Supporting Information S1

of the manuscript*: Soil fungal mycelia have unexpectedly flexible stoichiometric C:N and C:P ratios*

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Additional methodological details

Fungal isolates

Saprotrophic fungi were isolated from soil samples derived from a grassland site in northern Germany ("Oderhänge Mallnow" close to the town of Lebus, Germany; 52°13´N, 14°13´E) (Andrade-Linares *et al.* 2016). This fungal collection of overall 31 isolates has been characterized in detail in previous studies (Lehmann *et al.* 2019; Zheng *et al.* 2020), and covers Mucoromycota, Basidiomycota and Ascomycota (see Table S1).

Fungal growth media

For N manipulations we decided to add NH_4NO_3 as a universal fungal N source (Jennings 1995), for P manipulations NaH_2PO_4 since Na was previously shown to not affect fungal growth (Camenzind et al. 2018b). Levels of high N and P supply were based on expected fungal demands derived from published C:N:P contents (Mouginot et al. 2014; Zhang & Elser 2017), whereas values for low nutrient supply were based on lower limits reported in litter and soil (McGroddy et

al. 2004; Cleveland & Liptzin 2007). NH_4NO_3 or NaH_2PO_4 additions did not change the pH of the glucose base medium (~ 4.5). In case of manipulations of P supply, 16 g L^{-1} special purified agar was used (A7921, Sigma-Aldrich, Darmstadt, Germany; 0.04 mg P g^{-1} agar (ICP-OES analysis)).

Soil used for soil-extract agar (SEA) shows the following average characteristics (Horn *et al.* 2014): pH 5.77, C content 11.9 mg g^{-1}, N content 0.9 mg g^{-1} and P content 7.32 mg kg^{-1}. The pH of resulting SEA medium was determined as 7.35, which slightly decreased by N additions (7.14) and P additions (6.6), independently of C supply.

For all media prepared glucose/cellulose and phosphate were autoclaved separately, since glucose may caramelize in the presence of salts, and phosphate forms insoluble precipitates or provokes toxic conditions (Moore *et al.* 2011; Tanaka *et al.* 2014).

Enzymatic analyses

For enzymatic analyses, fungi were grown on different nutrient manipulated media with a cellophane layer. Due to the time requirement of the laborious method, only four fungal isolates were used (RLCS12, RLCS17, RLCS27, RLCS01; n=3) and partly only relevant treatment combinations included: the complete N and P gradient in glucose media, in cellulose media only high N (C:N 20) and low N conditions (C:N 200, in soil-extract agar only N and glucose manipulations (Ctr, +N, +Glu, +Glu+N). Four enzymes were tested for each isolate – laccase, beta-glucosidase, leucine-aminopeptidase and acid-phosphatase. In case of RLCS01, laccase was replaced by cellobiohydrolase, since no laccase activity was recorded. For each enzyme tested, a piece of mycelium was cut as a circle segment of the colony, in order to obtain enzymatic activity representative of the whole fungal colony. An additional circle segment per plate was cut and used

to determine dry weights. Detailed information on conditions and substrates are given in Table S3. Shortly, mycelial segments were kept in 100 µl buffer solutions, and 100 µl of substrate were added. An additional sterile control with no fungal material, as well as a control without substrate but fungal material was added, in order to account for unspecific colorimetric changes, also due to fungal material/spores. Enzymatic reactions were all performed at 25°C to allow for hyphal activity, while experimental duration was species-specific for each enzyme tested.

Table S1: Details on fungal strains used in the experiments: For each strain, information about phylum, class, order, family and taxon name and DSMZ accession numbers (German Collection of Microorganisms and Cell Cultures GmbH) are given.

strain ID	phylum	class	order	family	taxon name[1]	DSMZ accession number
RLCS10	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	*Alternaria alternata*	DSM100286
RLCS12	Ascomycota	Dothideomycetes	Pleosporales	Didymellaceae	Didymellaceae strain	DSM100405
RLCS22	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Paraphoma chrysanthemicola*	DSM100401
RLCS28	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Tricladium sp.*	DSM100323
RLCS13	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium solani*	DSM100290
RLCS18	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	*Gibberella sp.*	DSM100287
RLCS06	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Chaetomium angustispirale*	DSM100400
RLCS27	Ascomycota	Sordariomycetes	Sordariales	Chaetomiaceae	*Thielavia inaequalis*	DSM100326
RLCS16	Basidiomycota	Agaricomycetes	Agaricales	Pleurotaceae	*Pleurotus pulmonarius*	DSM100408
RLCS17	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Clitopilus sp.*	DSM100324
RLCS09	Basidiomycota	Agaricomycetes	Polyporales	Coriolaceae	*Trametes versicolor*	DSM100406
RLCS15	Mucoromycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella elongata* strain 1	DSM100402
RLCS01	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	*Mucor fragilis*	DSM100293

[1]best resolved tree annotation passing 80% threshold of bootstrap approach

Table S2: Growth ability of different fungal isolates with cellulose as carbon source, tested by growth responses to increasing cellulose supply (fungal biomass [mg]; mean ± standard deviation).

Cellulose content [g L^{-1}]	RLCS10	RLCS12	RLCS09	RLCS17	RLCS16	RLCS28	RLCS27	RLCS01
0	2.3 ± 0.3	2.4 ± 0.7	28.2 ± 26.5	2.1 ± 0.0	2.4 ± 0.1	2.1 ± 0.1	3.7 ± 0.1	2.2 ± 0.2
5[1]	20.8 ± 5.9	29.5 ± 0.4	34.7 ± 1.3	7.7 ± 0.5	28.7 ± 9.6	15.8 ± 1.3	23.9 ± 1.3	2.7 ± 0.7
20	32.5 ± 7.6	46.7 ± 1.9	40 ± 38	52.7 ± 21.8	27.5 ± 4.9	14.3 ± 3.0	29.5 ± 2.5	8.5 ± 4.1

[1]For the N manipulation experiment 4.5 g L^{-1} cellulose were used. The ability of fungi to increase biomass even further from 5 to 20 g L^{-1} indicates that no other elements in the medium were limiting (Camenzind *et al.* 2020).

Table S3: Conditions of analyses for enzymatic analyses.

Enzyme	substrate	substrate concentration	Tempe-rature	buffer solution	Absor-bance	RLCS01		RLCS17		RLCS12		RLCS27	
						circle segment	duration	circle segment	duration	circle segment	duration	circle segment	duration
Cellobio-hydrolase	para-nitrophenyl-cellobioside	2 mM	25 °C	acetate buffer (pH 5)	410 nm	1.41	8 h	-	-	-	-	-	-
laccase	2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS)	2 mM	25 °C	acetate buffer (pH 5)	405 nm	-	-	1.41	3 h	1.41	2 h	2.47	2 h
beta-glucosidase	2,4-nitrophenyl-β-d-glucopyranosidase	2 mM	25 °C	acetate buffer (pH 5)	400 nm	1.41	8 h	1.41	3 h	1.41	22 h	2.47	22 h
leucine-amino-peptidase	leucine-p-nitroanilide	5 mM	25 °C	tris buffer (pH 8)	405 nm	1.41	10 min	1.41	15 min	1.41	1 h	2.47	15 min
acid-phospha-tase	para-nitrophenyl phosphate	5 mM	25 °C	acetate buffer (pH 5.5)	410 nm	1.41	8 h	1.41	3 h	1.41	22 h	2.47	22 h

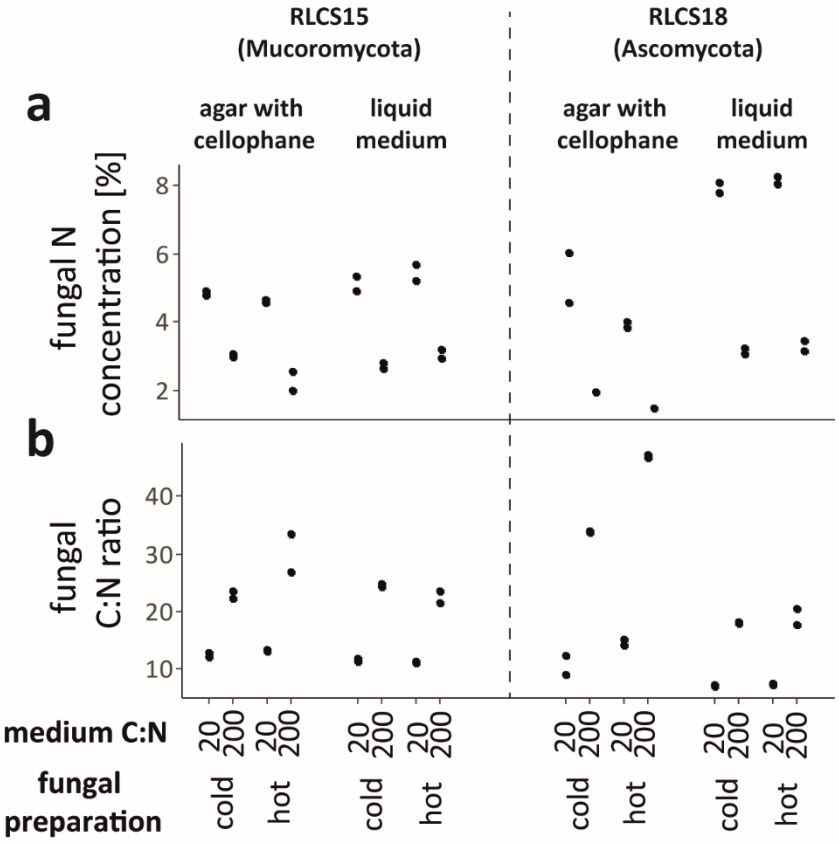

Fig. S1: Evaluation of the effects of media types and fungal preparation on fungal nitrogen (N) concentration [%] and corresponding C:N ratios, with dots representing individual data points. Two fungal isolates (two repitions each) were either grown in liquid media or on agar separated with a cellophane layer, using described glucose media with high N (C:N = 20) or low N supply (C:N = 200). At the end of the growth period of 12 days mycelium was either prepared as described in the methods, using a microwave and 1 L of 90° dest. H_2O (hot) or just rinsed with 1 L of cold dest. H_2O (cold). The effect of "hot" versus "cold" fungal preparation was non-significant when analyzed by factorial analysis of variances, treatment effects were most relevant and clearly distinguishable.

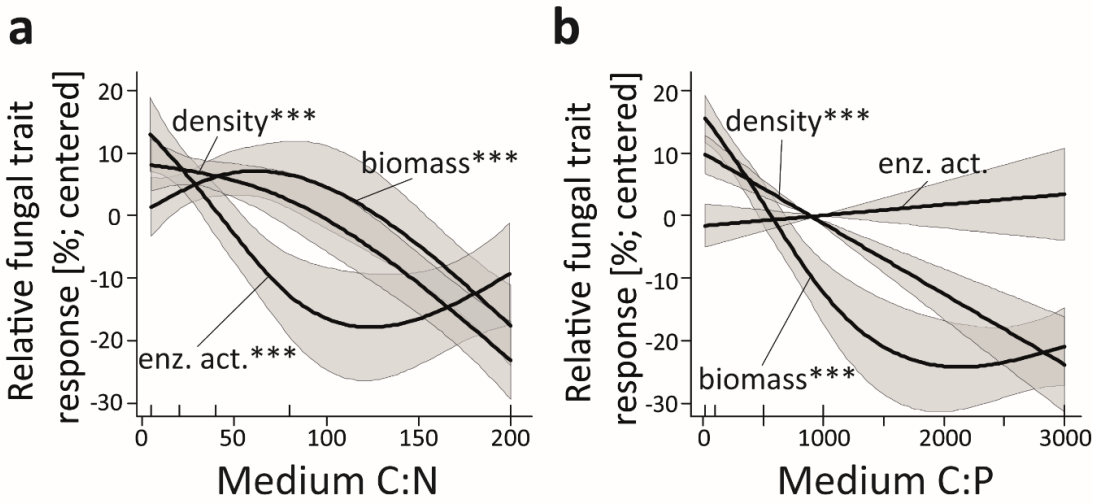

Fig. S2: Fungal growth responses to varying N and P supply in glucose medium. Non-linear response curves were analyzed by generalized additive mixed models using the function *gamm()* (package mgcv (Verbeke 2007)). This function is specified non-parametrically as "smooth function", adding isolate and repetition as random factors, respectively, as well as enzyme in case of enzymatic activities (enz. act.). For the analyses of fungal enzymatic activity responses in enzyme types related to the element tested (i.e. leucine aminopeptidase or phosphatase) were excluded from analyses, due to potential increases in deficient element supplies as compensatory mechanism (Sinsabaugh *et al.* 2008). In order to compare effect sizes among growth traits, and to standardize for differences among isolates, relative deviations in fungal growth responses (% deviation from maximum values for each isolate) were analyzed and plotted. Shaded areas represent respective 95% confidence intervals.

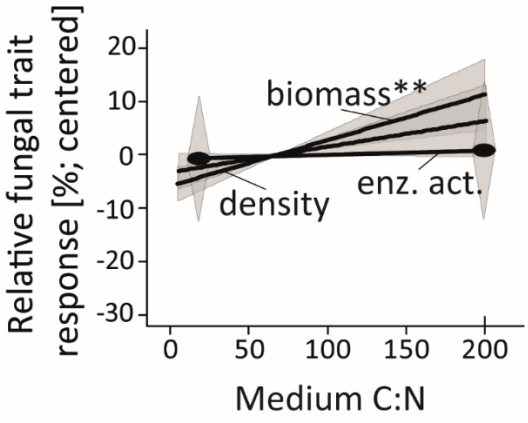

Fig. S3: Fungal growth responses to varying N supply in cellulose medium. Trait response curves were analyzed by generalized additive mixed models using the function *gamm()* (package mgcv (Verbeke 2007)). This function is specified non-parametrically as "smooth function", adding isolate and repetition as random factors, respectively. In order to compare effect sizes among growth traits, and to standardize for differences among isolates, relative deviations in fungal growth responses (% deviation from maximum values for each isolate) were analyzed and plotted. Differences in enzymatic activity (enz. act.; leucine-aminopeptidase activity was excluded from analyses) among the two levels assessed were analyzed by linear-mixed effects models. Shaded areas represent respective 95% confidence intervals.

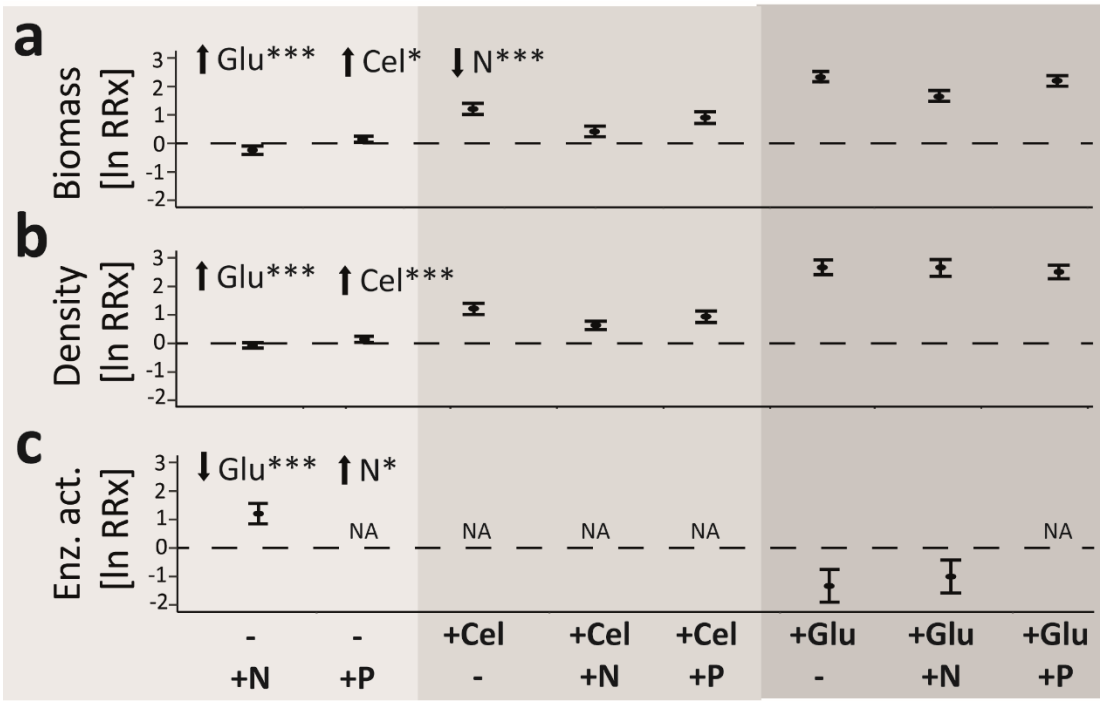

Fig. S4: Fungal growth responses to nitrogen (N), phosphorus (P), cellulose (Cel) and glucose (Glu) manipulations in soil extract agar. Deviations of fungal biomass [mg] (**a**), mycelial density [mg cm^{-2}] (**b**) and enzymatic activity [activity mg^{-1} fungal biomass] (**c**) are presented as normalized deviation from control values (depicted as dashed lines) to standardize among isolates. Dots represent average values, lines respective standard errors (8 fungal isolates, 2 repetitions each). Arrows in each graph depict significant effects of element additions, based on two-way linear-mixed effects models testing the interaction of N and P with cellulose and glucose addition, respectively, including isolate as random factor – no significant interaction terms were detected (* $P < 0.05$, *** $P < 0.001$). Data were log transformed due to non-normality. For the analyses of fungal enzymatic activity in certain treatments (*NA* = not available), responses in respective enzyme types related to the element tested (i.e. leucine aminopeptidase or phosphatase) were excluded from analyses, due to potential increases in deficient element supplies as compensatory mechanism (Sinsabaugh *et al.* 2008).

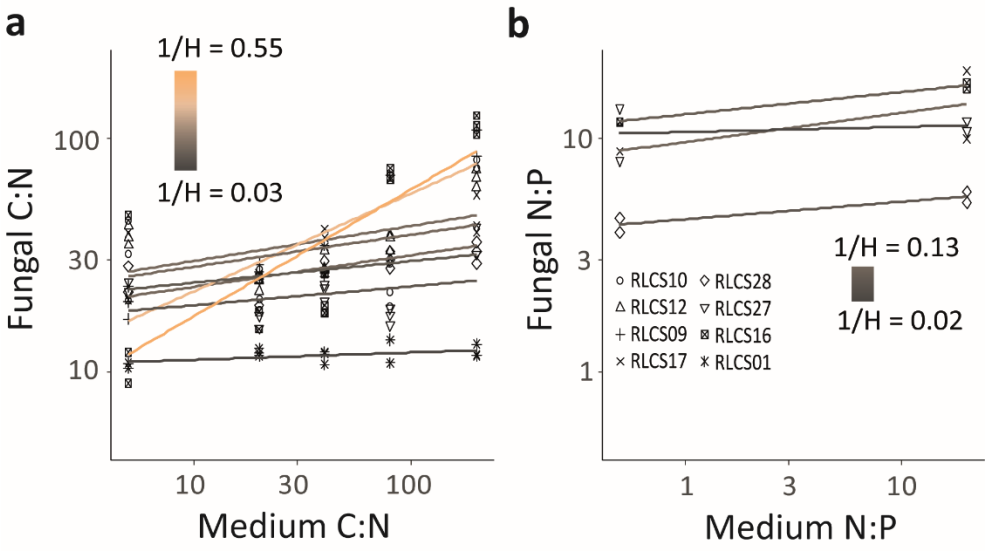

Fig. S5: Stoichiometric responses in fungal C:N:P ratios to varying N supply in defined cellulose media. **a** and **b** depict shifts in fungal C:N and N:P ratios in response to respective varying media supply ratios (axes are log10 transformed). Lines represent the linear response of fungal stoichiometry, symbols data points of individual isolates (see legend). Line colors depict 1/H values calculated for each isolate, with maximum and minimum values shown in the respective color legend.

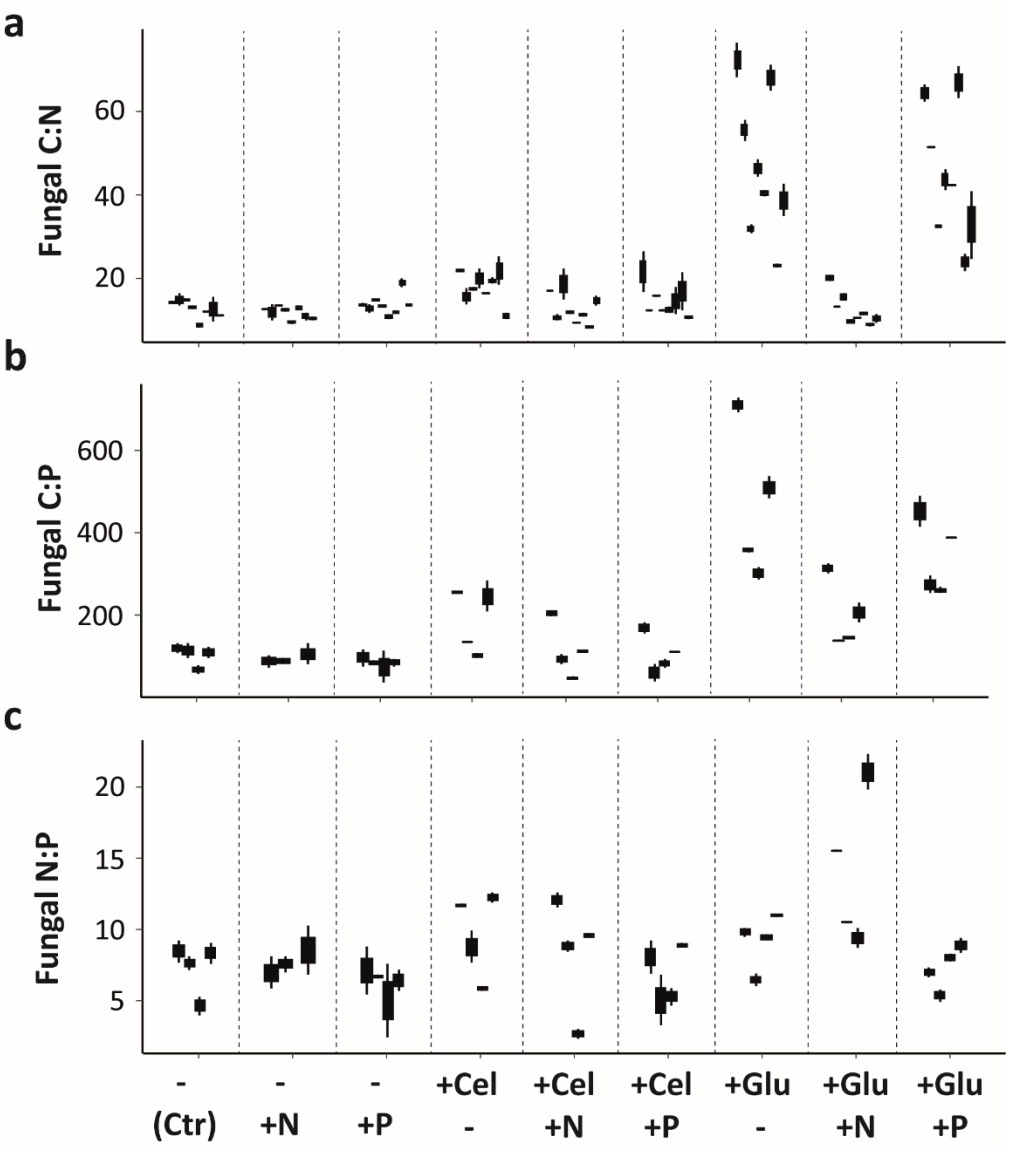

Fig. S6: Isolate-specific stoichiometric responses to nitrogen (N), phosphorus (P), cellulose (Cel) and glucose (Glu) manipulations in soil extract agar. Fungal C:N (**a**), C:P (**b**) and N:P (**c**) values are illustrated as individual box-and-whisker plots for each isolate (in (**a**) left to right: RLCS12, RLCS22, RLCS28, RLCS27, RLCS17, RLCS17, RLCS09, RLCS01; in (**b**) and (**c**) left to right: RLCS12, RLCS22, RLCS28, RLCS27; n=2).

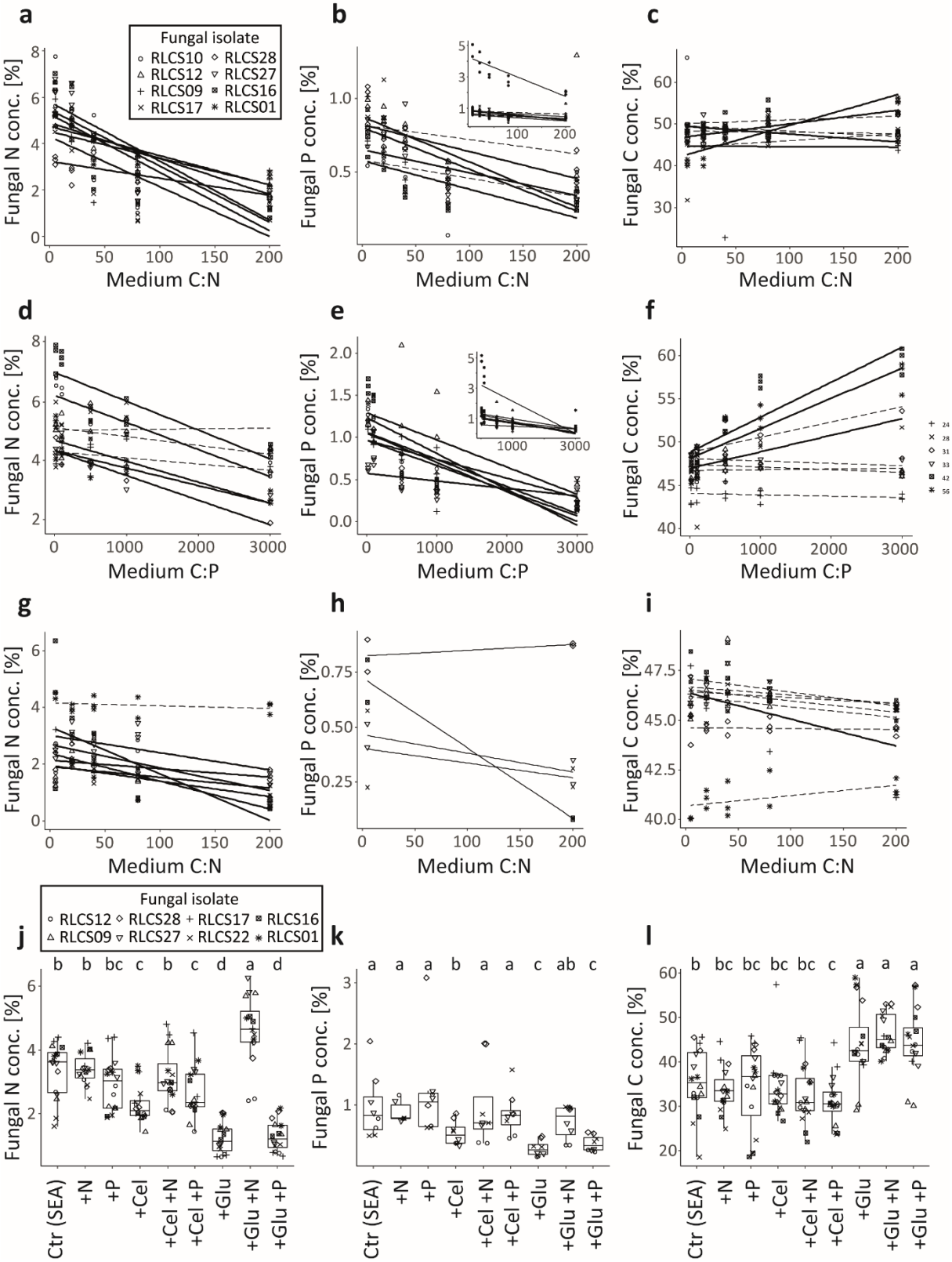

Fig. S7: Responses of fungal element concentrations [%] to nutrient manipulations in defined glucose media (**a-f**), defined cellulose medium (**g-i**) and soil-extract agar (SEA) (**j-l**). Data points of individual isolates are illustrated by respective symbols (see legends). Lines represent linear responses of individual isolates (**a-i**) based on linear mixed-effects models, with solid lines indicating a correlation

(*P* < 0.05), while dashed lines indicate no response (*P* > 0.05). In case of P concentrations analyzed in cellulose media (**h**), too few data points were available for statistical analyses. The insert in **b** and **e** depict high P concentrations observed in the fast growing isolate RLCS01, which were excluded from the main graph. Responses of element concentrations to nutrient manipulation in SEA are illustrated by box-and-whisker plots, with single data points of each fungal isolate depicted by different symbols. Letters indicate differences among groups (analysis of variances; *P* < 0.05). C: carbon; N: nitrogen; P: phosphorus; Cel: cellulose; Glu: glucose.

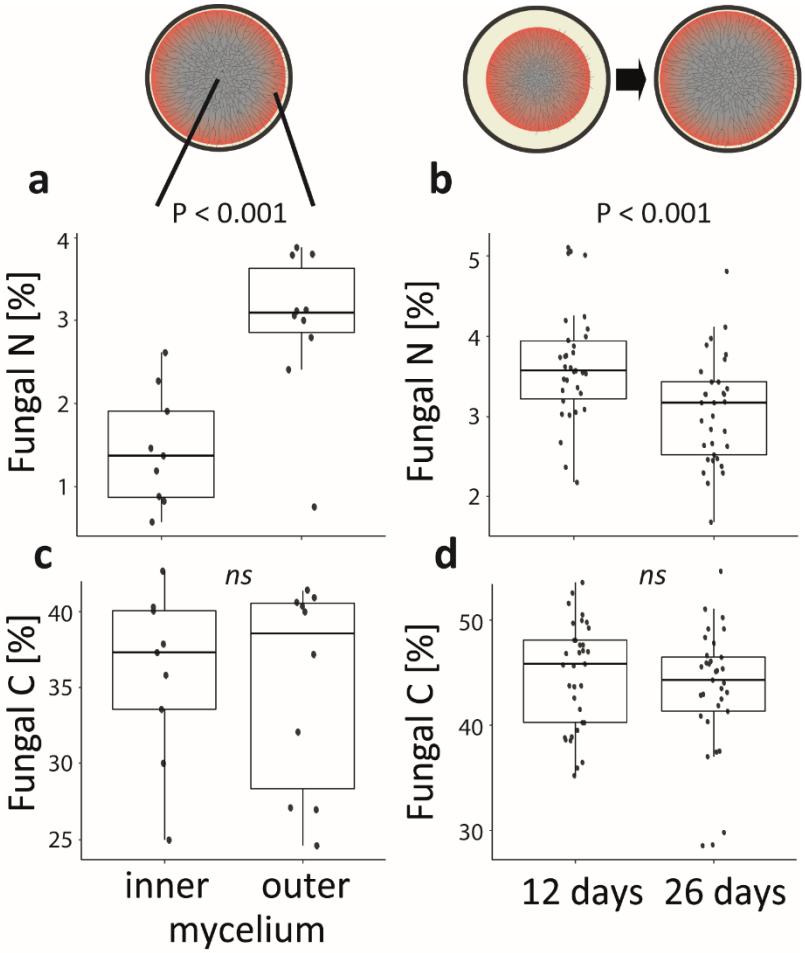

Fig. S8: Spatial and temporal shifts in fungal nitrogen (N) (**a, b**) and carbon (C) (**c, d**) concentrations [%] are depicted. Element concentrations were compared in inner versus outer parts of the mycelium (**a, c**), and in fungi grown for 12 or 26 days (**b, d**) on soil-extract agar. Dots represent data points underlain by box-and-whisker plots. *P*-values derive from paired t-tests.

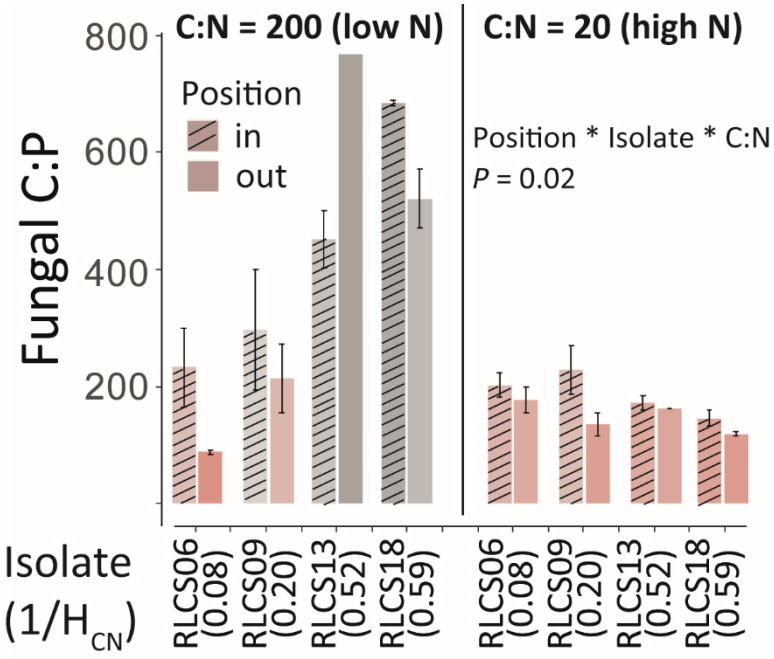

Fig. S9: Stoichiometric C:P ratios measured in inner (hatched bars) and outer (filled bars) parts of fungal mycelia grown in low and high N supply glucose media. Tested fungal isolates differ in $1/H_{CN}$ values, previously determined along a larger gradient of C:N (see Fig. 1). Bars illustrate mean values, error bars respective standard errors. Presented sums of squares and *P*-values are based on three-way type III analyses of variances. Colors illustrate respective P concentrations in fungal mycelia (red:high, grey: low).

Table S4: Predictive power of stoichiometric ratios in media, fungal tissues and the deviation among both for fungal growth responses to differing nitrogen supply in glucose media (linear mixed-effects models; isolate and repetition as random effects for fungal biomass and density, enzyme and isolate in case of enzymatic analyses).

Response variable	Fixed factor	*P*-value	R^2 (marginal[1])	R^2 (conditional[2])	Estimate	Std. Error	t-value
Fungal biomass (mg; log)	C:N (fungus)	*ns*					
	P:N (fungus)	*ns*					
Fungal density (mg cm^{-2} mycelium; log)	C:N (fungus)	**<0.001**	**0.02**	**0.82**	-0.0084	0.0024	-3.49
	P:N (fungus)	**<0.01**	**0.08**	**0.79**	-2.12	0.77	-2.77
Enzymatic activity (activity mg^{-1} mycelium; log)	C:N (fungus)	**<0.001**	**0.12**	**0.78**	-0.043	0.0085	-5.05
	P:N (fungus)	**<0.001**	**0.33**	**0.66**	-8.9	2.2	-4.04
Phosphatase / Leucine-Aminopeptidase activity	C:N (fungus)	**<0.001**	**0.26**	**0.9**	-0.01	0.0017	-6.06
	P:N (fungus)	**<0.01**	**0.52**	**0.95**	-3.27	0.7	-4.68

[1] variance explained by fixed effects only

[2] variance explained by fixed effects and random effects due to varying intercepts among isolates

Table S5: Predictive power of stoichiometric ratios in media, fungal tissues and the deviation among both for fungal growth responses to differing phosphorus supply in glucose media (linear mixed-effects models; isolate and repetition as random effects for fungal biomass and density, enzyme and isolate in case of enzymatic analyses).

Response variable	Fixed factor	*P*-value	R^2 (marginal[1])	R^2 (conditional[2])	Estimate	Std. Error	t-value
Fungal biomass (mg; log)	C:P (fungus)	**<0.001**	**0.08**	**0.48**	-0.0007	0.0002	-3.95
	N:P (fungus)	**<0.001**	**0.09**	**0.49**	-0.01	0.003	-3.84
Fungal density (mg cm^{-2} mycelium; log)	C:P (fungus)	**<0.001**	**0.05**	**0.84**	-0.001	0.0002	-5.27
	N:P (fungus)	**<0.001**	**0.04**	**0.82**	-0.013	0.003	-4.37
Enzymatic activity (activity mg^{-1} mycelium; log)	C:P (fungus)	*ns*					
	N:P (fungus)	*ns*					
leucine-aminopeptidase / phosphatase activity (log)	C:P (fungus)	**0.025**	**0.18**	**0.38**	-0.002	0.0009	-2.24
	N:P (fungus)	**0.04**	**0.18**	**0.3**	-0.028	0.014	-2.09

[1] variance explained by fixed effects only

[2] variance explained by fixed effects and random effects due to varying intercepts among isolates

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