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Nutrient stoichiometry of aquatic hyphomycetes: Interstrain variation and ergosterol conversion factors

Magali Brosed [a, b, c, *], Jérémy Jabiol [c, 1], Mark O. Gessner [a, c, d, e]

[a] Department of Aquatic Ecology, Eawag, Dübendorf, Switzerland
[b] Université des Sciences et Techniques de Franche-Comté, Besançon, France
[c] Department of Experimental Limnology, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Stechlin, Germany
[d] Department of Environmental Science, ETH, Zurich, Switzerland
[e] Department of Ecology, Berlin Institute of Technology (TU Berlin), Berlin, Germany

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ABSTRACT

Ecological stoichiometry is a powerful concept. Rarely, however, has it been applied to fungi, despite their pivotal role in ecosystems. In view of the paucity of stoichiometric data, we grew 16 fungal isolates from streams in liquid culture (C:N:P = 160:16:1) and analysed them for nitrogen (N), phosphorus (P) and ergosterol as a fungal biomass marker. Interspecific differences explained up to 60% of the variation in N, P and ergosterol concentrations, and variation between strains of the same species accounted for up to another 16%. We found an average C:N:P of 136:10:1 in mycelia, while N:ergosterol and P:ergosterol ratios were 9.5 and 2.5, respectively. These ratios are an important step towards establishing reliable conversion factors to estimate the contribution of fungi to litter nutrient contents in complex field samples. Estimates could be further improved by applying the species-specific conversion factors we obtained. Additional analyses of fungal strains in conditions reflecting field situations are needed to strengthen the basis of such estimates of fungal nutrient pools in ecosystems; however, inherent variation within species limits the accuracy and precision that can be achieved.

1. Introduction

Ecological stoichiometry is a powerful concept that hinges on the repartition of chemical elements within and between organisms as well as between organisms and the physical environment (Sterner and Elser, 2002). The concept has proved extremely useful to explain a wide range of processes and patterns across levels of biological organization, from genes to ecosystems (Elser et al., 2000). In particular, the elemental composition of organisms provides information about resource requirements and thus helps predicting fundamental ecological variables such as rates of resource consumption and growth, the dynamics of populations and communities, or the outcome of competitive species interactions (e.g. Daufresne and Hedin, 2005; Hillebrand et al., 2014). For many primary producers and groups of metazoans, the elemental composition and its degree of variation are relatively well known (e.g. Persson et al., 2010; Borer et al., 2013). However, although information on microorganisms is also improving (e.g. Makino et al., 2003; Persson et al., 2010; Godwin and Cotner, 2015), the range of variation and level in the elemental composition of fungi is poorly known (Danger et al., 2016). This is a major knowledge gap given the omnipresence and essential roles of fungi in the cycling of carbon (C) and nutrients, such as nitrogen (N) and phosphorus (P), in ecosystems.

A polyphyletic group of fungi known as aquatic hyphomycetes (Bärlocher, 2016) assumes particular importance in forested headwater streams, where they play a central role in the decomposition of leaf litter (Gessner et al., 2007), which serves as an important source of C, energy and nutrients to the food web of these ecosystems (Wallace et al., 1997, 2015). Leaf litter of most trees and shrubs is strongly depleted in nutrients compared to metazoan consumers such as macroinvertebrate detritivores (Cross

* Corresponding author. Present address: Asconit-Consultants, Ramonville-St-Agne, France.
E-mail address: magali.brosed@gmail.com (M. Brosed).
[1] Present address: Laboratoire écologie fonctionnelle et environnement (EcoLab), Université de Toulouse, CNRS, INPT, UPS, Toulouse, France.

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et al., 2005; Hladyz et al., 2009; Danger et al., 2016). This results in marked nutrient imbalances between food and consumer requirements. Accordingly, stoichiometric analyses focusing on resource-detritivore relationships have found strong effects of the elemental composition of food resources on processes such as consumer growth and litter decomposition (Cross et al., 2003; Hladyz et al., 2009; Frainer et al., 2016). Because aquatic hyphomycetes assimilate dissolved nutrients from stream water (Suberkropp, 1998), their activity in submerged litter can immobilize large amounts of nutrients such as N and P (Kaushik and Hynes, 1971; Gessner, 1991; Webster et al., 2009), thereby diminishing the nutrient imbalance between the leaf resource and detritivores (Cornut et al., 2015; Frainer et al., 2016). This change in nutrient availability for consumers has important consequences for detritivore feeding (Danger et al., 2013; Cornut et al., 2015), in addition to the direct effect of nutrient immobilization at the ecosystem level (Lin and Webster, 2014).

Although several studies suggest that aquatic hyphomycete species are functionally redundant (Dang et al., 2005; Gessner et al., 2010), there is growing evidence that differences among species (e.g. in the efficiency of nutrient uptake and storage) could influence both the degradative activity of fungal communities (Duarte et al., 2006) and detritivore feeding activities (Lecerf et al., 2005; Jabiol et al., 2013b) and preferences (Arsuffi and Suberkropp, 1984, 1985). In addition, aquatic hyphomycetes show considerable intraspecific genetic diversity (Peláez et al., 1996; Letourneau et al., 2010; Seena et al., 2012), with potential consequences for the composition and performance of these fungal communities (Charcosset and Gardes, 1999; Fernandes et al., 2011). It remains unknown, however, to what extent such intraspecific genetic variability translates into variation in elemental composition and, thus, what repercussions are to be expected on the functional diversity of aquatic hyphomycete communities and ecosystem processes.

Filamentous fungi exploit their resources as a result of pervasive expansion of hyphae within their substratum (Newell et al., 1996). As a consequence, the mycelium of aquatic hyphomycetes is tightly embedded within the colonized plant tissue. This intimate association makes it virtually impossible to distinguish between nutrients in leaves and leaf-associated fungi, thus hampering assessments of the contribution of aquatic hyphomycetes to litter nutrient contents and stoichiometric ratios, which is crucial information to quantify microbially mediated nutrient fluxes. A possible remedy is to determine fungal biomass by means of a biomarker and relate the estimated biomass to fungal N and P contents. One such biomarker is ergosterol, a membrane sterol specific to fungi that has proved particularly useful as a biomass marker and is now widely used for this purpose (Gessner and Newell, 2002). If nutrient concentrations in fungal hyphae vary within similarly modest ranges, the same approach could serve to assess N and P contents of filamentous fungi growing in plant litter and other solid substrates.

The present study aimed to fill a large information gap in ecological stoichiometry by assessing the magnitude, sources and extent of variation in N and P concentrations among aquatic fungi and to provide conversion factors relating fungal nutrient (N and P) and ergosterol contents. To this end, we analysed 16 fungal strains belonging to 8 species of aquatic hyphomycetes that commonly occur in submerged leaf litter in streams. We hypothesized that variation in nutrient and ergosterol concentrations, as well as ratios of these variables, are mainly accounted for by species identity and to a lesser extent by the specific strain of a species and by growth stage. If so, the accuracy of estimates of fungal nutrient contents would notably improve when species-specific factors are applied to convert ergosterol to fungal N and P.

2. Materials and methods

Single-spore isolates of 16 aquatic hyphomycete strains were obtained from foam collected in 5 circumneutral softwater streams draining forests in southwestern France (Table 1). Sixteen strains, two each of 8 different species were selected: *Alatospora acuminata* Ingold (A.a), *Articulospora tetracladia* Ingold (A.t), *Clavariopsis aquatica* De Wild (C.a), *Lemonniera terrestris* Tubaki (L.t), *Neonectria lugdunensis* (Saccardo & Therry) L. Lombard & Crous (N.l), *Tetracladium marchalianum* De Wild (T.m), *Tricladium chaetocladium* Ingold (T.c) and *Tricladium splendens* Ingold (T.s). All cultures were maintained on standard 1% malt agar at 15 °C in the dark.

Five agar plugs (6 mm diameter) were cut from the leading edge of 10-d old growing colonies (4–9 cm diameter), submerged in 10 mL of sterile water and homogenized for 30 s with an Ultra-Turrax tissue homogenizer (Typ TP 18-10). A 0.4 mL aliquot of the resulting homogenate was used to inoculate 40 mL of mineral solution in 200-mL Erlenmeyer flasks with baffles (Fig. 1). Baffles in the flasks served to create turbulence and ensure adequate oxygen supply. The mineral solution consisted of 2.5409 g KNO_3, 0.1513 g KH_2PO_4, 0.0814 g $Na_2PO_4 \cdot 2H_2O$, 0.5000 g $MgSO_4 \cdot 7H_2O$, 0.1500 g $CaCl_2 \cdot 2H_2O$, 2.0 mg $FeCl_3 \cdot 6H_2O$, 1.0 mg $MnSO_4 \cdot H_2O$, 25 μg $CoCl_2 \cdot 6H_2O$, 25 μg $NiCl_2 \cdot 6H_2O$ and 5 g glucose in 1 L of nanopure water (Gessner and Chauvet, 1993). Glucose was the sole C source, and the C:N:P ratio of the medium was chosen to reflect the Redfield atomic ratio of 106:16:1.

The culture flasks were placed on a rotary shaker (100 rpm) in a climate chamber at 15 °C in the dark. Mycelial pellets were collected when the maximum biomass was reached. The flask content was passed over a 10-μm nylon mesh-screen, suspended in 10 mL of nanopure water and homogenized for 1 min with an Ultra-Turrax tissue homogenizer. The homogenized mycelium was partitioned into 8 portions by inoculating 40 mL of mineral solution with 0.4 mL aliquots of the mycelial suspension. Cultures were grown in the same conditions as above until four replicate flasks were randomly sampled after 5–6 d (early stage) and again after 10–12 d (late stage). The mycelium was collected on a 10-μm nylon-mesh screen, rinsed with normal saline solution (0.9% NaCl in nanopure water) and stored at −20 °C for later analyses of total N, total P and ergosterol.

Total C concentrations of mycelium were assumed to be 45% of dry mass (Grimmett et al., 2013). Total N and P concentrations of the mycelium were determined on subsamples of freeze-dried mycelium (ca. 1 mg) from each replicate ($n = 4$ per strain and sampling date). Samples were digested with peroxodisulfate (20 g $K_2S_2O_8$ and 3 g NaOH in 1 L of nanopure water) (Ebina et al., 1983) and autoclaved (121 °C) for 40 min before spectrophotometrically quantifying nitrate and phosphate according to standard procedures. The same method was used to determine total N and P concentrations in 50 mL of filtered (GF/F; Whatman) culture medium. Carbon concentrations of the medium preserved with phosphoric acid were determined on a Shimadzu TOC-5000 analyser.

Ergosterol was extracted from mycelial pellets and quantified to establish conversion factors relating fungal biomass to C, N and P contents in complex samples such as leaf litter. Frozen mycelium was freeze-dried for 24 h, weighed to the nearest 0.01 mg, and subsamples (1–3 mg) stored in 10 mL of alkaline methanol (8 g KOH L^{-1}) at −20 °C. The ergosterol analysis consisted of a lipid extraction and saponification of lipid esters by heating (80 °C) for 30 min, followed by solid-phase extraction and reverse-phase high performance liquid chromatography (HPLC) (Gessner, 2005).

Hierarchical ANOVAs were used to test for differences in N, P and ergosterol concentrations and ratios between species, strains and growth stages. The factor strain was nested in species identity, which was included in the model as a random factor. The