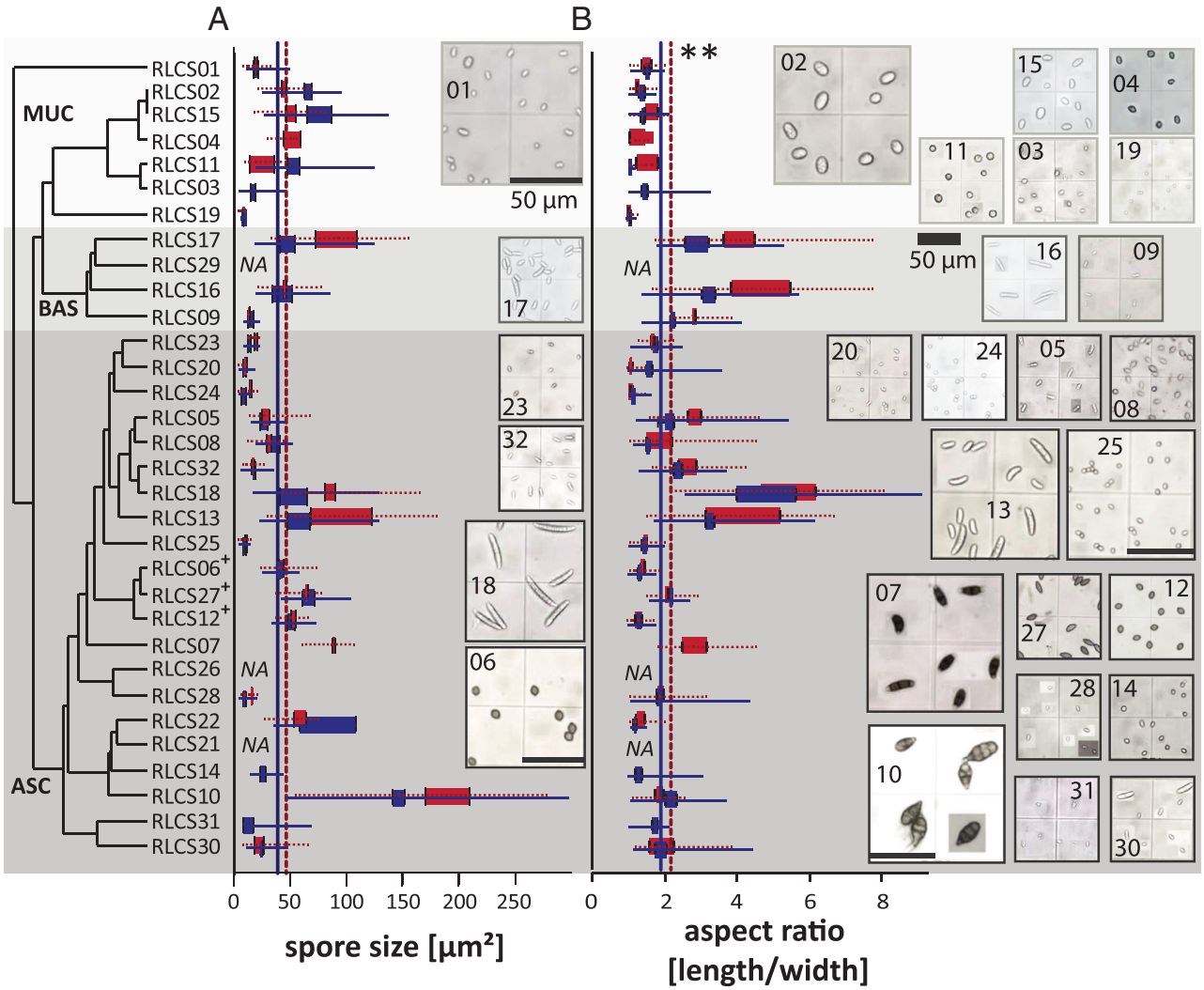

Fig. 3. Variation in spore size (µm^2) (A) and spore aspect ratio (length/width) (B) of individual fungal isolates grown on 1% (red, dotted lines) and 100% (blue, solid lines) potato-dextrose agar (PDA). For each fungal isolate and medium type, three samples (repetitions) were analysed, with (mostly) 20 spores surveyed per sample (for detailed counts of examined spores see Fig. S2). Thick bars illustrate the observed data range of average values of the three repetitions, lines visualize the full data range of variation observed in size and aspect ratio of all fungal spores analysed (see also Fig. S2 and S3). *NA* (not available) labels fungal isolates for which no spore was available for measurements in neither 1% nor 100% PDA. Coloured vertical lines represent mean values for each medium type, asterisks significant differences among treatments (**$P < 0.01$). The added microscopic pictures depict spores of fungal isolates, with the different scale bars depicting 50 µm (numbers relate to RLCS identities of isolates). Grey shadings differentiate fungal phyla – MUC: Mucoromycota, BAS: Basidiomycota, ASC: Ascomycota. Plus signs identify isolates of the genus *Chaetomium*, which only formed sexual ascospores and were excluded from statistical analyses.

Spore size and also shape (aspect ratio) varied widely among but also within isolates. The average values of spore sizes detected in different isolates ranged from 7.6 to 143.8 µm^2 in 100% PDA and 7.4 to 188.4 µm^2 in 1% PDA (Table 1, Fig. 3A) and size ranges of individual spores across the entire dataset ranged from a minimum size of 3.65 µm^2 to a maximum of 298.28 µm^2 (Fig. 3A, S2). Similarly, the aspect ratio (spore length/width) of spores varied widely – on average between 0.95 to 4.81 in 100% PDA and 0.97 to 5.45 in 1% PDA (Fig. 3b and S3). Spore aspect ratio was correlated with spore size in both media types (Fig. S6), indicating that larger spores have more elongated forms (Fig. 3).

In comparison to variation in spore numbers or biomass, the growth medium type only had little impact on spore size resulting in an overall non-significant response (Table 1, Fig. 3A). In contrast to spore size, spore shape was greatly influenced by growth medium type: many isolates expressed higher aspect ratios in 1% PDA leading to a shift of average aspect ratios from 1.94 to 2.24 ($P < 0.001$; Table 1). This shift was predominantly driven by more elongated spores (i.e. higher spore lengths),

Table 1. Average growth and sporulation traits of fungal isolates grown on 1% and 100% PDA medium, presenting raw data (mean ± standard deviation), treatment effects (for details on statistical models used see Table S2) and the phylogenetic signal of trait distributions in both media.

Response variable	100% PDA[a]	1% PDA	Medium effects	Medium: isolate	phylo. Signal[b] 100% PDA	phylo. signal 1% PDA
Biomass (mg)	86.65 ± 43.6	1.64 ± 2.0	$P < 0.001$	$P < 0.001$	$\lambda = 0$	$\lambda = 0.59$
Extension rate (cm^2 day^{-1})	2.93 ± 1.9	3.33 ± 1.9	$P < 0.001$	$P < 0.001$	$\lambda =$ **0.76** $^{(0.06)}$	$\lambda =$ **0.88** $^{(0.07)}$
Density (mg cm^{-2})	1.94 ± 1.8	0.03 ± 0.05	$P < 0.001$	$P < 0.001$	$\lambda =$ **0.98** $^{(<0.001)}$	$\lambda = 0$
Total spores	3.2e9 ± 8.2e9	3.6e7 ± 1.2e8	$P < 0.001$	$P = 0.09$	$\lambda = 0.62$	$\lambda =$ **0.78** $^{(0.07)}$
Spores mg^{-1} biomass	6.0e7 ± 1.2e8	2.7e8 ± 1.2e9	$P < 0.001$	$P < 0.001$	$\lambda =$ **0.82** $^{(0.09)}$	$\lambda = 0.70$
Spore size (μm^2)	34.9 ± 32	44.5 ± 42	$P = 0.39$	$P < 0.001$	$\lambda = 0$	$\lambda = 0$
Spore shape (length/width)	1.94 ± 0.9	2.24 ± 1.3	$P < 0.001$	$P < 0.001$	$\lambda = 0.32$	$\lambda = 0$

[a]Values in grey indicate significantly higher trait expressions in that medium.
[b]As phylogenetic signal Pagel's λ (lambda) is presented, with P values indicating significant deviations from models assuming $\gamma = 0$. Bold values indicate phylogenetic signals found in respective trait expressions ($P < 0.1$).

while spore width was not affected (data not shown). Despite the phylogenetic signal detected in the correlation of both traits (Fig. S6), neither the trait spore size nor shape showed a phylogenetic signal (Table 1).

Sporulation strategies over time in 1% and 100% PDA media

The analysis of fungal growth and relative spore production over time in five fungal isolates revealed different strategies in sporulation patterns among fungal isolates. The amount of spores produced varied at different time points on both media, which also caused shifts in treatment (growth medium type) effects. Still, the strategy of spore production remained similar within isolates on both media, even though the timing and amount of spores produced shifted. For example, the isolate RLCS01 (*Mucor fragilis*) that grows rapidly on both media and produces biomass quickly (Fig. 4A) also produced spores rapidly but steadily over time, leading to a linear increase in relative spore production (spores mg^{-1} biomass) in both media (Fig. 4b). In contrast, RLCS15 (*Mortierella sp*., also belonging to Mucoromycota) and RLCS17 (*Clitopilus sp*., Basidiomycota) showed a clear peak with a short time window of high relative spore production in both media types, best fitted by a skew-normal distribution (Fig. 4D and F, S7a and b). RLCS25 (*Gliomastix sp*.) expressed slower growth and also sporulated relatively late on both media (Fig. 4J), with a sudden onset of high spore numbers that was visible on mycelia by a switch from whitish to dark green cultures due to the sporangia formed. Here, strategies varied among media (skew-normal vs. log distributions, Fig. 4J). Still, the data suggest that in 100% PDA sporulation may also decrease again over even longer time periods than assessed here. Similarly, the correlation of relative spore production with time in RLCS05 (*Fusarium sp*.) showed a logistic distribution on both media (Fig. S7c and d); however, in 100% PDA, this distribution did not show a significantly better fit than a linear distribution and was thus rejected (Fig. 4H).

These described variations in spore production over time also caused treatment effects to shift at different time points, indicated by non-random deviations in treatment responses (Fig. 4). The difference in spore abundances between the two media types correlated with time, with distributions following either skew-normal or log-distributions. Consequently, treatment effects were only apparent after a certain time period and partly disappeared over time again. For example, RLCS17 showed clearly higher relative spore production in 100% PDA at days 8 and 12 (Fig. 4F), a pattern that was not detectable in the main experimental duration of 6 weeks (Figs. 2 and 4F). In contrast, in RLCS05 and RLCS25, the treatment effects observed here were similarly detected in the main experiment (Figs. 2 and 4H and J) but were not present before 12 days. For the fungal isolate RLCS01, no treatment effects were observed analysing the whole dataset, confirming results from the main experiment (Figs. 2 and 4B). RLCS15 showed similar patterns in both experiments also characterized by few replicates having no spore formation, which also explains the lack of treatment response at day 39 in time measurements.

Regarding extension rate, most fungi reached spatial margins (the edge of the plate) within the first half of the experimental period (Fig. 4). Following this maximum mycelium extension and a peak in biomass produced, these time measurements demonstrate that under spatial limitation fungal biomass was partly reduced again (Fig. 4A, C and G), potentially related to fungal recycling. In general, the measured biomass and mycelium size were no strong explanatory variables for the timing of spore production (Fig. 4). In RLCS01 maximum colony size and biomass were reached quickly, while relative spore production still increased linearly in both media types (Fig. 4A and B); the timing of mycelium extension rate and maximum biomass production in RLCS15 and

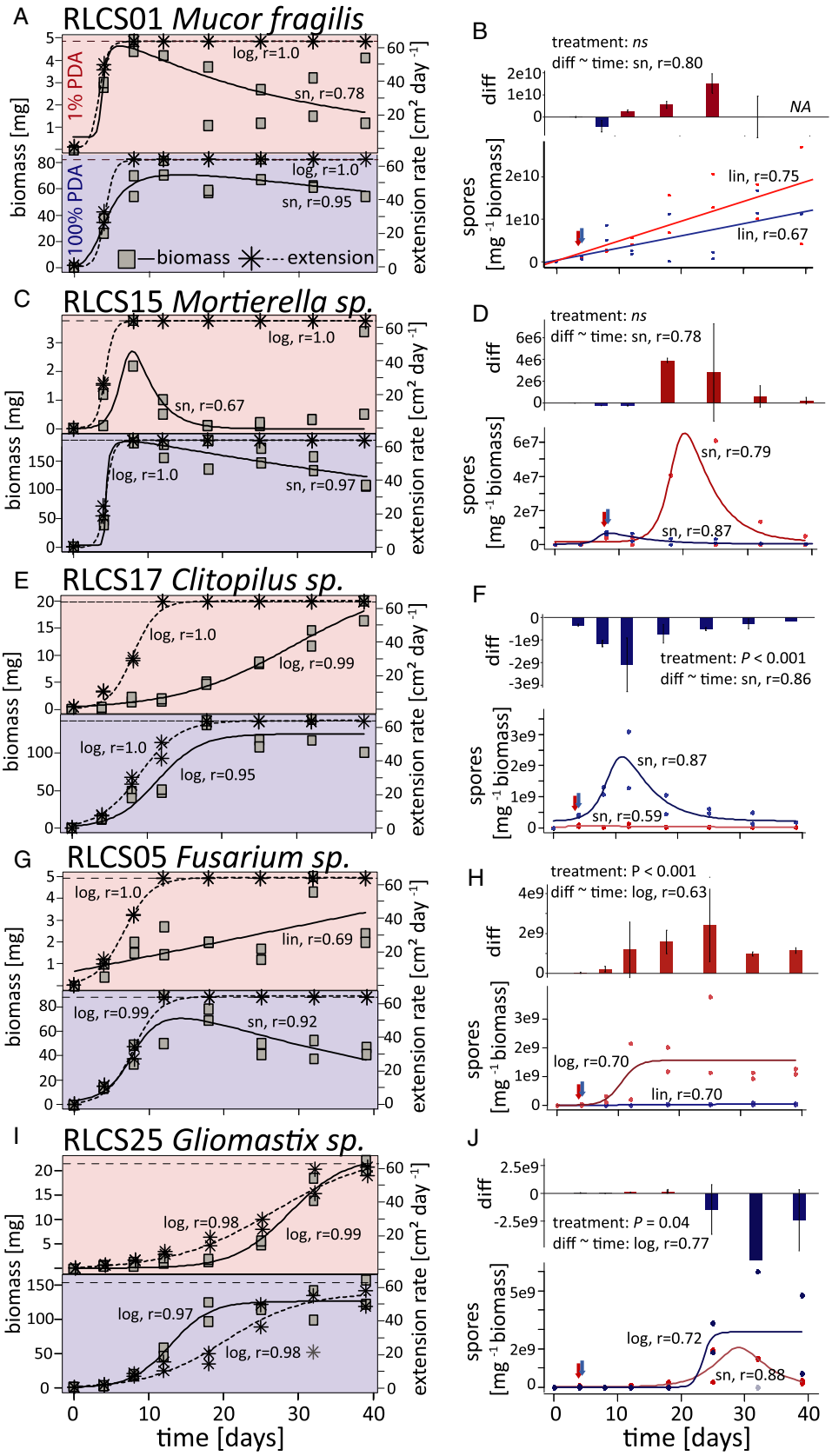

Fig. 4. Fungal growth traits and sporulation patterns observed in 'time measurements' on 1% and 100% potato-dextrose agar (PDA). Fungal growth is captured by increases in fungal biomass (mg) and extension rate (mg cm^{-2}) over time on each medium type (A, C, E, G, I), and parallel sporulation patterns are presented for each isolate, measured by relative fungal spore production (spores mg^{-1} biomass) and its absolute differences in 1% compared to 100% PDA (diff) (B, D, F, H, J). Data presented in each graph relate to one fungal isolate – RLCS01 (A, B), RLCS15 (C, D), RLCS17 (E, F), RLCS05 (G, H) and RLCS25 (I, J). Dots represent trait data measured in individual fungal samples (square: biomass, asterisk: extension rate, circles: relative spore abundance), lines the fitted distributions over time with the type of function and Pearson correlation coefficient indicated (lin: linear, log: logistic, sn: skew-normal; dashed lines show curves fitted to extension rates). Bars illustrate the average absolute difference in relative spore production, lines respective standard deviations. Red colours visualize data values measured in 1% PDA, with red bars indicating higher values in 1% PDA, while blue colours show the same for 100% PDA. *P* values of treatment effects are based on repeated measures analyses of variances. Shifts in the absolute differences in spore abundances were additionally modelled dependent on time, with the best-fitting function and Pearson correlation coefficients reported. Little arrows mark the first observation of spores produced. Detailed illustrations of curves with low visibility (F, H) are given in Fig. S7.

RLCS17 was not affected by medium type, still, relative spore production peaked at different time points (Fig. 4C–F). The slow-growing isolate RLCS25 started sporulation also relatively late, but still before reaching spatial limitations (Fig. 4I and J).

Trade-offs and correlations

PCA-based correlation analyses of the different traits analysed in the main experiment revealed a clear relationship among the same traits assessed on differing media (1% vs. 100%), despite overall treatment effects detected (Table 1, Fig. 5A and B). The relative spore production (spores mg^{-1} biomass) on the two different media was correlated ($R^2 = 0.4$, Fig. 5C), showing that isolates consistently produced high or low spore numbers irrespective of media type.

Clear trade-offs in the investment to spores versus biomass, or the size of spores and its numbers were only detected in 1% PDA medium: Here, relative spore production was negatively correlated with fungal biomass (Fig. 5E), and spore size also weakly negatively correlated with relative spore production (Fig. 5F). In contrast, no such correlations were detected in 100% PDA (Fig. 5D and F). When analysing the total amount of spores produced these general patterns did not change (Fig. S8).

Discussion

Our results reveal how reproductive allocation of soil fungi overall increases in response to resource scarcity, while observed isolate-specific strategies highlight potential niche-differentiation mechanisms in the heterogeneous resource environment of soils. Resource-poor media (1% PDA) led to a strong decrease in fungal biomass and total spore numbers, but the relative investment into spores (in terms of number of spores produced per gram of fungus) increased under resource scarcity, supporting our first hypothesis. In contrast, spore size was not affected on average, only the aspect ratio increased in low resource supply towards more elongated spores. However, these patterns were not universal for all isolates: as hypothesized observed effects were highly isolate-specific, indicating differential strategies among fungal strains in response to resource supply (that were not phylogenetically conserved) – in fact even isolates of the same genera showed variations in responses. Similarly, isolates responded differently to reductions in C, N or P supply indicating isolate-and nutrient-specific variations, since even within isolates responses differed depending on the element reduced (Supporting Information S2). Despite these observed complex resource-dependent shifts, spore abundance, size and shape appeared to be a useful trait characterizing fungal isolates, as indicated by clear correlations of spore traits in 1% and 100% PDA. Sporulation strategies (as revealed by time measurements) proved to be an additional isolate-specific trait: As hypothesized, despite shifts among media types the sporulation strategy and resource allocation over time was characteristic for the tested isolates. These isolate-specific strategies provide experimental evidence for models predicting differential life-history strategies present in fungal taxa (Gilchrist *et al*., 2006; Boddy and Hiscox, 2016). In line with this, observed trait trade-offs also support a differentiation of isolates along life-history gradients of differential resource allocation towards spores or biomass, though these trade-offs were only apparent in low resource supply.

Increased fungal investment to sporulation under resource scarcity

This large dataset sheds new light on the unresolved discussion whether resource limitation (i) reduces the ability of fungi to invest into spores (Heaton *et al*., 2016) or (ii) triggers sporulation with asexual spores as a 'rapid escape' under suboptimal conditions (Dahlberg and Etten, 1982). Here, we found that in high resource supply indeed all fungi (except two) were able to produce higher numbers of asexual spores. However, regarding the relative investment of resources into spores (in relation to biomass), we detected a general strategy among most isolates to increase relative spore production under low resource supply compared to high resource supply. This predominant strategy would allow saprobic fungi in heterogeneous soil environments to use resource-rich patches efficiently with high growth rates followed by investment in spore production, whereas in low resource environments the little available resources must be invested efficiently into spore production and dispersal over high growth rates enabling colonization of potentially more suitable patches (Fricker *et al*., 2008). Still, we also detected eight isolates with the opposite response: higher investment in spore production under high resources compared to low resources, isolate-specific patterns that have been reported before (Ritz, 1995; Gao *et al*., 2007). This detailed trait assessment also indicates that the choice of trait measurements is highly relevant to elucidate such ecological strategies – while total spore number is a good indicator of fungal fitness (Gilchrist *et al*., 2006), the relative spore investment is more relevant for understanding fungal resource allocation strategies. Many studies investigating sporulation under varying resource conditions measure total spore numbers only, while biomass also differs with changing nutrient supply (e.g. Olutiola, 1976; Ritz, 1995; Gao *et al*., 2007). Similarly, the standardization of spore numbers by

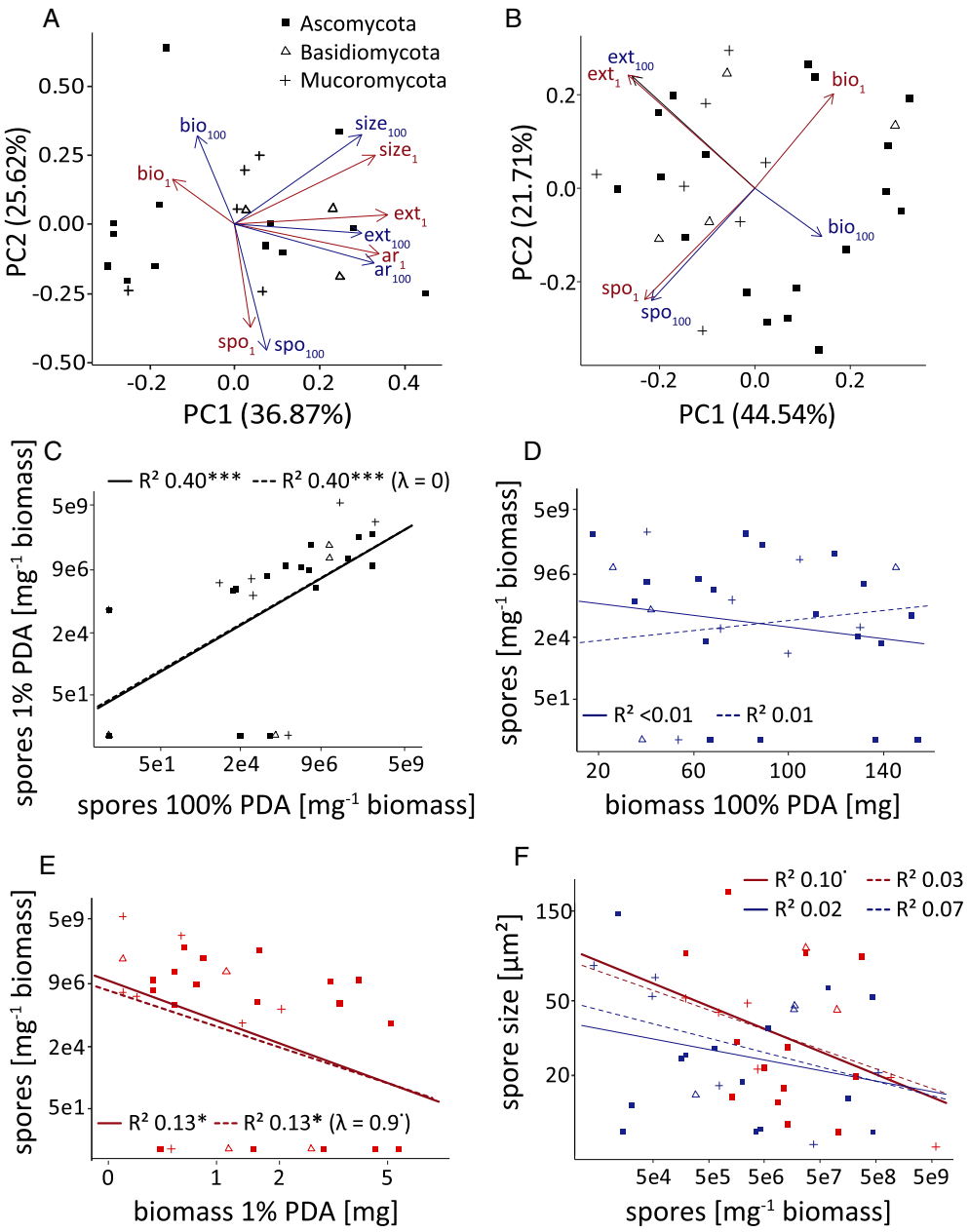

Fig. 5. Correlations among traits relevant in fungal sporulation patterns. The relation among all traits is visualized by principal component analyses (PCA; A,B), and relevant correlations among individual traits are presented as linear models (C–F). The PCA of all measured traits (A) led to the exclusion of certain isolates with missing values for spore size and shape (Fig. 3). Thus, a second PCA was conducted for traits available for all isolates (29, isolates with sexual spores only were excluded), to support the observed patterns of correlation (B).
A, B. PCA biplots display the scores of individual fungal isolates (dots) and loadings of included variables as arrows. Numbers in brackets indicate the explained variance by respective PC axes.
C–F. Linear regressions based on linear models (solid lines) and phylogenetic generalized linear models (pgls; dashed lines). Correlation coefficients (R^2 – values) and significance levels for each correlation are given ($P < 0.1$, * $P < 0.05$), and respective λ (lambda) values for pgls models and significant deviations from $\lambda = 0$ provided in brackets. Thick lines indicate relevant correlations ($P < 0.1$). Red colours indicate values assessed in 1% PDA medium, blue in 100%, respectively. Dots represent average values of individual fungal isolates (squares: Ascomycota, triangles: Basidiomycota, plus characters: Mucoromycota). *bio*: biomass (mg), *ext*: extension rate (cm^2 day^{-1}), *spo*: relative spore abundance (mg^{-1} biomass), *size*: spore size (μm^2), *ar*: aspect ratio (spore length/width), $trait_1$: trait assessed in 1% PDA medium, $trait_{100}$: trait assessed in 100% PDA medium.

mycelium area may be irrelevant when analysing resource contrasts (Sharma and Pandey, 2010; Chan *et al*., 2020), since as also shown here fungal mycelia switch towards explorative growth in nutrient-scarce environments, resulting in a decoupling of mycelium size and biomass (Camenzind *et al*., 2020).

Regarding spore size, it is a general assumption that larger spores give rise to higher fitness but are more costly (Kauserud *et al*., 2008; Norros *et al*., 2014; Aguilar-Trigueros *et al*., 2019). Thus, we hypothesized a decrease in spore size in resource-poor conditions, since previous studies also indicate a potential intraspecific variation in spore size in response to environmental conditions (Williams, 1959; Andrew *et al*., 2016). Spore size was variable within isolates and the average values of individual isolates shifted in response to resource supply, but there was no overall response detected. These results are in accordance with reproductive allocation patterns observed in other taxonomic groups (e.g. plants or birds) that showed that resource limitation strongly influences offspring production (by several orders of magnitude in plants) while offspring structure size (e.g. seeds) shows little to no variation (Stearns, 1992). In contrast, the spore aspect ratio shifted towards more elongated spores in resource-poor media. Spore shape is a trait that is rarely analysed, especially in asexual spores of soil saprobic fungi despite having large variation among fungal groups (Domsch *et al*., 2007). For sexual spores, it is known that the aspect ratio is affected by fungal nutritional mode (Kauserud *et al*., 2008) and more elongated shapes predominate in resource-depleted environments (Halbwachs *et al*., 2017). These shifts in spore shape in resource scarce environments may potentially relate to improved dispersal abilities as elongated shapes are easier to release in the air, at least in certain types of spore releasing structures (Pringle *et al*., 2015). Generally, spore size and shape observed in this study were more variable than in taxonomic descriptions by Domsch *et al*. (2007). Still, values clearly differed among isolates and the direction of responses to media type were isolate-specific, reflecting again the differential isolate-specific strategies.

Isolate-specific life-history strategies

These isolate-specific traits and strategies in response to resource limitation were present in all measured response variables. Thus, universal predictions of responses to changes in resource availability are difficult to make for this large and diverse group of saprobic soil fungi (Hawksworth and Lücking, 2017). Isolate-specific variability may translate to relatively little and heterogeneous effects on overall fungal abundance, compared to more pronounced shifts in fungal community composition and function (Rousk *et al*., 2011; Jiangwei *et al*., 2020; Moore *et al*., 2021b). Already the subset of saprobic fungi analysed in this study showed a multiplicity of different growth strategies. Not only did isolates vary in the number of asexual spores produced, mycelial extension rate and biomass production. Additionally, some isolates responded to limiting resources by increased hyphal extension which can be interpreted as explorative growth (Veresoglou *et al*., 2018), while others reduced hyphal extension; most isolates intensified relative investment into spores, but some did the opposite; some fungi sporulated only at high N supply, but low P supply, others needed both, high N and high P supply, and some showed no responses to N, P or C supply (Supporting Information S2). These strategies were so manifold that within this subset of fungi no clear groups or correlations among differential response types could be established. However, it impressively highlights the diversity of life-history strategies in saprobic fungi, which most likely allows the co-existence in diverse soil habitats and reflects the process of niche differentiation (Crowther *et al*., 2014). Fungal response strategies were not explained by phylogeny, rather additional information on fungal lifestyle and life-history strategies may explain some of the observed variability. Especially the resource use of fungi will be relevant, and the adaptation to abiotic stress or disturbed environments (Boddy and Hiscox, 2016; Bittleston *et al*., 2021). The differential shifts in extension rate and spore production under resource limitation further give support to the model of Fricker *et al*. (2008), that differentiates saprobic fungi by their spread between resource patches either via mycelia or spores.

Time-course measurements allow additional insights into fungal growth strategies (Heaton *et al*., 2016). Here, comparisons among the two media types revealed a relatively conserved strategy of sporulation in isolates, which is in agreement with genetic studies that show a tight regulation of sporulation in fungal mycelia (Park and Yu, 2012; Moore *et al*., 2021a). The sudden onset of high sporulation in most isolates is in agreement with the 'bang-bang resource allocation strategy': Following a lag phase of (predominantly) vegetative growth and resource uptake, asexual spores are produced (Damgaard and ØStergärrd, 1997; Gilchrist *et al*., 2006). However, similar to observations of Chan *et al*. (2020) in pycnidia of a saprotrophic species, our data suggest that internal resource levels represent more relevant cues for spore formation than spatial limitations. In case of RLCS01 (*Mucor fragilis*), the strategy differed and relative investment into spores was linearly increasing over time, with constantly novel sporangia formed in overgrown mycelial parts. This isolate is also special regarding other traits, namely rapid mycelial extension and unusually high P contents and respiration rates (Camenzind *et al*., 2020), which may point towards a ruderal lifestyle with adaptations to disturbed environments by rapid acquisition of simple sugars and high reproduction rates (Boddy and Hiscox, 2016; Bittleston *et al*., 2021). Genetic studies also revealed different triggers of sporulation for fungal

species, which fits to our observation that low or high internal nutrient levels differently affect the timing and especially the extent of spore production of isolates (Park and Yu, 2012; Ruger-Herreros and Corrochano, 2020). All these findings illustrate the relevance to also monitor spore formation over time, since single measurements do not always capture the complex responses of fungal resource allocation over time and are less informative regarding life-history strategies and responses to environmental variables.

Asexual spore production as a trait – challenges and novel opportunities

Spore number, size and shape are relevant traits to understand fungal growth strategies (Aguilar-Trigueros *et al*., 2015; Dawson *et al*., 2019). But more importantly, these spore traits are highly relevant to complete the picture of fungal responses to environmental change, which may not only be reflected in changes in fungal growth and activity but also increasing resource allocation towards reproduction. However, the complexity revealed here regarding not only the timing but also the differential impact of resource levels and type demonstrate the challenges related to this trait. Also, some important factors could not be included in this study. For example, further environmental triggers may be needed for some isolates to induce sporulation. Especially light is important, but also aeration in submerged cultures, which may both be highly relevant for fungi growing in soil pores (Morton and Brian, 1961; Steyaert *et al*., 2010). Additionally, in heterogeneous fluctuating, soil environments abrupt nutrient scarcity may exert a different trigger than the uniformly low resource supply applied here (Dahlberg and Etten, 1982). Similarly, here we mainly showed differences in fungal resource allocation under two distinct resource conditions, though in the light of shifting soil C:N:P ratios by environmental change the analysis of patterns along ecosystem-relevant gradients will be interesting in future studies (see also Figs. S10 and S11).

Regarding conditions in soil, it is interesting to note that trade-offs in resource allocation of fungal isolates towards spore numbers, spore size or biomass were only detectable in low resource media. That is, 100% PDA media contains such a surplus of available resources that allocation trade-offs cannot be observed in fungi. We think that these high levels of easily available C and nutrient sources, although common to grow microorganisms in the laboratory, rarely occur in the natural environment of fungi. Thus, our results illustrate that microbial life-history strategies may only be detected in natural environments, or growth media more closely resembling these conditions (Crowther *et al*., 2018). Despite these known limitations of experimental studies, the elimination of some more complex conditions in soil, for example, heterogeneity, competition and predation, allows to reveal simple mechanisms and trade-offs in response to different resource levels and enables the assessment of all relevant fungal traits, which is currently not possible in soil.

Conclusions

In conclusion, our data provide a comprehensive overview of spore formation as an ecological trait in saprobic soil fungi, the effects of resource limitations on this trait and the diversity of isolate-specific strategies that may reflect niche differentiation mechanisms. Sporulation patterns and resource allocation mechanisms are especially relevant when analysing differences in fungal growth strategies, which provides novel insights into saprobic fungal ecology especially when combined in future studies with data on fungal resource use, disturbance tolerance or responses to environmental change drivers. The responses to low-nutrient environments detected here suggest a relevant role of reproduction in fungal adaptation to differing resource levels in soil, with expected community shifts in changing environments due to varying isolate-specific strategies. Such knowledge on fungal asexual reproduction will also add substantially to the increasing number of studies and modelling approaches of mycelial growth and activity in soil, enabling more accurate predictions of changes in fungal activity under different environmental conditions, including global change.

Experimental procedures

Fungal isolates

All fungal strains analysed in this study were isolated from soil samples taken to 20 cm depth from a grassland site in northern Germany in 2014 ('Oderhänge Mallnow' close to the town of Lebus, Germany; 52°28′N, 14°29′E). Isolation protocols were adjusted to increase the likelihood to extract active fungal hyphae and reduce the predominance of spores and fast-growing fungi adapted to simple sugars (for details see Andrade-Linares *et al*., 2016). Fungal cultures were kept on potato-dextrose agar (PDA, full-strength) plates at low temperatures (4 or 12°C) and transferred to new plates infrequently (once or twice a year) to keep fungi alive. For taxonomic classification, long sequence reads of ITS1, 5.8S, ITS2 and partial LSU were established for all fungal cultures (deposited at DSMZ, German Collection of Microorganisms and Cell Cultures GmbH). Individual marker regions were compared to respective databases – ITS to Unite database (Nilsson *et al*., 2018) and LSU to RDP LSU dataset (Cole *et al*., 2014) – using the function

assignTaxonomy() in the R package dada2 (Callahan *et al*., 2016) with a bootstrap threshold of 80% to relate sequences to species identity. In case different markers resulted in diverging species names, only the genus was accepted. The 32 fungal isolates used in this study included 21 Ascomycota, 4 Basidiomycota and 7 Mucoromycota, covering a wide range of different orders and classes within these phyla (Fig. 1, see details in Table S1 and Fig. S1). The phylogenetic relatedness among taxa was determined using an alignment of the full sequence reads (AlignSeqs(), R package *DECIPHER*; Wright, 2016), genetic distance was calculated (dist.ml(), *phangorn*; Schliep, 2010) and a phylogenetic tree constructed using the unweighted pair group method with arithmetic mean (upgma(), *phangorn*).

Experimental design

Fungal sporulation patterns were analysed with fungi growing under nutrient-rich and -poor conditions. As nutrient-rich media we used full-strength PDA (100%; composition stated by the manufacturer 4 g L^{-1} potato infusion, 20 g L^{-1} glucose, 15 g L^{-1} agar, Carl Roth GmbH + KG, Karlsruhe, Germany). For nutrient poor media, we used 1% of the nutrient composition of that media while keeping the total amount of agar (0.04 g L^{-1} potato infusion, 0.2 g L^{-1} glucose, 15 g L^{-1} agar). These two concentrations were chosen to apply two very distinct resource conditions for all fungal isolates included: either clearly (carbon) limited growth (1% PDA) or ample resource supply (100% PDA). PDA was used since it is a universal growth substrate for saprobic fungi, and these fungal isolates have previously been characterized using PDA. To capture spore formation in all isolates within the experimental period and ensure comparable spore maturity, fungi were grown for 6 weeks on PDA plates (Ø 9 cm) overlain with cellophane sheets (Bio-Rad Laboratories, California, USA), since experiments demonstrated that within shorter periods only few of the fungi formed spores (see Supporting Information S2). Spore number, size and shape as well as mycelial biomass and extension rate were determined as response variables. To analyse also the timing of sporulation in response to different nutrient conditions in detail, fungal growth and spore formation was monitored in an additional experiment during the whole experimental period for a subset of five selected isolates [RLCS01 (*Mucor fragilis*), RLCS15 (*Mortierella sp*.), RLCS17 (*Clitopilus sp*.), RLCS05 (*Fusarium sp*.), RLCS25 (*Gliomastix sp*.); Fig. S1] on 1% and 100% PDA at days 4, 8, 12, 18, 25, 32 and 39. These isolates were selected based on their affiliation to different phyla, the presence of spores in both treatments in the main experiment and a varying direction and magnitude of treatment responses. This experiment will hereafter be referred to as 'time measurements'. In addition, spore production of fungal isolates was further assessed in experiments manipulating only N or P supply, to provide information about fungal niche spaces in the context of different element limitations, as opposed to the general resource scarcity with predominant C limitation imposed by 1% PDA (Methods and Results are presented in Supporting Information S2).

Prior to all experiments, three repetitions of each fungal isolate were freshly grown on PDA with antibiotics to eliminate any bacterial contaminants. Thereafter, fungi were transferred to water agar (1.5%) to restrict nutrient storage in fungal tissues. These three separate repetitions of each fungal isolate were treated and analysed individually hereafter. Only for time measurements two repetitions per isolate were used, of which individual plates were prepared for each measurement day. At the experimental start, a mycelial plug (Ø 5 mm) was cut from the active outer part of a fungal colony growing on water agar and transferred to the centre of the experimental plate. Fungal cultures were kept in the dark at 20°C during the experimental period.

Measurements

The size of the fungal mycelium was measured on several days (before spatial limitation), and its area determined with ImageJ (Schneider *et al*., 2012). Based on these data, mycelium extension rate was determined during the linear growth phase of the mycelium (cm^2 day^{-1}). In case of time measurements, mycelium area was determined at each measurement day.

For the analyses of sporulation and fungal biomass, mycelium was collected by removing the complete fungus from the cellophane surface and transferring it into 50 ml tubes. The 16 ml of PBS buffer was added, which was prepared with 0.25 g L^{-1} KCl, 1.8 g L^{-1} Na_2HPO_4, 0.3 g L^{-1} KH_2PO_4 and 10 g L^{-1} NaCl added to 800 ml H_2O, adjusted to a pH of 7.4 and subsequently replenished to 1 l volume with H_2O. The tubes were covered with Miracloth filter paper (EMD Millipore Corp., Darmstadt, Germany), and vortexed for 15 s. Subsequently, the filtrate was transferred into a new tube. This washing step was repeated three times with each sample: A pre-experiment showed that in the third washing step relative to the first one only 1%–5% of spores were extracted, and three iterations were optimal to extract all spores. The remaining fungal biomass was refilled with 10 ml H_2O and briefly microwaved to dissolve the agar plug, transferred to a filter paper and dried at 60°C to determine fungal biomass (mg). Fungal density was specified as the ratio of fungal biomass and final mycelium area (mg cm^{-2}).

In case of the 'time measurements' experiment, only two washing steps were included for spore extractions. Also, the initial agar plug and attached mycelium were removed before spore extractions, to avoid the inclusion of spores formed prior to the experimental period.

The spore filtrate was kept at 4°C before counting spore contents within 4 days. 2 ml of the suspension was centrifuged (12 500 rpm for 5 min) to concentrate spores in 200 μl. From this solution, 12 μl were transferred to a Neubauer chamber either directly or after diluting the solution in case of high spore concentrations. Spores were counted at 400× magnification using the Neubauer counting scheme, and its total abundance per sample calculated (total spores), as well as its abundance relative to biomass formation (spores mg^{-1} biomass), referred to as relative spore production.

To determine respective spore size (μm^2) [(spore length/2) × (spore width/2) × pi] and aspect ratio (length/width), microscopic pictures were taken during counting (Leica DFC 290; Leica Microsystems, Heerbrugg, Switzerland). For each sample, 20 spores were selected randomly and their length and width determined in ImageJ, focusing on the longest and widest position of the spore, respectively. When fewer than 20 spores were present within the counting scheme of the Neubauer chamber, spores outside this area were included, which also led to spore size values in few samples with no spores detected. In some samples, the number of spores measured was lower than 20 due to low spore abundances (see Figs. S2 and S3).

Statistical analyses

All statistical analyses were conducted in R version 4.1.0 (R Core Team, 2021). Respective commands and packages are given in brackets.

The overall differences in fungal response variables to 1% versus 100% PDA were captured using the response ratio RRx [natural logarithm (trait expression in 1% PDA/trait expression in 100% PDA)], statistical differences were determined by linear mixed-effects models [lme(), *nlme*; Pinheiro *et al*., 2021] and generalized linear mixed models [glmmPQL(), *MASS*; Venables and Ripley, 2002]. These treatment effects were analysed by one-way models, taking isolate identity nested within phylum as random factors into account. In addition, the interaction of isolate and treatment was determined by two-way models, using generalized least squares (gls(), *nlme*) or generalized linear models (glm()). In case of non-normality, data were either log-transformed or gamma distribution of data with a logarithmic link function used in generalized linear models, while for zero-inflated data of spore abundances quasi-Poisson distributions were assumed. For the analyses of fungal biomass and density, an unequal variance structure of the data was included with the 'weights' argument and the respective variance function varIdent() in linear mixed-effects and generalized least square models (for a detailed overview of models used for each response variable, see Table S2).

The phylogenetic signal detected in response variables for fungal isolates growing on 1% and 100% PDA was used to estimate whether trait expressions were more similar in closely related isolates. Following Münkemüller *et al*. (2012) Pagel's λ (lambda) was calculated, and its significant deviation from models assuming $\gamma = 0$ determined by likelihood ratio tests [fitContinuous(), *geiger*; Pennell *et al*., 2014]. Comparative models without phylogenetic signal ($\lambda = 0$) were based on Brownian motion models using a rescaled tree (rescale(), fitContinuous(), *geiger*). In case of spore numbers, data were log-transformed prior to analyses.

In order to assess sporulation patterns over time, fungal mycelium area, biomass and relative spore production in time measurements were correlated with time, resulting in mostly non-linear correlations. Thus, beside linear models also other distributions were fitted to the data, and the best fitting model and its goodness of fit were determined. Model fitting was performed using the nonlinear least squares method (nls()), assigning model formulas of linear, logistic and skew-normal functions to the data. In addition to logistic functions, Gompertz and exponential models were applied, but did not result in significantly better fits (Tjørve and Tjørve, 2017). Each response variable was analysed this way, for individual isolates separately. The detailed model formulas applied can be found in the Supporting Information S1. Likelihood ratio tests were used to compare model fits and select for the function best representing response curves [lrtest(), *lmtest*; Zeileis and Hothorn, 2002]. To accept a model function as best fit, a significant deviation ($P < 0.05$) from null models needed to be given, as well as significant deviations from more simple models (level of complexity: null < linear < logistic < skew-normal). Goodness of fit of respective models was determined via Pearson correlations of observed data with respective model estimates.

For time measurements, deviations in fungal investment to spores in 1% and 100% PDA medium was calculated as the absolute difference in relative spore production (mg^{-1} biomass) at the respective days among the same repetition of one fungal isolate. Since again observed deviations were non-linearly correlated with time, the shifts in treatment effects were analysed accordingly. First, overall medium (treatment) effects on relative spore production were analysed based on repeated measures one-way analyses of variances, adding day (as factor) as error stratum. In case of non-normality, data were log-transformed. Second, the

nonlinear shift in treatment effects over time was analysed by fitting respective distributions as described above to the calculated differences in relative spore production, determining its significant deviations from null models (= no shift in treatment responses over time) via likelihood ratio tests and the respective goodness of fit was identified by Pearson correlations.

The correlations among different fungal traits were analysed via multivariate analyses using principal components analyses (PCA), testing relevant traits additionally with univariate linear models. Prior to analyses, all non-normal data were log-transformed. PCAs were first conducted with all selected traits (prcomp()): Since this led to a reduction of isolates due to missing values, PCA was repeated with spore abundance and fungal growth traits alone, in order to assess relations among these traits in 1% and 100% media for all isolates. Individual correlations emerging from PCA either showing interesting positive correlation or trade-offs among traits were analysed separately using linear models. Here, normal linear regression analyses were complemented with phylogenetic generalized linear models [pgls(), *caper*; Orme *et al*., 2018] to assess the impact of phylogenetic dependence on observed relationships. Phylogenetic structure was incorporated by allowing lambda and delta transformations to be maximized by maximum likelihood methods. In case of correlations using spore abundances (zero-inflated data), the observed significant correlations in linear models were additionally confirmed using generalized linear models with quasi-Poisson distributions [glmmPQL(), glm()], considering phylum as random factor to account for phylogenetic structure.

Three fungal isolates formed only sexual ascospores (Fig. 1, S1). Since the initiation of sexual spore production may differ ecologically from asexual spore production, these isolates were excluded from all statistical analyses. An inclusion of these data slightly changed the observed phylogenetic signals, while treatment effects were not affected (Table S3).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1 Details of the analysed fungal isolates including taxonomic classification based on long sequence reads of ITS1, 5.8S, ITS2 and partial LSU regions, including DSMZ accession numbers (German Collection of Microorganisms and Cell Cultures GmbH)

Fig. S1: Phylogenetic classification of fungal isolates and the related phylogenetic tree as depicted in Fig. 1, with an additional table displaying information on the subset of fungal isolates that were used in different parts of the experiments.

Fig. S2: Overall variation in spore size within fungal isolates grown on 100% and 1% potato-dextrose agar (PDA), including data of all individual spores analysed per sample, for three repetitions per isolate. Some isolates had only few spores that could be analysed, thus, the quantity of individual spores considered for analyses are given below the box- and whisker plots. Isolates of the genus *Chaetomium* which only formed sexual ascospores are marked with a plus sign.

Fig. S3: Overall variation in spore aspect ratio [spore length/width] within fungal isolates grown on 100% and 1% potato-dextrose agar (PDA), including data of all individual spores analysed per sample, for three repetitions per isolate. Some isolates had only few spores that could be analysed, thus, the quantity of individual spores considered for analyses are given below the box- and whisker plots. Isolates of the genus *Chaetomium* which only formed sexual ascospores are marked with a plus sign.

Table S2: Average growth and sporulation traits of fungal isolates grown on 1% and 100% PDA medium (same as Table 1) and the respective statistical functions used to determine treatment effects

Table S3: Results of fungal growth and sporulation patterns in 1% versus 100% PDA medium including isolates forming sexual ascospores (Fig. S1).

Fig. S4: Total spore numbers (log-scale) of individual isolates in 100% (**a**) and 1% potato dextrose agar (PDA; **b**), and the average response ratios RRx (**c**). **a, b** Dots indicate mean values of each isolate and medium ($n = 3$), bars the respective complete data range (non-log transformed data were used for the calculation of average values). Vertical lines mark the average values of all isolates. **c** Bars represent the response ratio RRx (natural logarithm (spores formed in 1% PDA/spores formed in 100% PDA)), with blue bars indicating higher spore numbers in 100%, red bars higher numbers in 1% PDA medium. The black vertical line indicates the average value of all isolates, asterisks the significant overall medium effects (*** $P < 0.001$). The phylogenetic signal calculated as Pagel´s λ (lambda) is shown for each trait, with *P*-values in brackets indicating significant model deviations from models assuming $\gamma = 0$. Grey shadings differentiate fungal phyla - MUC: Mucoromycota, BAS: Basidiomycota, ASC: Ascomycota. Plus signs identify isolates of the genus *Chaetomium*, which only formed sexual ascospores and were excluded from analyses.

Fig. S5: Responses in fungal biomass and mycelium extension rate (ext. rate) to 1% potato-dextrose agar (PDA) medium in comparison to growth on 100% PDA. Average response ratios (RRx) for each isolate are presented, calculated as the natural logarithm of the ratio of traits observed on 1% and 100% PDA. Red lines indicate the average response ratio for all isolates, asterisks significant treatment effects (* $P < 0.05$, *** $P < 0.001$). The tree indicates phylogenetic relations of the analysed fungal isolates ($n = 3$; Fig. 1), no phylogenetic signal was detected.

Fig. S6: Correlations among spore size and aspect ratio [spore length/width] in 1% (red) and 100% (blue) potato-dextrose agar (PDA). Linear regressions are based on linear models (solid lines) and phylogenetic generalized linear models (pgls; dashed lines). Correlation coefficients (R^2 - values) and significance levels for each correlation are given (* $P < 0.05$, ** $P < 0.01$), and respective λ (lambda) values for pgls models and significant deviations from $\lambda = 0$ provided in brackets. Thick lines indicate relevant correlations ($P < 0.1$). Dots represent average values of individual fungal isolates (squares: Ascomycota, triangles: Basidiomycota, plus characters: Mucoromycota).

Fig. S7: Detailed illustration of relative spore production over time assessed in the fungal isolates RLCS17 (*Clitopilus sp*.) (**a, b**) and RLCS05 (*Fusarium sp*.) (**c, d**) in 100% (**a, c**) and 1% PDA (**b, d**). Dots represent data measured in individual fungal samples, lines the fitted distributions over time with the type of function and Pearson correlation coefficient indicated (lin: linear, log: logistic). **c** In RLCS05 grown on 100% PDA, the logistic distribution showed no significantly better fit than the linearly fitted function (see Fig. 4).

Fig. S8: Correlations among fungal traits relevant in fungal sporulation patterns visualized by principal component

analyses (PCA), focusing on total spore numbers instead of relative spore production (see Fig. 5a,b). PCA biplots show the scores of individual fungal isolates (dots) and loadings of included variables as arrows. Numbers in brackets indicate the explained variance by respective PC axes. Red colours indicate values assessed in 1% PDA medium, blue in 100%, respectively. Dots represent average values of individual fungal isolates (squares: Ascomycota, triangles: Basidiomycota, plus characters: Mucoromycota). bio: biomass [mg], spo: total spore abundance, ext: mycelial extension rate [cm^2 day^{-1}], $trait_1$: trait assessed in 1% PDA medium, $trait_{100}$: trait assessed in 100% PDA medium.

Fig. S9 Relative spore production of fungal isolates in response to differing element limitations. Fungal spores were isolated from fungi cultured on media differing in nitrogen (N) (**a**), phosphorus (P) (**b**) and potato dextrose agar (PDA) concentrations (**c, d**), the latter used to induce carbon (C) limitations. In simplified terms, the graphs can be interpreted as on the right side spores are preferentially produced under limiting conditions, whereas on the left side under high resource supply. **a, b** Dots indicate the calculated optima of C:N and C:P ratios for relative spore production (spores mg^{-1} biomass) on media differing in N and P contents (represented as C:N and C:P ratios, with high values indicating low nutrient supply (for details see Camenzind *et al*. 2020)). Lines represent the respective niche breadth - defined as spore formation more than 50% of the maximum calculated based on fitted distributions (Fig. S10 and S11). The absence of dots indicates that spore abundance was not affected by the respective element manipulation, and no optima could be defined. Detailed data and fitted distributions can be seen in Fig. S10 and S11. Red crosses indicate isolates that were tested along the respective gradients, but did not form spores within the experimental period. All fungal isolates were cultured for 12 days, except the fast growing RLCS01, RLCS03, RLCS04 and RLCS05 (7 days) and few slow growing fungi – RLCS21, RLCS28, RLCS29 and RLCS31 (27 days). **c, d** For comparison purposes spore abundances of isolates in 1% versus 100% PDA are presented as response ratio RRx (natural log (spores mg^{-1} biomass in 1% PDA/spores mg^{-1} biomass in 100% PDA), with spore formation patterns measured after 6 weeks of growth (**d**). Additionally, in order to compare the experiments, data from time measurements at day 12 or day 8 (depending on growth periods on C:N and C:P media) are presented for the available five fungal isolates (**c**). Red bars indicate isolates that formed more spores in 1% PDA, blue bars in 100% PDA, respectively.

Fig. S10: Relative spore production [mg^{-1} biomass] of different fungal isolates in response to media differing in C:N (carbon: nitrogen) ratios, with high C:N ratios indicating low N supply and vice versa (Camenzind *et al*. 2020). Different distributions were fitted to the data as described in the methods part, and its Pearson correlation coefficient assessed (r-values). In case of RLCS05, no response to the N gradient was detected (no significant deviation to null models). lin: linear, log: logistic, sn: skew-normal, neg: negative decay function.

Fig. S11: Relative spore production [mg^{-1} biomass] of different fungal isolates in response to media differing in C:P (carbon: phosphorus) ratios, with high C:P ratios indicating low P supply and vice versa (Camenzind *et al*. 2020). Different distributions were fitted to the data as described in the methods part, and its Pearson correlation coefficient assessed (r-values). In case of RLCS27, only one sample formed spores, which may be regarded as outlier - more samples would need to be analysed to confirm the positive trend. Lin: linear function.