Short Communication

Elemental composition and degree of homeostasis of fungi: are aquatic hyphomycetes more like metazoans, bacteria or plants?

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A B S T R A C T

Ecological stoichiometry generally assumes that heterotrophs have a higher degree of elemental homeostasis than autotrophs. Differences between fixed consumer nutrient requirements and nutrients available in resources allow prediction of the intensity of nutrient recycling ensured by heterotrophs. Despite their fundamental role in detritus decomposition, extremely few data are currently available on fungal elemental composition. In this study, we quantified the degree of elemental homeostasis of aquatic hyphomycetes used as model organisms. Contrary to metazoans, but similar to plants, aquatic hyphomycetes exhibited highly plastic elemental compositions. Mycelium also reached far higher C/nutrient ratios than reported for bacteria. Our results suggest that non-homeostasis of fungi should be explicitly included in stoichiometric models dealing with nutrient recycling, and that the discrepancy in homeostasis between some bacterial strains and fungi should certainly be considered when investigating interactions between both groups of decomposers.

Keywords:
Decomposition models
Ecological stoichiometry
Elemental composition
Homeostasis
Microbial decomposers
Nutrient recycling

Introduction

Ecological stoichiometry is a recent and powerful theory aimed at studying the balance of multiple chemical elements in ecological interactions (Sterner and Elser, 2002). Some aspects of this theory rely on the assumption that heterotrophic organisms exhibit a higher degree of elemental homeostasis than autotrophic organisms, i.e. a higher resistance to change in their elemental composition when faced with resources of distinct elemental composition. For example, several experiments have shown that an imbalance between a quite constant consumer demand and variable element supply in resources can predictably affect metazoan's growth, reproduction and survival (Elser et al., 1996; Danger et al., 2013a). This imbalance also controls several ecosystem processes such as nutrient recycling, brought about by microorganisms (Enríquez et al., 1993) and metazoans (Vanni et al., 2002). Currently, most models dealing with microbial nutrient recycling from local to global scales assume fixed microbial elemental compositions (e.g., Tyrrell, 1999; Daufresne and Loreau, 2001).

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Table 1 – Composition of mineral culture media used for experiments 1 and 2

Compound	Experiments 1 and 2	Final medium concentration (mg l^{-1})								
		Experiment 1				Experiment 2				
		NP-rich	N-depleted	P-depleted	NP-depleted	N/P_1	N/P_2	N/P_3	N/P_4	N/P_5
$MgSO_4$, $7H_2O$	0.5									
CaCl2, $2H_2O$	0.15									
$FeCl_3$, $6H_2O$	2									
$MnSO_4$, H_2O	1									
H_3BO_3	1									
$AlSO_4$, $18H_2O$	0.1									
KI	0.1									
Na_2MoO_4, $2H_2O$	0.1									
$CoCl_2$, H_2O	0.025									
$NiCl_2$, $6H_2O$	0.025									
KNO_3		1 000	200	1 000	200	100	100	100	100	100
Na_2HPO_4		50	50	10	10	0.6	1.9	5	12.5	25
KH_2PO_4		50	50	10	10	0.6	1.9	5	12.5	25
Molar N/P ratio		13.8	2.8	68.9	13.8	110	37	13.8	5.5	2.7

While many data on bacteria elemental compositions are currently available in the literature, data concerning fungi remain scarce (Persson et al., 2010). Like metazoans, several bacterial strains are able to maintain their elemental composition at a quite constant level (Chrzanowski and Kyle, 1996; Makino et al., 2003; Danger et al., 2008). Nevertheless, due to competitive exclusion mechanisms and selection of bacterial strains, bacterial communities are likely to adjust their stoichiometry to that of their resources (Danger et al., 2008). Recent studies also reported a weak homeostasis for some bacterial taxa depending on resource availability (Scott et al., 2012). In contrast, data on fungal elemental composition and on their degree of homeostasis are extremely rare and incomplete. Yet, such data might be essential for understanding fungal community structures and the intensity of nutrient recycling. Levi and Cowling (1969) first showed that some species of fungi were able to reduce their nitrogen (N) content when facing low N resources. Later, Beever and Burns (1980) reviewed the phosphorus (P) uptake and storage capacities of several fungal species showing that some species were able to store P in excess, but only a few studies were carried out along nutrient gradients, limiting our abilities to generalize the lack of homeostasis in fungi. This study was thus aimed at: (1) testing the degree of elemental homeostasis of aquatic hyphomycetes, which are generally the most important fungal decomposers of plant detritus in aquatic ecosystems (Suberkropp and Klug, 1976; Bärlocher, 1992); and (2) giving ranges of variation in fungal mycelium elemental composition permitting, among others, proper parameterization of models.

Material and methods

Two distinct experiments were carried out. First, we investigated the variation in elemental composition of one aquatic hyphomycete species, *Tetrachaetum elegans*, during its growth in four conditions of nutrient availability (Experiment 1). Four liquid culture media containing glucose (5 g l^{-1}) as the sole carbon source and mineral solutions based on the modified GMS medium (Gessner and Chauvet, 1993) were used (Table 1). The four distinct nutrient levels were achieved by manipulating N and P inputs, leading to a NP-rich treatment, a N-depleted treatment, a P-depleted treatment, and a NP-depleted treatment. The experiment was carried out in 250 ml Erlenmeyer flasks containing 75 ml of sterilized culture medium for a total of 72 units, i.e. 4 nutrient levels × 6 dates × 3 replicates. Microcosms were agitated on an orbital shaker and maintained at 15 °C in the dark. Three Erlenmeyer flasks were randomly sacrificed at each sampling date (2, 4, 6, 8, 11 and 15 d). Mycelium was rinsed twice with sterile deionized water before being recovered on a pre-weighted GF/F filter (Whatman International Ltd., Maidstone, UK). Filters were then frozen at −20 °C before being freeze-dried. Mycelial biomass was estimated to the nearest 0.1 mg and elemental composition of mycelium was measured on manually ground subsamples. Mycelium C and N contents were determined using a CHN elementary analyzer (NA 1500 Series 2, Fisons, Manchester, UK) with acetanilide as a standard, and P contents were measured after persulfate digestion in alkaline conditions followed by colorimetric assessment (Danger et al., 2008).

In a second experiment (Experiment 2), the degree of homeostasis of three common aquatic hyphomycete species, *Lemonniera terrestris*, *Articulospora tetracladia* and *Tricladium chaetocladium* were assessed using mycelium cultures along a P-gradient in 45 Erlenmeyer flasks, i.e. 3 species × 5 P-levels × 3 replicates. Culture media were similar to those used in the first experiment (Table 1), except that P inputs were selected to reach a gradient of molar N/P ratios. This experiment lasted 16 d, i.e. just above the time necessary to reach steady state for *T. elegans* in the first experiment. Microcosms were agitated on an orbital shaker at 15 °C in the dark. Mycelium analyses were similar to those described for the first experiment.

For both experiments, flasks were initially inoculated with 1 ml of a suspension of mycelium grown in the NP-depleted medium. Briefly, single spore isolates of aquatic hyphomycetes were obtained from foam samples taken in headwater streams of southwestern France. Colonies were grown on 2 %

malt agar. After 15 d, agar plugs containing mycelium were cut, transferred in sterilized deionized water, and homogenized using a sterile Ultra-Turrax blender (Gessner and Chauvet, 1993). One ml of each mycelium homogenate was inoculated into 500 ml-Erlenmeyer flasks containing 150 ml of sterile NP-depleted medium, and grown for 6 d on an orbital shaker at 15 °C in the dark. Final mycelium inocula were obtained from water-rinsed mycelial pellets homogenized in sterile deionized water with the Ultra-Turrax blender.

In the first experiment, treatment effects on mycelium biomass, C/N, C/P, and N/P ratios were assessed using two-way ANOVA with nutrient level and time set as fixed factors. The condition of independence was achieved by destructively sampling microcosms on each date. For the second experiment, the degree of homeostasis of each species was estimated by quantifying the slopes of the regression between $\log(x_{medium})$ and $\log(x_{mycelium})$, x being either C/P or N/P ratios (Persson et al., 2010). Slopes tending to 1 indicated that organisms were plastic. Inverse values of the slope (coefficient of homeostasis H) were also calculated (Sterner and Elser, 2002). All statistical analyses were performed using Statistica (SAS Institute). The significance threshold was set at $P < 0.05$.

Results and discussion

Mycelium growth followed similar patterns in all culture media, i.e. initial exponential growth followed by a stationary phase (Fig 1A). Mycelial growth did not significantly differ among the four culture media (nutrient level: $F_{3,\ 56} = 2.14$, $P = 0.10$; time × nutrient level: $F_{18,\ 56} = 1.25$, $P = 0.25$). At the beginning of their growth, in nutrient unlimited conditions, mycelium exhibited similar C/P, C/N, and N/P molar ratios (Fig 1A–C, respectively). These molar ratios were low ($9.1 < C/N < 10.2$; $66.3 < C/P < 96.4$; $7.3 < N/P < 9.4$) and typical of organisms with elevated growth rates (Sterner and Elser, 2002; Makino et al., 2003). Indeed, high growth rates reduces C/P and N/P ratios of organisms through increases in their relative requirements of P compared to other elements due to synthesis of large quantities of P-rich RNA (heterotrophs: Elser et al., 2003; autotrophs: Ågren, 2004; microorganisms: Makino et al., 2003). In addition, mycelium growing in nutrient unlimited conditions might store P in excess in their own biomass, mainly in membranes or in vacuoles (Beever and Burns, 1980). After *ca.* 8 d of mycelial growth, nutrient significantly impacted elemental ratios of fungi (time × nutrient level: $F_{15,\ 48} = 21.1$, $F_{15,\ 48} = 68.9$, $F_{15,\ 48} = 53.6$, for C/P, C/N, and N/P, respectively; $P < 0.001$), fungi tending to match elemental composition of available resources. At stationary phase, mycelium elemental composition remained constant and fungal C/nutrient ratios reached far higher values (up to 1 499 for C/P ratio of *L. terrestris*) than other heterotrophic organisms, in particular bacteria. For example, bacterial C/P ratio never exceeded *ca.* 500 for both individual strains and non-homeostatic communities (Chrzanowski and Kyle, 1996; Makino et al., 2003; Danger et al., 2008; Scott et al., 2012). These abilities of fungi to live with very limited nutrient resources certainly explain, in addition to their specific enzymatic activities and production of inhibitory substances, their competitive advantage in comparison with bacteria on many nutrient-depleted detrital resources (Gulis and Suberkropp, 2003; Romani et al., 2006; Danger et al., 2013b). Such differences would be representative for organisms with slow (mainly fungi) *versus* fast growth (some bacterial strains), as proposed on stoichiometric basis by Arrigo (2005).

Homeostasis coefficients (1/H and H) of the three different aquatic hyphomycete strains tested (Table 2) tended to 1,

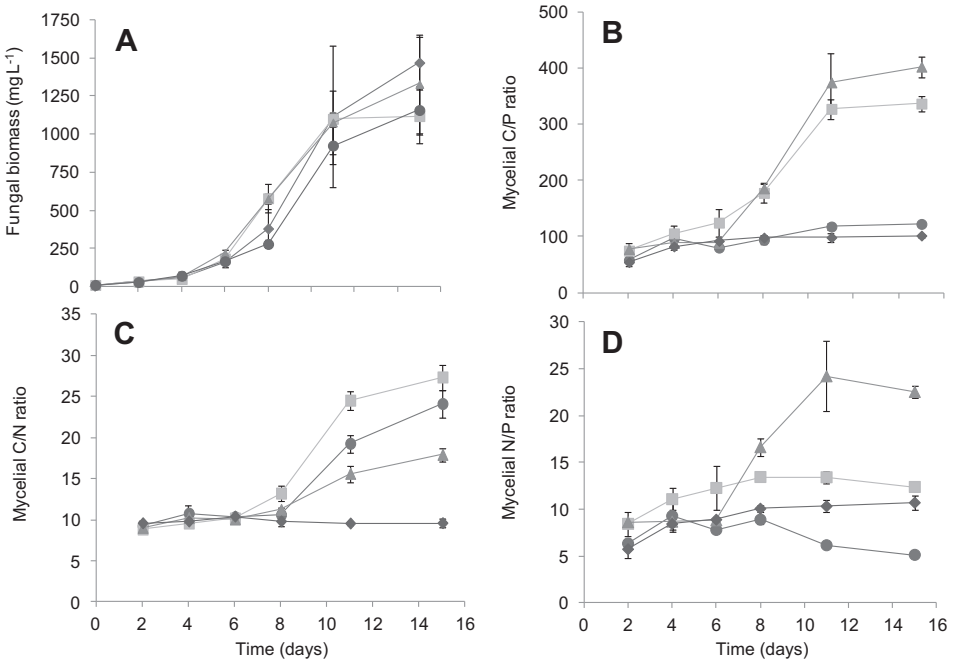

Fig 1 – Growth of the aquatic hyphomycete *Tetrachaetum elegans* (A) and elemental composition of mycelium (C/P (B), C/N (C), and N/P (D) molar ratios) during its growth in a batch experiment, in four nutrient conditions: NP-rich (◆), N-depleted (●), P-depleted (▲), and NP-depleted (■). Vertical bars represent standard errors ($n = 3$).

Table 2 – Degree of homeostasis of aquatic hyphomycete species. The coefficient of homeostasis 1/H corresponds to the slope of the relationship between log(x_{medium}) and log($x_{mycelium}$), x being the elemental ratios, i.e. C/P or N/P ratios, as proposed by Persson et al. (2010). All the regressions were significant ($P < 0.001$). Slopes and H values tending to 1 indicate that organisms are plastic (Persson et al., 2010). Minimal and maximal values of C/P and N/P molar ratios of mycelium are also displayed

		1/H	H	r^2	Range
Lemonniera terrestris	C/P	0.64	1.57	0.97	90–1 499
	N/P	0.55	1.80	0.94	4.5–52.5
Articulospora tetracladia	C/P	0.75	1.34	0.98	76–1 166
	N/P	0.71	1.41	0.93	3.6–53.4
Tricladium chaetocladium	C/P	0.61	1.63	0.92	89–1 175
	N/P	0.68	1.47	0.93	3.7–54.8

meaning that these species were highly plastic. For comparison, 1/H for C/P ratios varied between 0.16 and 0.34 for bacterial isolated strains and between 0.07 and 0.20 for homeostatic zooplankton (Danger et al., 2008; Persson et al., 2010). Similarly, 1/H for N/P ratios varied between 0.17 and 0.29 for isolated bacterial strains.

To conclude, contrary to what is commonly considered in most models dealing with nutrient recycling by decomposers and what has been demonstrated for metazoans (Sterner and Elser, 2002) and several bacterial strains (Danger et al., 2008; Makino et al., 2003; but see; Scott et al., 2012), aquatic hyphomycetes are clearly not homeostatic. It is now necessary to verify the elemental plasticity of other fungi and their physiological determinants in both terrestrial and aquatic contexts and when facing more refractory C substrates. Models predicting decomposer interactions and nutrient recycling should explicitly include fungal elemental variability and consider that overlap can exist between bacterial and fungal elemental compositions when resources are not nutrient limited, rather than simply stating that fungi exhibit higher C/nutrient ratios than bacteria. It would certainly help explain some inconsistencies observed in the outcome of competitive interactions between fungi and bacteria (e.g., Bengtsson, 1992; Mille-Lindblom et al., 2006), but also refine predictions of nutrient recycling models by providing biologically relevant lower and upper limits of fungal elemental composition.

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