Soil fungal mycelia have unexpectedly flexible stoichiometric C:N and C:P ratios

Tessa Camenzind[1], Kay Grenz[1], Johannes Lehmann[2], and Matthias Rillig[1]

[1]Freie Universität Berlin
[2]Cornell University

August 11, 2020

Abstract

Soil ecological stoichiometry provides powerful theories to integrate the complex interplay of element cycling and microbial communities into biogeochemical models. One essential assumption is that microbes maintain stable C:N:P (carbon:nitrogen:phosphorus) ratios independent of resource supply, although such homeostatic regulations have rarely been assessed in individual microorganisms. Here, we report an unexpected high flexibility in C:N and C:P values of saprobic fungi along nutrient supply gradients, overall ranging between 7-126 and 20-1488, respectively, questioning microbial homeostasis. Fungal N:P varied comparatively less due to simultaneous reductions in mycelial N and P contents. As a mechanism, internal recycling processes during mycelial growth and an overall reduced N and P uptake appear more relevant than element storage. The relationships among fungal stoichiometry and growth disappeared in more complex media. These findings affect our interpretation of stoichiometric imbalances among microbes and soils and are highly relevant for developing microbial soil organic carbon and nitrogen models.

Introduction

Ecological stoichiometry represents an important field originally developed in aquatic ecosystems, with its principles being now also applied to terrestrial soil systems (Sterner & Elser 2002). Models use stoichiometric theory for predicting soil nutrient cycles and carbon storage (Sardans *et al.* 2012). Based on the general observation that C:N:P (carbon:nitrogen:phosphorus) ratios of soil microbial communities are more narrow than resource C:N:P, critical ratios of C:N and C:P are defined to predict nutrient demands of soil microbes, mineralization versus immobilization patterns and C sequestration versus respiration (Manzoni *et al.* 2012; Zechmeister-Boltenstern*et al.* 2015). Thus, ecological stoichiometry facilitates the incorporation of extremely complex processes into global models and predictions, especially in the context of global change (Hall *et al.* 2011a).

These models make crucial assumptions that are critical for their validity (Mooshammer *et al.* 2014; Spohn 2016), one of them that heterotrophic soil microbes are homeostatic, i.e. maintain stable C:N:P ratios independently of the soil nutrient status (Persson *et al.*2010). Even though some models allow microbial C:N:P to vary slightly (McGill *et al.* 1981; Nicolardot *et al.* 2001), homeostatic flexibility is currently only attributed to microbial community shifts, not an actual stoichiometric flexibility in individuals (Buchkowski*et al.* 2019). Fixed microbial stoichiometric ratios are interpreted as an indicator of nutrient demands: If microbes need to maintain their narrow C:N ratios, N will be in limiting supply in substrates characterized by wider C:N ratios (Manzoni *et al.*2010). These assumptions of soil microbial homeostasis are supported by analyses of entire soil communities, which indeed show relatively little variation in microbial C:N:P ratios compared to soil resource variability (Cleveland & Liptzin 2007; Hartman & Richardson 2013). By contrast, we know astonishingly little about the stoichiometry of individual soil microbial groups.

Indeed, flexibility of C:N:P ratios in individual microbial species has rarely been analyzed, and the few

studies available mainly use aquatic isolates and partly result in contradictory results depending on the methods applied (Danger *et al.* 2016). Surprisingly, some of these studies suggest that microbial C:N:P ratios may be less homeostatic than assumed for heterotrophic organisms. For aquatic bacteria Scott et al. (2012) and Godwin and Cotner (2018) demonstrated high variation in C:P ratios for some but not all isolates (see also Makino *et al.*2003; Danger *et al.* 2008). In case of saprobic fungi, aquatic hyphomycetes responded to varying element supply with non-homeostatic adjustments in C:P ratios, while C:N remained stable (Danger & Chauvet 2013; Gulis *et al.* 2017). In soils there are also indications that fungal C:N:P may exceed common textbook assumptions of C:N ˜10-20 and C:P ˜100-300 (Jennings 1995; Strickland & Rousk 2010). A recent meta-analysis demonstrated wide C:N and C:P ratios in few fungal samples (Zhang & Elser 2017), as also reported for C:N in wood decomposing fungi under low N conditions (Levi & Cowling 1969), while other studies indicate again only little flexibility (Heck 1928; Egli & Quayle 1986; Mouginot *et al.*2014).

Physiological mechanisms causing non-homeostasis in microbes are still unknown. Most authors assume P storage, i.e. element uptake in excess as the exclusive mechanism (Scott *et al.* 2012; Danger & Chauvet 2013; Mooshammer *et al.* 2014), though this only explains stoichiometric shifts in response to high element supply. The significance of such stoichiometric shifts for fungal growth and activity also remain unresolved. Available data show that N or P uptake can be independent of fungal growth, potentially related to storage mechanisms or primary C limitations (Levi & Cowling 1969; Gulis*et al.* 2017).

In order to understand stoichiometric adjustments in soil saprotrophic fungi in detail, we analyzed fungal mycelial element concentrations in response to varying N, P and C supply in different growth media specifically developed for this question (varying from highly controlled to natural substrates). Derived from common assumptions in soil ecological stoichiometry, we tested the hypothesis that saprobic fungi are homeostatic, especially regarding their C:N ratios. Our results not only further question the general assumption of microbial homeostasis, but also provide insights into the physiological mechanisms of element allocation in mycelia, and the significance of stoichiometric shifts for fungal growth and activity under varying conditions.

Materials and methods

Fungal material

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 (Zheng *et al.* 2018; Lehmann *et al.* 2019), and covers Mucoromycota, Basidiomycota and Ascomycota (see Table S1).

Before the experiment, each isolate used here was divided into three repetitions and cultured separately on potato-dextrose agar with antibiotics to eliminate potential contaminants. Subsequently, repetitions were transferred to water agar (1.5 %), in order to reduce nutrient storage in fungal tissues. Hereafter, the three repetitions were analyzed separately for each fungal isolate and treatment.

Experimental design of nutrient manipulations

Nutrient availability was manipulated in growth media differing in complexity and C sources, namely defined glucose medium, defined medium with cellulose as C source and soil-extract agar (SEA) manipulated by N, P, glucose and cellulose additions. Eight fungal isolates covering all three phyla were used (RLCS10, RLCS01, RLCS16, RLCS12, RLCS27, RLCS17, RLCS28, RLCS09; see Table S1 for information on these isolates); in SEA medium RLCS10 was replaced by RLCS22, since its growth in SEA was too limited for further analyses.

Glucose medium . N and P availability were manipulated in defined medium with known growth conditions and nutrient limitation patterns. This medium was designed in accordance with the law of the minimum, providing all elements and conditions in non-limiting supply to ensure N or P limitation of fungal growth only (Camenzind *et al.* 2020). The resulting medium contained 5 g L^{-1} glucose, 0.5 g L^{-1} $MgSO_4$, 0.5 g KCl, 0.1 g NaFeEDTA, 5 mg L^{-1} $ZnSO_4$, 0.05 mg L^{-1} Na_2Mo_4, 0.05 mg L^{-1} $MnSO_4$, 0.05 mg L^{-1} H_3BO_4, 0.01

mg L^{-1} $CuSO_4$, 1 mg L^{-1}Thiamine HCl, 0.05 mg L^{-1} biotin and 20 g L^{-1} agar. N supply was manipulated by adding NH_4NO_3 in a defined quantity as universal fungal N source (Jennings 1995), five levels of N supply were tested (molar C:N = 5, 20, 40, 80 and 200 (C:P = 100)). P availability was manipulated similarly by adding NaH_2PO_4 (molar C:P = 20, 100, 5000, 1000, 3000 (C:N = 20)), 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 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)).

Cellulose medium . For this experimental approach glucose was replaced by cellulose (SigmaCell - highly purified fibers, Sigma-Aldrich, St. Louis, US) maintaining molar C contents. In a separate pre-test we confirmed that no other elements or conditions were limiting and fungi were able to use cellulose as a C source, though to varying degrees (Table S2). N was manipulated as described above.

Nutrient manipulation in SEA . In order to obtain a more natural fungal growth substrate, we prepared an additional growth medium based on soil extract. Soil sampled at the original grassland site was used (average soil characteristics: pH 5.77, C content 11.9 mg g^{-1}, N content 0.9 mg g^{-1}, P content 7.32 mg kg^{-1} (Horn *et al.* 2014)). Samples were autoclaved, mixed thoroughly with demineralized H_2O 1:3 (v:v) and sieved through 20 µm. The resulting liquid was supplemented with 20 g L^{-1} agar. For nutrient manipulation, only the addition of N and P is possible in uncontrolled organic substrate. However, by concomitantly increased C availability by adding glucose (Glu) or cellulose (Cel), respectively, nutrient limitation was induced experimentally. Consequently, treatments comprised a control (Ctr; non-manipulated SEA), +N, +P, +Glu, +Glu+N, +Glu+P, +Cel, +Cel+N and +Cel+P. Glucose and Cellulose were added in equal molar C quantities, mimicking conditions in controlled media – 5 g L^{-1}glucose and 4.5 g L^{-1} cellulose. For N addition, 1.28 g L^{-1} NH_4NO_3 was added, lowering soil C:N ratios of 15 experimentally to 5. P was added as 0.27 g L^{-1} $NaH_2PO_4$to shift soil C:P ratios of 1500 to 100 (based on original soil sample analyses). The parallel addition of C and N or C and P resulted in C:N ratios of ˜5.2 and C:P ratios of ˜75, respectively. pH of SEA was determined as 7.35, and slightly decreased by N additions (7.14) and P additions (6.6), independently of C supply.

For media preparation, 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).

In glucose and cellulose media, fungi were grown in petri dishes in the dark for 12 days at 20°C (Ø 9mm), only the fast-growing isolate RLCS01 was kept for 7 days in glucose media, while RLCS28 was grown for 27 days to obtain sufficient biomass. In SEA, all fungal strains were cultured for 26 days to ensure sufficient biomass formation for analyses.

Additional experiments evaluating N and P allocation in mycelia

Stoichiometric data derived from nutrient gradient tests suggested a differential allocation of N and P in fungal mycelia during growth. To test such patterns of element allocation, data of fungal strains growing on SEA and glucose media were analyzed in this context.

12 fungal strains (RLCS01, RLCS16, RLCS27, RLCS15, RLCS13, RLCS28, RLCS22, RLCS17, RLCS09, RLCS18, RLCS12, RLCS11; n=3) were grown on SEA, analyzing N contents after 12 and 26 days. Additionally, the N content of inner and outer mycelium of four strains (RLCS12, RLCS16, RLCS22, RLCS28; n=3) was analyzed after 26 days of growth. Therefore, the radius of fungal mycelia at four positions was determined, and partitioned by 2/3 (inner) and 1/3 (outer). Mycelia were cut and divided into inner and outer parts according to this separation, and element contents analyzed separately.

Similarly, in an additional nutrient manipulation experiment the inner and outer mycelium of fungi grown in

glucose media with high N (C:N 20) and low N content (C:N 200) were harvested separately using the same mycelial partitioning. Four fungal strains were selected based on large variation of $1/H_{CN}$ values see below), also using fungal strains previously characterized in another study (RLCS06, RLCS18, RLCS09, RLCS13; unpublished data).

Fungal growth and stoichiometric analyses

At the end of experiments, agar media were melted in a microwave and mycelium collected on a 20 µm mesh. To remove superficial element traces, mycelia were washed with 1 L of >90 °C demineralized H_2O, and cleaned with 0.1 M HCl for one minute. This washing method was inevitable to ensure complete removal of agar medium from the mycelium (Maynard *et al.* 2017), but we showed in a separate experiment that it did not alter element concentrations or treatment effects (Fig. S1). Fungal material was freeze-dried and kept at -20 °C. Fungal dry biomass was determined, as well as fungal density [mg cm^{-2} mycelium] based on the actual size of the mycelium, since mycelial density as a trait proved to be a good estimate of fungal fitness and indicator of nutrient limitations (Camenzind *et al.* 2020). Fungal enzymatic activity was assessed as an additional important response trait (for details see Supporting Information S1). For element analyses, fungi were milled and C and N contents determined with an Elemental Analyzer (EuroEA, HekaTech, Germany). P content was analyzed after aqua regia digestion (1:4 HCl:HNO_3) by ICP-OES analyses (Optima 2100 DV, Perkin Elmer, Germany). In cellulose media, P contents were only analyzed for four isolates (RLCS16, RLCS17, RLCS27 and RLCS28) in media with C:N of 5 and 200, respectively. Likewise, in SEA media P contents were only analyzed for four isolates (RLCS12, RLCS22, RLCS27 and RLCS28).

Statistical analyses

All statistical analyses were done in R version 3.6.1 (R Core Team 2019).

The homeostatic coefficient 1/H was calculated to analyze the response of fungal stoichiometric ratios to varying nutrient supply in glucose and cellulose media (Persson *et al.* 2010). For example, $1/H_{CN}$ represents the regression slope of fungal log10 (C:N) correlated with log10 (C:N) in media, and takes values between 0 (strictly homeostatic) and 1 (non-homeostatic) (for more details see also Sterner and Elser (2002)). The same was calculated for $1/H_{CP}$ and $1/H_{NP}$ values, for the latter using the whole gradient of N and P manipulation in glucose media. Differences in isolate-specific 1/H values among different element gradients were calculated by paired t-tests. Variations in fungal stoichiometric ratios in fertilized SEA media were analyzed by linear mixed-effects models, taking isolate as random effect into account (lme(); package *nlme* (Pinheiro *et al.* 2020)). Here, in case of non-normality, data were log-transformed.

Details of the results and analyses of fungal growth responses to varying nutrient supply in glucose, cellulose and SEA medium are shown in Fig. S2, S3 and S4, respectively.

Total fungal element masses [mg] were correlated with fungal biomass [mg] in each medium type, in order to understand the stability of different elements in fungal mycelia under varying resource conditions. In this case, data were normalized within isolates for each element and medium type, respectively, by standardizations between 0 and 1. Model outputs are based on linear random slope models, using isolate as random factor. Explanatory power of biomass versus nutrient manipulation on total fungal element masses were assessed by the comparison of sums of squares, evaluated by type III analyses of variances of model outputs (package *lmerTest* (Kuznetsova *et al.* 2017)).

To assess simultaneous decreases in fungal N and P concentrations [%] in both, N and P manipulations, linear correlations were done by linear-mixed effects models with isolate as random factor for each medium and nutrient manipulation, respectively. Relative deviations in element concentrations [% deviation from maximum values of each isolate] were used to compare effect sizes of element reductions among isolates. In SEA media, added carbon sources were included as fixed effect, since element concentrations were significantly affected by cellulose and glucose additions. Marginal R^2 values were calculated using function r.squaredGLMM() (package *MuMIn* (Barton 2019)).

Differences in fungal C:N values of inner versus outer mycelium in SEA medium, as well as of fungi growing

12 or 26 days, respectively, were determined by paired t-tests (one-sided). To evaluate the effects of treatment (low vs. high N), position (in vs. outer mycelium) and isolate on fungal C:N and C:P values in low and high N glucose media, three-way type III analyses of variances were applied.

The predictive power of stoichiometric ratios for fungal growth and activity was determined by linear correlations of growth responses (namely fungal biomass, density, enzymatic activity and the ratio of leucine-aminopeptidase and phosphatase activity) with stoichiometric ratios, applying linear mixed-effects models (lmer(); package*lme4* (Bates *et al.* 2012)). Marginal (variance explained by fixed effects only) and conditional (variance explained by fixed effects and random effects due to varying intercepts among isolates) R^2 values were calculated with function *r.squaredGLMM()* .

Results

Fungal C:N:P ratios in response to varying nutrient supply

Fungal C:N and C:P ratios were highly flexible for most isolates in all media types and nutrient manipulations, compared to more stable N:P ratios. Along a defined gradient of N and P supply using glucose as a C source, fungal C:nutrient ratios positively correlated with C:nutrient supply in the medium, with $1/H_{CN}$ and $1/H_{CP}$ values of isolates ranging from 0.16 to 0.65 (Fig. 1a,b). At high N supply (medium C:N = 5) fungal C:N only varied within 8 to 18, while reaching a maximum of 84 at low N supply (C:N = 200). Likewise, fungal C:P ranged from 23 to 199 at high P (C:P = 20), compared to values up to 1114 at low P supply (C:P = 3000) (Fig. 1). By contrast, shifts in N:P ratios resulted in lower $1/H_{NP}$than $1/H_{CN}$ and $1/H_{CP}$ ($P< 0.01$), with most isolates remaining relatively homeostatic (Fig. 1c).

In similarly controlled N and P manipulations with cellulose as a more costly C source, the C:N ratio was likewise non-homeostatic in some isolates, overall even reaching higher C:N values up to 126 (Fig. S5a), while again N:P remained relatively stable ($1/H_{NP}$ 0.02 – 0.13; Fig. S5b). Also in complex soil-extract agar (SEA) varying N, P and C supply strongly affected fungal C:N:P ratios. In non-manipulated SEA fungal C:N:P was on average 103:7:1. N and P additions alone did not lower ratios significantly, whereas the addition of cellulose and glucose significantly increased C:N and C:P ratios four- to fivefold, respectively (Fig. 2). Increased C availability consistently led to higher fungal biomass (in parallel with C uptake) (Fig. S4), while N and P concentrations remained low. The supplementary addition of N and P again reduced C:N and C:P ratios to levels of the control, without affecting biomass production (Fig. 2, S4). Increased availability of N and P both affected C:P and C:N ratios to a similar extent. Consequently, N:P remained more stable than C:nutrient ratios, only varying up to twofold (Fig. 2c).

Quantification and correlation of fungal C, N and P contents in nutrient manipulation experiments

In homeostatic fungal growth, not only would the ratio of elements but also the relative concentration of each element in mycelia have to remain constant. In this scenario, fungal size would be the only determinant of respective total element masses in mycelia, irrespective of nutrient supply, i.e. the larger a fungus grows the more elements it accumulates. This was the case for the total mass of fungal C, which showed a strong linear correlation with biomass (Fig. 3a). Comparing model sums of squares (see pie charts Fig. 3), biomass was the primary determinant for total C mass (Fig. 3d), except for a weak treatment effect of glucose fertilization in SEA (Fig. 3a, Fig. S7l). Total N and P masses, however, were less strongly coupled to fungal biomass production. The correlation with fungal biomass was also positive, but R^2 and slope values were much lower (Fig. 3b,c). Mostly, nutrient manipulations exerted stronger impacts on total fungal N and P masses than biomass production (see pie charts Fig. 3; Fig. 3e,f)). Thus, contrary to the homeostatic assumption that C, N and P concentrations remain constant within fungal mycelia independent of environmental conditions, fungal mycelia must have physiological mechanisms to reduce or increase P and N concentrations, while C remains relatively constant (Fig. S7).

Since fungal N:P ratios were more stable than C:nutrient ratios in all media types, fungal N and P must have responded to nutrient manipulations in a concerted way, even though only the availability of one element changed (Fig. 1, 2). Correlating the relative reductions of N and P concentrations in fungal mycelia within

the different applied nutrient gradients supported this conclusion (Fig. 4). For example, along a gradient of C:N manipulated in defined glucose medium, under conditions of low N supply the proportion of N in fungal mycelia decreased on average to 31 % of its maximum values. In parallel, even though the P supply in the medium was not experimentally manipulated, the mycelium P concentrations was reduced to an average of 41 % of its highest value, resulting in a correlation with an R^2 of 0.66 and a slope as high as 0.67 between fungal N and P concentrations (Fig. 4a). In case of N manipulations, the parallel shift in P concentration was comparably strong for all media tested (Fig. 4a, c, e, Fig. S7), whereas in P manipulated media the parallel shift in N was slightly weaker, indicated by lower slope values (Fig. 4b, d). Still, correlations were also very clear. None of the elements positively correlated with C concentrations, which slightly increased in N and P limited conditions in some isolates in glucose media (Fig. S7c,f) and raised from 35.5 ± 1.9 % (mean ± SE) in SEA to 44.3 ± 2.2 % in SEA + Glu ($P < 0.001$) (Fig. S7l).

Element allocation in fungal mycelia

The ability to reduce or increase N and P concentrations (in parallel) within the fungal mycelium may be related to spatial patterns (e.g. retranslocation/recycling) or a general decline throughout the mycelium. In fungal mycelia grown on SEA we indeed observed different C:N ratios in inner versus outer regions of mycelia (Fig. 5a) due to twofold increases in relative N concentrations in outer parts (Fig. S8). Since high N concentrations were only present in the outer, more recently produced and likely more active mycelium portion, the relative amount of N decreased during fungal growth causing also temporal shifts in C:N ratios (Fig. 5b, Fig. S8). We tested whether this simple element allocation pattern also explained non-homeostatic shifts in C:N ratios due to limiting N supply (Fig. 1), namely a simple spatial reduction of the outer N-rich zone (Fig. 6). Again, all fungal isolates had lower C:N (and also C:P) ratios in outer mycelial parts, however, this differential allocation was not affected by N supply rates (Fig. 5c, Fig. S9). Shifts in fungal C:N ratios occurred equally in inner and outer mycelial parts (Fig. S9).

Relation of stoichiometric shifts with fungal growth and activity

In a defined glucose medium characterized by N or P limitations alone, varying fungal C:N:P values were good linear predictors of fungal growth and activity (Fig. S2, Table S4 and S6). In parallel to shifts in element concentrations, biomass production, fungal density and enzymatic activity were positively affected by higher N supply, as well as biomass and density by increasing P supply (Fig. S2). Thus, models examining the explanatory power of fungal C:N and C:P ratios distinguished them as good predictors of fungal growth, activity and the ratio of phosphatase and leucine-aminopeptidase production (Table S4 and S5). By contrast, in complex media with more diverse C sources, observed strong shifts in C:N and C:P values did not allow to predict N or P limitations. Along varying N supply with cellulose as a C source, fungal traits did not respond positively to increasing N availability, showing no indication of N limitation despite high fungal C:N values (Fig. S3). Likewise, in soil extract agar the addition of N and P alone did not affect biomass production or fungal density, nor did it show a significant interaction with the addition of cellulose or glucose (Fig. 2b, c). By contrast, cellulose and glucose additions shifted fungal biomass from 7.8 mg (± 2.7) to 19.9 mg (± 2.1) and 64.4 mg (± 10.6), respectively. Only enzymatic activity responded positively to N additions, while glucose strongly suppressed fungal enzymatic activity also in the presence of additional N (Fig. 2d). Consequently, stoichiometric ratios did not correlate with fungal growth or activity in cellulose and SEA media.

Discussion

Contrary to the proposed hypothesis of element homeostasis, we observed high flexibility in fungal C:P and even C:N ratios, reaching values far beyond common estimates of microbial stoichiometry (Cleveland & Liptzin 2007; Strickland & Rousk 2010), with maxima of 1488 and 126, respectively. Induced N and P limitations under controlled conditions reduced the relative amount of fungal N and P concentrations on average by 69 and 81%, respectively, causing wide fungal C:nutrient ratios, while increasing C availability in more natural substrate (i.e. SEA) allowed fungi to build up on average eight times more biomass despite strongly widening C:N and C:P ratios. These results show that soil fungi can adjust C:nutrient ratios much

more flexibly than expected. Fungal N:P also showed variations, but the consistent parallel shift in N and P concentrations resulted in more homeostatic fungal N:P than C:nutrient ratios. This may be a general pattern in fungi: Gulis et al. (2017) also reported lower 1/H values for N:P than C:P in aquatic hyphomycetes, while Zhang and Elser (2017) realized that fungal N:P was closer to the canonical Redfield ratio than C:N and C:P average values (Redfield 1958). However, potential underlying mechanisms and general stoichiometric patterns revealed here have not been described before.

Mycelial internal retranslocation and shifted nutrient uptake explain stoichiometric flexibility

Strict homeostasis is typically assumed for heterotrophs, for example in metazoans with a determined body shape, whereas in addition to other mechanisms discussed below the modular indeterminate growth form of autotrophic plants allows more flexible adjustments (Güsewell 2004; Persson *et al.* 2010). In soil fungi, an indeterminate mycelial lifestyle may also permit stronger stoichiometric flexibility than expected. Fungi cannot only dynamically translocate elements within their mycelium depending on element demands (Watkinson *et al.*2006), but also internally recycle elements and cytoplasm. Mycelial tip growth is sustained by cytoplasm transport towards active hyphal tips (Moore *et al.* 2011), and recycling mechanisms of hyphal autolysis by intracellular degrading enzymes allow efficient mycelial expansion (Reyes *et al.* 1990; Lilly *et al.* 1991; Pusztahelyi*et al.* 2006). Given that N and P are more abundant in active cytoplasm, while C is also mainly bound in the hyphal wall structure, variable C:nutrient ratios in contrast to more stable N:P appear to be the logical outcome of this mycelial growth (only about 2 % of N is bound in cell walls compared to 60-70 % in proteins (Paustian & Schnürer 1987a; Peter 2005)) (Fig. 6). Not only the parallel shifts in fungal N and P, but also the spatial and temporal variability of C:N and C:P ratios in fungal mycelia observed here lend strong support to this idea, since N and P accumulated in the outer "active growth" zone and proportionately decreased with progressing mycelial expansion. Previous reports of more active outer mycelia (Zheng 2015) and temporal relative reductions in RNA and N and P concentrations (Levi & Cowling 1969; Newell & Statzell-Tallman 1982; Grimmett *et al.* 2013) also support this result. Whether the inner mycelium is dead or simply less active (and still involved in transport; Fricker *et al.* 2017) is currently unclear.

As a mechanism for non-homeostasis in microbial individuals, mainly P storage has been discussed so far (Fricker *et al.* 2008; Scott*et al.* 2012). In plants, by contrast, diverse additional processes are known, including shifted element uptake, nutrient resorption and investment in different tissues (Frost *et al.*2005). Our data as well as previous stoichiometric assessments in fungi also provide evidence for more complex mechanisms in fungi (Fig. 6; Levi & Cowling 1969). Resorption and efficient use of N and P is supported by the spatial stoichiometric flexibility (here retranslocation). However, the response to low N conditions (after only 12 days) was not driven by a simple reduction of the outer active zone, but may rather relate to small-scale spatial processes throughout the young growing mycelium (Fig. 6; see also Klein and Paschke (2004)). Beside a spatial reduction in active cytoplasm, the parallel shift in fungal N and P concentrations may also relate to reduced uptake (as described for plants) and a decline of both elements throughout the mycelium. Here, homeostatic N:P ratios simply relate to a tight coupling of the synthesis of N-rich proteins with P-demanding ribosomal activity (Loladze & Elser 2011).

Regarding element storage, the parallel reduction in N and P concentrations lend little support to N or P storage as the major mechanism of non-homeostasis. Only along the applied P gradient may polyphosphate stores have been depleted before reducing internal P (and N) concentrations below optima (Scott *et al.* 2012), which may also explain the lack of enzymatic responses to P limitation (Fig. S2). On the other hand, C storage may enhance wide C:element ratios, for example in glucose supplemented SEA media (Wilson *et al.* 2010). Future studies need to apply detailed chemical and microscopic analyses to reveal the contribution of these physiological mechanisms to mycelial non-homeostasis (Hall *et al.* 2011b).

In complex growth substrates fungal C:N:P values do not indicate nutrient demands

Shifts in stoichiometric ratios can be useful to predict organismic nutrient demands, as applied for variable leaf N:P ratios in plants (Güsewell 2004). However, the observed shifts in C:N:P ratios only allowed to infer fungal N or P demands in controlled media with the respective element as the only limitation (Camenzind

*et al.*2020). In complex growth substrates, i.e. more "costly" C sources and unknown limitation patterns, stoichiometric ratios were not related to responses in fungal growth or activity to N and P supply. In complex media (co-)limitation by various elements may impede simple causalities (Kaspari & Powers 2016). The parallel shifts in C:N and C:P ratios reported here, which were also observed for other elements (unpublished data) complicate the interpretation of stoichiometric shifts: Wide C:P ratios, for example, are indicative of both, limitations of N and P and potentially also other nutrients. Here, N:P may be explored as a preferential stoichiometric indicator of nutrient limitations (Reich & Oleksyn 2004; Persson *et al.* 2010). In addition, C limitation in soil appears to primarily control fungal biomass production - as shown in SEA media - most likely due to the investment of C into hyphal wall structures compared to the efficient (re)use of cytoplasmic N and P contents (Paustian & Schnürer 1987b; Camenzind *et al.* 2018a).

Implications for soil carbon sequestration and nutrient cycling

What are the implications of these findings, and why do analyses of soil microbial communities primarily show homeostasis despite this apparent flexibility in individuals? Our results imply that most saprobic fungal species do adjust their stoichiometry and nutrient use efficiency to resource supplies, a finding not only giving interesting new insights into mycelial growth dynamics but also affecting our view of microbially driven element cycles. Since stoichiometric models in soil are based on whole microbial communities, our data on fungal individuals do not directly indicate flexible C:N:P ratios at the community level. Still, if many individuals in a microbial community were non-homeostatic, this would subsequently also allow for non-homeostatic adjustments in soil microbial communities. So far, occasionally observed C:N:P plasticity in soil microbes was only explained by community shifts (Fanin *et al.* 2013; Mooshammer *et al.* 2014). However, in case of the "true non-homeostasis" described here, interpretations of soil microbial C:N:P may be reevaluated: the commonly observed low microbial C:N:P would not be indicative of high N and P demands, but rather of C limitation but sufficient nutrient supply. Differences in stoichiometric flexibility among bacteria and fungi may also result in homeostasis at the soil community level, since bacteria are assumed to be homeostatic (even though aquatic studies also start to challenge this view (Scott*et al.* 2012; Godwin & Cotner 2018)), but are still relevant for models starting to recognize different decomposer groups (Waring*et al.* 2013; Riley *et al.* 2014). Regarding biogeochemical cycles, not only the lack of homeostasis but also the suggested differential use of C versus nutrients in fungi is relevant. High structural C demand for biomass buildup compared to an efficient use of nutrients by internal recycling not only shape fungal nutrient use efficiency, but especially subsequent C sequestration (Liang *et al.* 2019). It is likely that fungal necromass is enriched in C, as indicated by our stoichiometric analyses; which suggests that C sequestration by fungi may occur without large nutrient losses (van Groenigen *et al.* 2017).

Acknowledgments

We thank Laura Córdoba, Chiara Bordier, Raul Machado and Anja Wulf for technical support in the lab, and Anika Lehmann, Marie Spohn and Alexander Tischer for their valuable input for the development of the experimental design. Philip Hoelzmann and Manuela Scholz helped with elemental analyses. MCR acknowledges funding from an ERC Advanced Grant (694368).

References

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

Barton, K. (2019). MuMIn: Multi-Model Inference. R package version 1.43.6, https://CRAN.R-project.org/package=MuMIn.

Bates, D., Maechler, M. & Bolker, B. (2012). lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0, http://CRAN.R-project.org/package=lme4.

Buchkowski, R.W., Shaw, A.N., Sihi, D., Smith, G.R. & Keiser, A.D. (2019). Constraining Carbon and Nutrient Flows in Soil With Ecological Stoichiometry. *Frontiers in Ecology and Evolution* , 7, 382.

Camenzind, T., Hättenschwiler, S., Treseder, K.K., Lehmann, A. & Rillig, M.C. (2018a). Nutrient limitation of soil microbial processes in tropical forests. *Ecol. Monogr.* , 88, 4-21.

Camenzind, T., Lehmann, A., Ahland, J., Rumpel, S. & Rillig, M. (2020). Trait-based approaches reveal fungal adaptations to nutrient-limiting conditions. *Environmental Microbiology* , doi: 10.1111/1462-2920.15132.

Camenzind, T., Lehmberg, J., Weimershaus, P., Álvarez-Garrido, L., Andrade Linares, D.R., Súarez, J.P. *et al.* (2018b). Do fungi need salt licks? No evidence for fungal contribution to the Sodium Ecosystem Respiration Hypothesis based on lab and field experiments in Southern Ecuador. *Fungal Ecol.* , 32, 18-28.

Cleveland, C.C. & Liptzin, D. (2007). C : N : P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass?*Biogeochemistry* , 85, 235-252.

Danger, M. & Chauvet, E. (2013). Elemental composition and degree of homeostasis of fungi: are aquatic hyphomycetes more like metazoans, bacteria or plants? *Fungal Ecol.* , 6, 453-457.

Danger, M., Daufresne, T., Lucas, F., Pissard, S. & Lacroix, G. (2008). Does Liebig's law of the minimum scale up from species to communities?*Oikos* , 117, 1741-1751.

Danger, M., Gessner, M.O. & Barlocher, F. (2016). Ecological stoichiometry of aquatic fungi: current knowledge and perspectives.*Fungal Ecol.* , 19, 100-111.

Egli, T. & Quayle, J.R. (1986). Influence of the Carbon: Nitrogen Ratio of the Growth Medium on the Cellular Composition and the Ability of the Methylotrophic Yeast Hansenula polymorpha to Utilize Mixed Carbon Sources. *Microbiology* , 132, 1779-1788.

Fanin, N., Fromin, N., Buatois, B. & Hattenschwiler, S. (2013). An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant littermicrobe system. *Ecology Letters* , 16, 764-772.

Fricker, M.D., Heaton, L.L.M., Jones, N.S. & Boddy, L. (2017). The Mycelium as a Network. *Microbiology Spectrum* , 5, FUNK-0033-2017.

Fricker, M.D., Lee, J.A., Bebber, D.P., Tlalka, M., Hynes, J., Darrah, P.R. *et al.* (2008). Imaging complex nutrient dynamics in mycelial networks. *Journal of Microscopy* , 231, 317-331.

Frost, P.C., Evans-White, M.A., Finkel, Z.V., Jensen, T.C. & Matzek, V. (2005). Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos* , 109, 18-28.

Godwin, C.M. & Cotner, J.B. (2018). What intrinsic and extrinsic factors explain the stoichiometric diversity of aquatic heterotrophic bacteria? *ISME Journal* , 12, 598-609.

Grimmett, I.J., Shipp, K.N., Macneil, A. & Barlocher, F. (2013). Does the growth rate hypothesis apply to aquatic hyphomycetes? *Fungal Ecol.* , 6, 493-500.

Gulis, V., Kuehn, K.A., Schoettle, L.N., Leach, D., Benstead, J.P. & Rosemond, A.D. (2017). Changes in nutrient stoichiometry, elemental homeostasis and growth rate of aquatic litter-associated fungi in response to inorganic nutrient supply. *ISME Journal* , 11, 2729–2739.

Güsewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytol.* , 164, 243-266.

Hall, E.K., Maixner, F., Franklin, O., Daims, H., Richter, A. & Battin, T. (2011a). Linking Microbial and Ecosystem Ecology Using Ecological Stoichiometry: A Synthesis of Conceptual and Empirical Approaches.*Ecosystems* , 14, 261-273.

Hall, E.K., Singer, G.A., Polzl, M., Hammerle, I., Schwarz, C., Daims, H. *et al.* (2011b). Looking inside the box: using Raman microspectroscopy to deconstruct microbial biomass stoichiometry one cell at a time. *ISME Journal* , 5, 196-208.

Hartman, W.H. & Richardson, C.J. (2013). Differential Nutrient Limitation of Soil Microbial Biomass and Metabolic Quotients (qCO(2)): Is There a Biological Stoichiometry of Soil Microbes? *Plos One* , 8, e57127.

Heck, A.F. (1928). A study of the nature of the nitrogenous compounds in fungous tissue and their decomposition in the soil. *Soil Sci.* , 27, 1-47.

Horn, S., Caruso, T., Verbruggen, E., Rillig, M.C. & Hempel, S. (2014). Arbuscular mycorrhizal fungal communities are phylogenetically clustered at small scales. *ISME Journal* , 8, 2231-2242.

Jennings, D.H. (1995). *The Physiology of Fungal Nutrition* . Cambridge University Press, Cambridge, UK.

Kaspari, M. & Powers, J.S. (2016). Biogeochemistry and Geographical Ecology: Embracing All Twenty-Five Elements Required to Build Organisms.*American Naturalist* , 188, 62-73.

Klein, D.A. & Paschke, M.W. (2004). Filamentous Fungi: the Indeterminate Lifestyle and Microbial Ecology. *Microbial Ecology* , 47, 224-235.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* , 82, 1-26.

Lehmann, A., Zheng, W., Soutschek, K., Roy, J., Yurkov, A.M. & Rillig, M.C. (2019). Tradeoffs in hyphal traits determine mycelium architecture in saprobic fungi. *Scientific Reports* , 9, 14152.

Levi, M.P. & Cowling, E.B. (1969). Role of nitrogen in wood deterioration. 7. Physiological adaptation of wood destroying and other fungi to substrates deficient in nitrogen. *Phytopathology* , 59, 460-469.

Liang, C., Amelung, W., Lehmann, J. & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter.*Glob. Change Biol.* , 25, 3578-3590.

Lilly, W.W., Wallweber, G.J. & Higgins, S.M. (1991). Proteolysis and amino acid recycling during nitrogen deprivation inSchizophyllum commune. *Current Microbiology* , 23, 27-32.

Loladze, I. & Elser, J.J. (2011). The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio. *Ecology Letters* , 14, 244-250.

Makino, W., Cotner, J.B., Sterner, R.W. & Elser, J.J. (2003). Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry. *Funct. Ecol.* , 17, 121-130.

Manzoni, S., Taylor, P., Richter, A., Porporato, A. & Agren, G.I. (2012). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* , 196, 79-91.

Manzoni, S., Trofymow, J.A., Jackson, R.B. & Porporato, A. (2010). Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecological Monographs* , 80, 89-106.

Maynard, D.S., Crowther, T.W. & Bradford, M.A. (2017). Fungal interactions reduce carbon use efficiency. *Ecology Letters* , 20, 1034-1042.

McGill, W.B., Hunt, H.W., Woodmansee, R.G. & Reuss, J.O. (1981). PHOENIX, a model of the dynamics of carbon and nitrogen in grassland soils. *Ecological Bulletins* , 33, 49-115.

McGroddy, M.E., Daufresne, T. & Hedin, L.O. (2004). Scaling of C : N : P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios. *Ecology* , 85, 2390-2401.

Moore, D., Robson, G. & Trinci, A. (2011). *21st Century Guidebook to Fungi* . Cambridge University Press, Cambridge, UK.

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S. & Richter, A. (2014). Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. *Frontiers in Microbiology* , 5, 22.

Mouginot, C., Kawamura, R., Matulich, K.L., Berlemont, R., Allison, S.D., Amend, A.S. *et al.* (2014). Elemental stoichiometry of Fungi and Bacteria strains from grassland leaf litter. *Soil Biol. Biochem.* , 76, 278-285.

Newell, S.Y. & Statzell-Tallman, A. (1982). Factors for conversion of fungal biovolume values to biomass, carbon and nitrogen: variation with mycelial ages, growth conditions, and strains of fungi from asalt marsh. *Oikos* , 39, 261-268.

Nicolardot, B., Recous, S. & Mary, B. (2001). Simulation of C and N mineralisation during crop residue decomposition: A simple dynamic model based on the C : N ratio of the residues. *Plant and Soil* , 228, 83-103.

Paustian, K. & Schnürer, J. (1987a). Fungal growth response to carbon and nitrogen limitation - a theoretical model. *Soil Biol. Biochem.* , 19, 613-620.

Paustian, K. & Schnürer, J. (1987b). Fungal growth response to carbon and nitrogen limitation - application of a model to laboratory and field data. *Soil Biol. Biochem.* , 19, 621-629.

Persson, J., Fink, P., Goto, A., Hood, J.M., Jonas, J. & Kato, S. (2010). To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* , 119, 741-751.

Peter, M. (2005). Chitin and Chitosan in Fungi. In: *Biopolymers Online* (ed. Steinbüchel, A). Wiley-VCH Verlag GmbH & Co. KGaA Weinheim, Germany.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team (2020). nlme: Linear and nonlinear mixed effects models. R package version 3.1-147, http://CRAN.R-project.org/package=nlme.

Pusztahelyi, T., Molnar, Z., Emri, T., Klement, E., Miskei, M., Kerekgyarto, J. *et al.* (2006). Comparative studies of differential expression of chitinolytic enzymes encoded by chiA, chiB, chiC and nagA genes in Aspergillus nidulans. *Folia Microbiologica* , 51, 547-554.

R Core Team (2019). R: A language and environment for statistical computing. Vienna, Austria, http://www.R-project.org/.

Redfield, A.C. (1958). The biological control of chemical factors in the environment. *American Scientist* , 46, 205-221.

Reich, P.B. & Oleksyn, J. (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* , 101, 11001-11006.

Reyes, F., Villanueva, P. & Alfonso, C. (1990). Nucleases in the autolysis of filamentous fungi. *FEMS Microbiology Letters* , 69, 67-72.

Riley, W.J., Maggi, F., Kleber, M., Torn, M.S., Tang, J.Y., Dwivedi, D. *et al.* (2014). Long residence times of rapidly decomposable soil organic matter: application of a multi-phase, multi-component, and vertically resolved model (BAMS1) to soil carbon dynamics. *Geosci. Model Dev.* , 7, 1335-1355.

Sardans, J., Rivas-Ubach, A. & Penuelas, J. (2012). The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review and perspectives. *Biogeochemistry* , 111, 1-39.

Scott, J.T., Cotner, J.B. & LaPara, T.M. (2012). Variable stoichiometry and homeostatic regulation of bacterial biomass elemental composition. *Frontiers in Microbiology* , 3, 42.

Spohn, M. (2016). Element cycling as driven by stoichiometric homeostasis of soil microorganisms. *Basic and Applied Ecology* , 17, 471-478.

Sterner, R.W. & Elser, J.J. (2002). *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere* . Princeton University Press, Oxford, UK.

Strickland, M.S. & Rousk, J. (2010). Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications.*Soil Biol. Biochem.* , 42, 1385-1395.

Tanaka, T., Kawasaki, K., Daimon, S., Kitagawa, W., Yamamoto, K., Tamaki, H. *et al.* (2014). A hidden pitfall in the preparation of agar media undermines microorganism cultivability. *Applied and environmental microbiology* , 80, 7659-7666.

van Groenigen, J.W., van Kessel, C., Hungate, B.A., Oenema, O., Powlson, D.S. & van Groenigen, K.J. (2017). Sequestering Soil Organic Carbon: A Nitrogen Dilemma. *Environmental Science & Technology* , 51, 4738-4739.

Waring, B.G., Averill, C. & Hawkes, C.V. (2013). Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. *Ecology Letters* , 16, 887-894.

Watkinson, S., Bebber, D., Darrah, P., Fricker, M., Tlalka, M. & Boddy, L. (2006). The role of wood decay fungi in the carbon and nitrogen dynamics of the forest floor. In: *Fungi in Biogeochemical Cycles*(ed. Gadd, GM). Cambridge University Press, Cambridge, UK, pp. 151-181.

Wilson, W.A., Roach, P.J., Montero, M., Baroja-Fernandez, E., Munoz, F.J., Eydallin, G. *et al.* (2010). Regulation of glycogen metabolism in yeast and bacteria. *FEMS Microbiology Reviews* , 34, 952-985.

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Penuelas, J., Richter, A., Sardans, J. *et al.* (2015). The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. *Ecol. Monogr.* , 85, 133-155.

Zhang, J. & Elser, J.J. (2017). Carbon:Nitrogen:Phosphorus Stoichiometry in Fungi: A Meta-Analysis. *Frontiers in Microbiology* , 8, 1281.

Zheng, W. (2015). Soil stability and filamentous fungi. *Institute of Biology, Freie Universitat Berlin* , Berlin, Germany.

Zheng, W., Lehmann, A., Ryo, M., Valyi, K. & Rillig, M.C. (2018). Growth rate trades off with enzymatic investment in soil filamentous fungi. In: *bioRxiv* , p. 360511.

Figure Legends

Fig.1: Stoichiometric responses in fungal C:N:P ratios to varying N and P supply in defined glucose media. **a, b** and **c** depict shifts in fungal C:N, C:P and N:P ratios in response to 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 fungal isolate, with maximum and minimum values shown in the respective color legend.

Fig. 2: Fungal 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 violin plots, a mirrored density plot showing overall data distribution, with average values illustrated by dots (8 (**a**) or 4 (**b,c**) isolates, 3 repetitions each). Letters indicate deviations among treatments (linear mixed-effects model), red dots deviations from control values ($P < 0.05$). Large variations are mainly driven by distinct differences among isolates (see Fig. S6).

Fig. 3: Correlations of total fungal (**a**) carbon (C), (**b**) nitrogen (N) and (**c**) phosphorus (P) mass [mg] with fungal biomass [mg] in different nutrient manipulation experiments (both standardized [0,1] for comparisons among elements and fungal isolates). Lines show predicted model outputs from linear mixed effects models, with grey areas illustrating 95% confidence intervals. Line types indicate respective nutrient manipulation experiments (solid: N manipulation in glucose medium; dashed: P manipulation in glucose medium; dotdashed: N manipulation in cellulose medium; longdashed: fertilization experiment in soil extract agar). For correlations conducted in each medium respective formulas of regression lines as well as R2 values are given. Pie charts illustrate the relative variation in element masses determined by fungal biomass (dark

grey) versus the respective nutrient manipulation treatments applied (light blue). 3D correlation graphs (**d-e**) further illustrate the relative contribution of fungal biomass versus nutrient manipulation to total element mass, exemplarily shown for N manipulations in defined glucose media.

Fig. 4: Positive correlations of relative fungal nitrogen (N) and phosphorus (P) concentrations [% deviation from maximum values] in defined glucose media (**a, d**), soil-extract agar (**b,e**) and defined cellulose medium (**c**). The relative deviation of fungal concentrations of the manipulated element is depicted on the x-axis, while the relative concentrations of the respective other element (unchanged in media) is plotted on the y-axis. The chosen relative values allow to assess effect sizes in element reductions, and reduce isolate-specific variability by data standardization. Colors and symbols depict respective treatments applied (see legends), black lines predicted model outputs from linear mixed effects models, with grey areas illustrating 95% confidence intervals (*** $P < 0.001$).

Fig. 5: Spatial and temporal shifts in fungal C:N ratios are depicted, with colors illustrating fungal nitrogen concentrations (red: high, grey: low). Stoichiometry was compared in (**a**) inner versus outer parts of the mycelium (4 fungal isolates, 3 repetitions each), and in fungi grown for 12 or 26 days (**b**) on soil-extract agar (12 fungal isolates, 3 repetitions each), as well as in (**c**) inner versus outer parts of mycelium grown in glucose media with low (C:N = 200) and high (C:N = 20) N supply (4 fungal isolates, 3 repetitions each). (**a, b**) Dots represent data points underlain by box-and-whisker plots. P -values are based on paired t-tests. Illustrations depict potential element distributions in fungal mycelia (see Fig. 6). (**c**) Bars illustrate mean values of fungal C:N ratios measured in inner (hatched bars) and outer (filled bars) parts of the fungal mycelium. Tested fungal isolates differ in $1/H_{CN}$ values, previously determined along a larger gradient of C:N (see Fig. 1). Given P -values are based on three-way type III analyses of variances.

Fig. 6: Illustration of potential mechanisms in fungal mycelia allowing flexible carbon:nutrient (C:X) adjustments to nutrient limited conditions. Colors indicate element contents throughout different mycelial parts (red: low C:X, i.e. high nutrient contents; grey: wide C:X, i.e. low nutrient contents). Little grey dots illustrate the distribution of C throughout mycelia, red dots nutrients, e.g. nitrogen and phosphorus. Mycelial growth form depicts typical adaptations to reduced nutrient supply (decreased biomass, density and activity (Fig. S2; Camenzind *et al.* 2020)).