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***Microbial Stoichiometry and Homeostasis of Nutrient  
Ratios in Fungi***

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**Abstract**

Stoichiometry is the ratio of elements in a substance or microbial biomass. This paper investigates microbial stoichiometry and the responses of microbes to dissolved nutrient concentrations and ratios. The current work assesses both autotrophic and heterotrophic responses to nutrient enrichment: autotrophic and heterotrophic states are defined mainly by ecosystem primary production and respiration. In ecosystems dominated by autotrophic microbes, nutrient enrichment can lead to increased biomass and biomass-specific rates of primary production. Heterotrophic ecosystems rely on subsidies of organic carbon from outside the system. Their enrichment with N and/or P can accelerate microbial respiration rates and result in carbon losses from detritus-based systems.

The Redfield ratio is relatively unknown for major groups of heterotrophic organisms, especially fungi. The degree to which heterotrophic bacteria and fungi are homeostatic given variable N and P concentrations and ratios in the medium is also unknown. A bacterial community can be more homeostatic in terms of biomass C:P and N:P than a single strain culture, though there is some evidence of non-homeostasis in some bacterial communities.

In this experiment, we tested if aquatic fungi are homeostatic, i.e. if their biomass C:N, C:P and N:P ratios change with varying dissolved nutrient concentrations and ratios. We also determined fungal “Redfield ratio”, which appeared to be  $C_{90}N_9P_1$ , on average. Overall, aquatic fungi were capable of maintaining nearly constant stoichiometric ratio of C:N regardless of changes in their resource stoichiometry or concentrations, while fungal C:P ratio varied to some degree depending on medium C:P ratio and P concentration. The

1/H values obtained in this study indicate that fungal biomass is weakly homeostatic with respect to C:P and homeostatic with respect to C:N and N:P.

Such studies can provide information on the effects of nutrient enrichment on biogeochemical cycling and energy flow in microbially mediated food webs.

## **Introduction**

A limitation of growth by nutrient deficiency is known as nutrient limitation. All microbes are nutrient limited, meaning that microorganisms will never be able to obtain the full complement of nutrients needed for their optimal growth. This applies for both autotrophic and heterotrophic microorganisms. Common factors that would affect the availability of nutrients for autotrophic and heterotrophic microorganisms are geology, human activities such as fertilizing, natural and environmental disasters, and changes in microbial community structure. All these factors could potentially lead to changes in microbial abundance, community structure and activity which would affect microbes and larger organisms at all trophic levels.

Recent studies attempted to assess and measure how these particular factors affect nutrient ratios of microbial biomass and nutrient homeostasis. The studies of microbial elemental ratios and homeostasis are known as microbial stoichiometry. Stoichiometry denotes a particular branch of science that deals with the relations between the masses or molar ratios of the components. The word “stoichiometry” derives from the Greek root “stoicheion” for element. Thus “stoichiometry” means measuring elements such as C, H, N, P, and O, etc. and their ratios (Sterner and Elser, 2002). Microbial denotes a particular field of biology that deals with organisms at a microbial level. Thus, microbial stoichiometry is the study of the balance of multiple chemical substances in microbial

biomass. By investigating the stoichiometry of microbes we can determine the quantitative relationship of these nutrients and the factors that can affect these nutrient ratios.

The Redfield ratio is an important reference point in microbial stoichiometry and describes the consistency of nutrient ratios of C, N, and P from different chemical fractions. The Redfield ratio was developed and primarily applied to autotrophic microbes, such as algae. The ratio indicates that there are 106 atoms of C for 16 atoms of N for every one atom of P ( $C_{106}N_{16}P_1$ ). Consistent nutrient ratios (homeostasis), combined with data linking specific patterns of microbial stoichiometry with direct evidence of microbial nutrient limitation, suggest that measuring the ratios of C, N, and P in the microbial biomass may represent a useful tool for assessing nutrient limitations of microbes.

### **Autotrophic response to nutrient enrichment**

Autotrophic and heterotrophic states are determined by the relative importance of ecosystem primary production and respiration. Both biomass and biomass specific activity can be influenced by biotic and abiotic controlling factors. Autotrophic and heterotrophic state can be controlled by different factors; however, inorganic nutrients often stimulate heterotrophic and/or autotrophic activity, with nitrogen (N) and phosphorus (P) being the most important nutrients.

Initial studies with primary producers have shown that autotrophic microbes are nutrient limited. Many scientists have proved this theory of nutrient limitation of autotrophs by the studying photosynthetic microorganism (e.g. algae). Photosynthetic biota (autotrophs hereafter) are frequently limited by supplies of N and P in freshwater, marine,

and terrestrial environments (Elser 2007). More recent studies have focused on conditions that can cause these limitations. These studies indicated a diverse set of geochemical and ecological factors that influence the concentrations or bioavailability of N and P elements in particular ecosystems. Autotrophic state is dependent upon photosynthesis so is controlled by light and nutrients. Often biomass of primary producers (chlorophyll a) is used to indicate trophic state, but both the biomass and activity of that biomass determine autotrophic state.

A study conducted in a terrestrial environment indicated that photosynthetic biota may be limited by P concentrations due to soil age. “P becomes increasingly sequestered because of mineralogical transformations over time scales of  $10^3 - 10^5$  years” (Elser 2007). In addition, fire regimes volatilized some N while leaving P behind (Elser 2007). In marine environments, P is limited by its sequestration in calcareous sediments, while N by planktonic  $N_2$  fixation due to insufficient light (Elser 2007). Past studies have indicated a variety of factors that may affect the supplies of N and P in lakes and streams; those are redox-dependent P retention in sediments, the intensity of denitrification, watershed land use patterns, and internal food web structure (Elser 2007).

Current studies have begun to question these generalizations, implying that there is an equivalence of N and P limitations in different ecosystems. Elser et al (2007) reported results of a study that revealed uniformity in autotrophic response to N and P concentrations. They also conducted a large meta-analysis of data from marine, freshwater and terrestrial ecosystems, investigating the effects of N: P concentrations in each environment. Their studies revealed that N and P limitations in autotrophic microbes

were often simultaneous (i.e. co-limitation) within all ecosystems. Later research has been focused towards determining the nutrient criteria policy in US and other countries.

Studies have also focused on carbon pathways that are affected by the increased carbon fixation from primary production (Dodds and Cole, 2007). The standing crop and flux rate of carbon are fundamental properties of ecosystems because they affect the potential food base in food webs. Studies have shown that human activities, such as fertilizing, croplands, watersheds, disturbances, and sewage releases, influence large river ecosystems by increasing inorganic nutrient loading in rivers and streams. The consequences of such human activities can be substantial to the stoichiometry and activity of autotrophic (and heterotrophic) organisms *via* effects on their production rates.

### **Heterotrophic response to nutrient enrichment**

A wealth of information has been amassed for autotrophic organisms, but how nutrient concentrations and ratios affect heterotrophic pathways is presently not well understood. Current research indicates that heterotrophic pathways (those based on heterotrophic microbial utilization of detrital carbon) may be profoundly affected by nutrient enrichment in aquatic systems. In aquatic systems, heterotrophic response to nutrient enrichment is determined by organic carbon availability from both within and outside of the stream (e.g. leaf input). The removal of riparian vegetation decreases allochthonous C inputs while increasing the potential for autochthonous production by increasing light input (Dodds and Cole, 2007).

Heterotrophic and autotrophic responses to nutrient enrichment differ in their effect on trophic state, or the energy available to the food web. As noted previously, for autotrophic microbes, nutrient enrichment can lead to increased biomass and biomass-

specific rates of primary production. In heterotrophic ecosystems, nutrient enrichment can accelerate microbial respiration rates and result in carbon losses from detritus-based systems (Dodds 2007). Heterotrophs are limited by carbon availability given their reliance upon the assimilation of organic carbon to build cells and conserve energy (Dodds 2007).

A few studies have examined detrital nutrient dynamics at a whole ecosystem scale. Evidence suggest that in streams, nutrient enrichment results in dramatically increased carbon loss due to downstream export and increased respiration when compared to a corresponding reference conditions (Benstead et al 2009). A two year study was conducted at the Coweeta Hydrologic Laboratory, North Carolina, USA by enriching one stream with low levels of inorganic nitrogen and phosphorous. The results indicated 340% increase in fine particulate organic matter (FPOM) export in the treatment stream. The respiration rate of heterotrophs, which was the dominant carbon output of this system had also increased, although not as dramatically as FPOM exports (Benstead et al 2009). Nitrogen and phosphorous were added at approximately Redfield ratio of N:P (16:1). This may or may not have corresponded to elemental ratios of dominant heterotrophs. So, while positive responses were noted, the concentrations and ratios at which maximum responses would be elicited are unknown.

### **Homeostasis of heterotrophic microorganisms**

The Redfield ratio is relatively unknown for heterotrophs, and the degree to which heterotrophic bacteria and fungi are homeostatic under varying inputs of N and P is also unknown. Elemental homeostasis is crucial to understanding the response of detrital pathways to nutrient enrichment. Consequently, a few studies have addressed

homeostasis of heterotrophic microorganisms. Makino and Cotner (2004) conducted a study that asked whether bacterial communities are homeostatic in terms of C:N:P stoichiometry. Their results revealed that the bacterial community at Lake Owasso was homeostatic in terms of biomass C:P and N:P. Moreover, the degree of homeostasis in the bacterial community of Lake Owasso was similar to that of single bacterial strains.

Some evidence also suggests non-homeostasis in some bacterial communities. An earlier study conducted at Lake Biwa in Japan (Tezuka 1990 as cited by Makino and Cotner, 2004), indicated a lack of homeostasis in C:N:P stoichiometry in the bacterial communities. Lake water nutrient ratios varied from 50 to 1200 for C:P supply and from 7 to 60 for N:P supply. Stoichiometric ratios of bacterial biomass were found to range from 31 to 464 and from 7 to 41 for C:P and N:P, respectively (Tezuka 1990). The bacterial communities at Lake Owasso (Makino and Cotner, 2004) were collected across a similar range of C:P ratios (93 to 933), and a broader range of N:P ratios (72 to 721). “This created a 3-fold variation in biomass C:P (55 to 175) and biomass N:P (11 to 31), a much smaller variation than the nearly 10-fold variation in Lake Biwa” (Makino and Cotner, 2004). Makino and Cotner speculated that variability in C:P and N:P homeostasis in different bacterial communities may be due to the differences in the nutrient content of individual species and changes in their relative abundances in microbial communities (e.g. seasonal variations).

## **Experiment**

The objective of this experiment was to find out if aquatic fungi are homeostatic at broad range of dissolved N and P concentrations and ratios. Aquatic fungi were

cultivated in liquid media in tissue culture flasks on a shaker that simulated stream conditions with respect to dissolved C:P, C:N and N:P ratios. Fungal growth after a period of three weeks was assessed by determining their biomass, C, N and P content, and C:N, C:P & N:P ratios. I hypothesized that fungi are homeostatic under varying concentrations and ratios of dissolved N and P.

### **Materials and Methods**

Base liquid medium used in this experiment contained a source of carbon (carboxymethylcellulose) and salts, micronutrients or vitamins necessary for fungal growth. The recipe (per 1 L) is as follows: carboxymethylcellulose (sodium salt) 8.38 g; CaCl<sub>2</sub> 0.1 g; MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g; MnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.004 g; FeCl<sub>3</sub> x 6 H<sub>2</sub>O 0.01 g ; thiamine 0.01 mg. MnSO<sub>4</sub> and CaCl<sub>2</sub> were prepared as higher concentration stocks (10 g/L) and autoclaved separately; micronutrients and thiamine were filter sterilized (0.22 µm membrane filter).

Inorganic nutrients (N and P) were provided as NaNO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub>, respectively, at 14 combinations of concentrations and ratios (see Table 1, Fig. 1).

Table 1. Fourteen combinations of nutrient concentrations and ratios (=treatments) used in the experiment.

| Treatment | N:P ratio (molar) | Series        | N (mM) | P (mM) | N (mg/L) | P (mg/L) | NaNO <sub>3</sub> (mg/L) | KH <sub>2</sub> PO <sub>4</sub> (mg/L) |
|-----------|-------------------|---------------|--------|--------|----------|----------|--------------------------|--|
| 2L        | 2                 | Low nutrient  | 0.44   | 0.22   | 6.19     | 6.85     | 37.57                    | 30.05                                  |
| 4L        | 4                 | Low nutrient  | 0.63   | 0.16   | 8.75     | 4.84     | 53.13                    | 21.25                                  |
| 8L        | 8                 | Low nutrient  | 0.88   | 0.11   | 12.37    | 3.43     | 75.13                    | 15.03                                  |
| 16L       | 16                | Low nutrient  | 1.25   | 0.08   | 17.50    | 2.42     | 106.25                   | 10.63                                  |
| 32L       | 32                | Low nutrient  | 1.77   | 0.06   | 24.75    | 1.71     | 150.26                   | 7.51                                   |
| 64L       | 64                | Low nutrient  | 2.50   | 0.04   | 35.00    | 1.21     | 212.50                   | 5.31                                   |
| 128L      | 128               | Low nutrient  | 3.54   | 0.03   | 49.50    | 0.86     | 300.52                   | 3.76                                   |
| 2H        | 2                 | High nutrient | 1.77   | 0.88   | 24.75    | 27.40    | 150.26                   | 120.21                                 |
| 4H        | 4                 | High nutrient | 2.50   | 0.63   | 35.00    | 19.38    | 212.50                   | 85.00                                  |
| 8H        | 8                 | High nutrient | 3.54   | 0.44   | 49.50    | 13.70    | 300.52                   | 60.10                                  |
| 16H       | 16                | High nutrient | 5.00   | 0.31   | 70.00    | 9.69     | 425.00                   | 42.50                                  |
| 32H       | 32                | High nutrient | 7.07   | 0.22   | 98.99    | 6.85     | 601.04                   | 30.05                                  |
| 64H       | 64                | High nutrient | 10.00  | 0.16   | 140.00   | 4.84     | 850.00                   | 21.25                                  |
| 128H      | 128               | High nutrient | 14.14  | 0.11   | 197.99   | 3.43     | 1202.08                  | 15.03                                  |

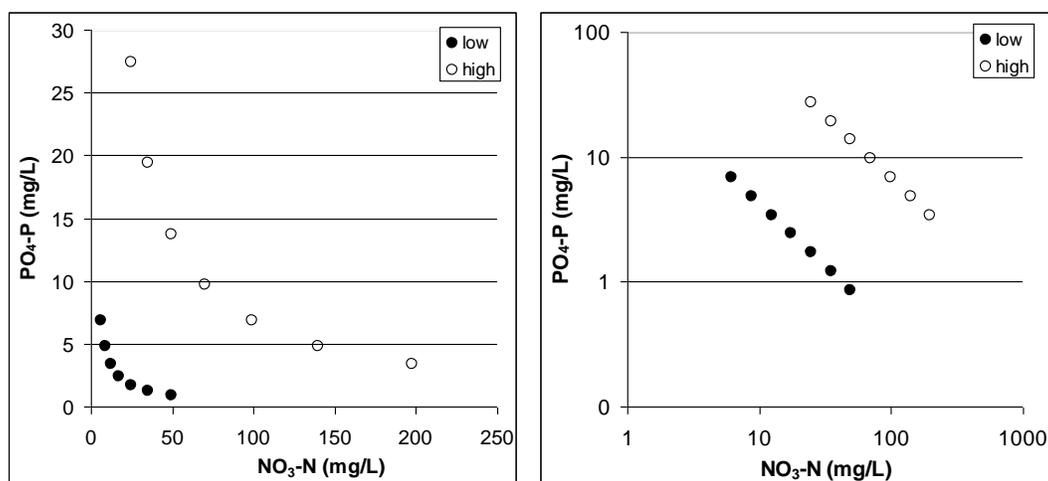


Fig. 1. Concentrations of inorganic N and P in 14 nutrient treatments of the experiment. The same treatments are shown on a regular scale (left panel) and log-log scale (right panel).

The base media (carboxymethylcellulose plus appropriate amounts of N and P for each of the 14 treatments) were autoclaved. After autoclaving and cooling, CaCl<sub>2</sub> stock, MgSO<sub>4</sub> stock and micronutrient/thiamine stock were added aseptically. Prepared nutrient media were dispensed aseptically into tissue culture flasks (25 mL per flask; 9 tissue culture flasks per nutrient level). Flasks were inoculated with *ca.* 1000 conidia per flask (see below). Thus, experiment included 14 nutrient levels x 3 fungal inocula x 3 replicates that gave 126 flasks total. The flasks were placed on an orbital shaker and incubated at 125 rpm at 20 °C (16/8 hours light/dark cycle) in an environmental chamber for 24 days (Fig. 2).



Fig. 2. Experimental setup.

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Conidia of two species of aquatic hyphomycetes and a mixture of conidia of seven species were used as inocula (i.e., 3 treatments, Table 2). Conidia production was

induced by placing slivers of actively growing 4 week-old fungal colonies (malt extract agar) in 40 mL of sterile distilled water and incubating for 3 days on a shaker at 20 °C. Conidia suspensions were used to inoculate tissue culture flasks (*ca.* 1000 conidia per flask, see above).

Table 2. Aquatic hyphomycetes used in the experiment.

| Species                          | Isolate | Treatment |
|----------------------------------|---------|-----------|
| <i>Tricladium chaetocladium</i>  | 27-1    | Tc        |
| <i>Heliscus lugdunensis</i>      | 62-1    | H         |
| <i>Tricladium chaetocladium</i>  | 27-1    | } Mix     |
| <i>Heliscus lugdunensis</i>      | 62-1    |           |
| <i>Anguillospora filiformis</i>  | 23-4    |           |
| <i>Articulospora tetracladia</i> | 24-4    |           |
| <i>Dimorphospora foliicola</i>   | 62-4    |           |
| <i>Flagellospora minuta</i>      | 31-4    |           |
| <i>Tetrachaetum elegans</i>      | 24-1    |           |

After experiment was terminated on day 24, the content of flasks was filtered to capture fungal mycelium and spores for further analyses. Before filtering, glass fiber filters (Whatman 934-AH) were combusted in the muffle furnace at 500 °C for 2 hours and then weighed (to 0.01 mg). Culture liquids with fungal biomass were filtered by vacuum filtration to prepare two filters per flask (one to be used for fungal N analysis and the other for P analysis). Filters were placed in drying oven at 60 °C to dry for at least 24 hours. Then the filters were weighed to determine fungal biomass and analyzed for N or P.

### *N analysis*

Carbon and nitrogen contents of fungal biomass were determined by CHN elemental analyzer at the Odum School of Ecology, University of Georgia. NIST C and N standards were used as a reference.

### *P analysis*

Phosphorus content of fungal biomass was determined using a modification of the combustion and hot HCl extraction procedure of Andersen (1976).

Pyrex borosilicate glass tubes (16 x 100 mm) and Teflon-lined phenolic caps were left in an acid bath (5% HCL) for at least 24 hours. After acid washing the glass tubes and caps were rinsed 6 times with diH<sub>2</sub>O. Tubes were placed in a metal wire rack, and glass-fiber filters with mycelium were placed inside the tubes. Four reference samples were included with each batch (also prepared in acid-washed tubes). They were 4 glass fiber filters spiked with 1.0, 2.5, 5.0, and 10.0 µg of P per filter, respectively (see P standards). Tubes with filters/mycelium and reference tubes with spiked filters were combusted in the muffle furnace at 500 °C for at least 2 hours and then allowed to cool to room temperature. 4.0 mL of 1.0 N HCl was added using the Eppendorf Repeater Plus pipettor to each tube. Tubes were capped tightly and vortexed for 15 seconds. Caps were then loosened and tubes were autoclaved for 20 minutes at 121 °C. After cooling, caps were tightened and tubes vortexed for 15 seconds. Samples were allowed to sit overnight for glass fibers to settle. Next day, 5 ml of ultrapure water (17 MΩm) was pipetted into labeled 14-mL (17 x 100 mm) plastic tubes. Next, 0.5 ml of digest was pipetted into each corresponding tube, 0.5 ml of 1.0 N HCl was pipetted into additional tube to serve as a blank sample. 0.90 mL of combined reagent (see below) was added to each tube. Samples

were vortexed briefly and incubated for 20 minutes for color to develop. Absorbance was determined at 880 nm using Beckman DU 520 spectrophotometer. One mL of each sample was pipetted into disposable plastic cuvette and absorbances were recorded. The instrument was blanked and reference samples were run with each batch of 20 samples. A standard curve was plotted using the 4 reference samples. The P content of 0.5 mL of each initial sample extract was calculated and multiplied by 8 (since 0.5 mL out of 4.0 mL was subsampled) to determine the P content (in  $\mu\text{g}$ ) per sample/filter. The P content of fungal biomass was calculated by dividing P content per filter by the corresponding weight of mycelium.

**P standards.** For the master stock P solution, potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) was dried in an oven at  $105^\circ\text{C}$  for one hour. A master stock (10.0 g/L) was prepared in 100 mL volumetric flask (0.1 mL of chloroform was added to prevent microbial growth). Note that 10.0 g/L of  $\text{KH}_2\text{PO}_4$  contains 2.28 g of P per 1 L. For the standard solution (1.0 mL = 10  $\mu\text{g}$  P), the master stock was diluted as follows: 219  $\mu\text{L}$  of master stock was added to 49.78 mL of ultrapure water in 50 mL centrifuge tube. 1-2 drops of chloroform were added. Four reference samples (above) were prepared by adding known amounts of P to filters in acid-washed tubes (Table 3). In addition, 8 subsamples of NIST standards (NIST 8437, hard red wheat spring flour, 0.137% P and NIST 8414, bovine muscle, 0.836% P) were weighed and extracted as described to determine P extraction efficiency. Since the recovery was high (*ca.* 103%), the results were not corrected.

Table 3. Amounts of P and corresponding volumes of standard P solution (10  $\mu\text{g}$  P per mL) used to prepare reference samples.

| P per filter ( $\mu\text{g}$ ) | Standard solution added ( $\mu\text{L}$ ) |
|--------------------------------|---|
| 1.0                            | 100                                       |
| 2.5                            | 250                                       |
| 5.0                            | 500                                       |
| 10.0                           | 1000                                      |

**Solutions.** For the 1 *N* HCl solution, 83.3 mL of concentrated (~37%) hydrochloric acid was diluted to 1.0 L with diH<sub>2</sub>O and stored in glass container till further use. The solutions to prepare combined reagent for spectrophotometric P analysis were prepared as follows (according to APHA):

- (a) Sulfuric acid (5 *N*): 70 mL of concentrated (~96%) H<sub>2</sub>SO<sub>4</sub> was diluted to 500 mL with diH<sub>2</sub>O and stored in a glass container.
- (b) Potassium antimonyl tartrate: 0.274 g was dissolved in 100 mL diH<sub>2</sub>O. Solution was stored in a glass bottle.
- (c) Ammonium molybdate: 10.0 g was dissolved in 250 mL diH<sub>2</sub>O and stored in a brown plastic bottle.
- (d) Ascorbic acid (prepared daily before analysis): 0.264 g was dissolved in 15 mL diH<sub>2</sub>O. The amount of both ascorbic acid and water were doubled if more than 45 samples were taken per day).

The combined reagent needed to be prepared just before the analysis (stable for 4 h only).

For 50.0 mL of combined reagent (enough for 40-50 samples), 25.0 mL of solution (a), 2.5 mL of solution (b), 7.5 mL of solution (c) and 15.0 mL of solution (d) were mixed in

a glass bottle. The solution was mixed after the addition of each reagent and mixed in the order given.

### Data Analysis

Molar (atomic) C:P, C:N and N:P ratios of media were calculated directly by dividing molar amounts of respective nutrients (see Table 1, carbon concentration for all treatments was 38.5 mM). Molar nutrient ratios of fungal biomass were estimated similarly, but first I had to calculate molar amounts of C, N and P for each sample (by dividing C, N or P content in  $\mu\text{g}$  by the element atomic weight).

H is the homeostatic coefficient used to determine the degree of stoichiometric homeostasis in a given species. It is defined as follows:

$$H = \frac{\log_{10}(x)}{\log_{10}(y) - \log_{10}(c)}$$

, where x is the resource (medium) nutrient stoichiometry (e.g. C:P, C:N or N:P ratio), y is the fungal nutrient stoichiometry (same units as the resource) , and c is a constant (Persson, 2010). 1/H is the slope of regression between  $\log(x)$  and  $\log(y)$  and varies between 0 and 1. This regression slope was used in all analyses. Microorganisms with  $1/H=0$  are considered ‘strictly homeostatic’ and those with  $1/H=1$  are not homeostatic (Persson, 2010). These 1/H values, with significant regressions, were classified in the following manner: “ $0 < 1/H < 0.25$  ‘homoeostatic’,  $0.25 < 1/H < 0.5$  ‘weakly homeostatic’,  $.05 < 1/H < 0.75$  ‘weakly plastic’,  $1/H > 0.75$  ‘plastic’” (Persson, 2010).

Regression slopes ( $1/H$ ), with respect to C:P, C:N, and N:P, were calculated separately for *Tricladium chaetocladium* (Tc), *Heliscus lugdunensis* (H), and a mixture of 7 species of aquatic hyphomycetes (Mix, see Table 2 for species).

## Results

Fig. 3 shows the fungal biomass accrual of aquatic fungi in relation to C:P ratio in the medium. We observed a clear trend of lower fungal biomass with higher C:P ratios that suggest some degree of P limitation (with high C:P ratio, P is low, and as such there is a P deficiency). *T. chaetocladium* (Tc-High series) experienced significant drop in fungal biomass as the C:P ratio increased. In fact, the fungal biomass of this species for some samples was so low that we were unable to calculate corresponding fungal nutrient ratios (see below) due to insufficient amount of mycelium collected.

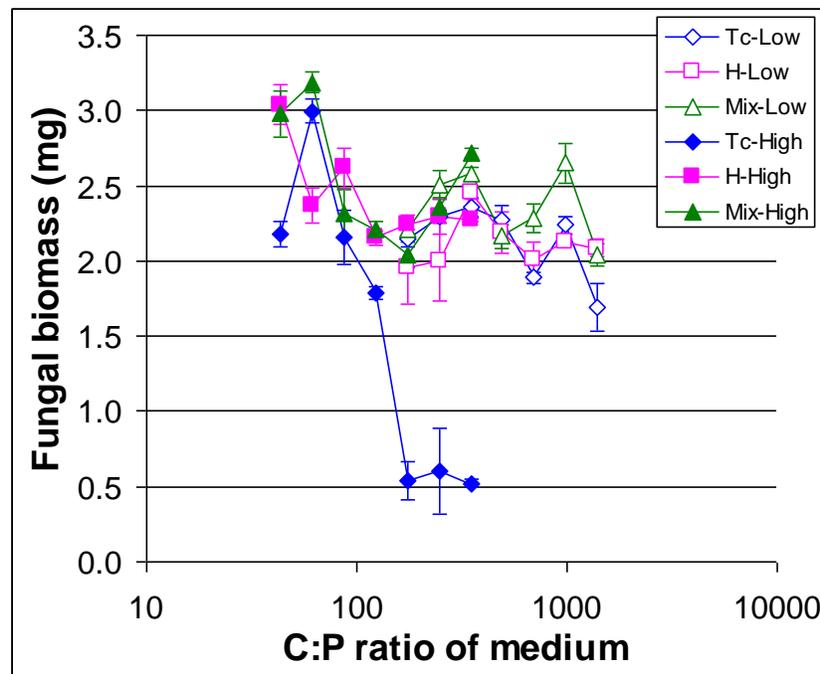


Fig. 3. Fungal biomass accrual of aquatic hyphomycetes at day 24 in relation to C:P ratio of liquid medium. Means  $\pm 1$  SE are shown.

No clear pattern was obvious for the relation between C:N ratio of the medium and fungal biomass (Fig. 4). It is thought that low fungal biomass of *T. chaetocladium* (Tc-High series) was not due to low C:N ratio (i.e. plentiful supply of N) but rather P limitation (see above).

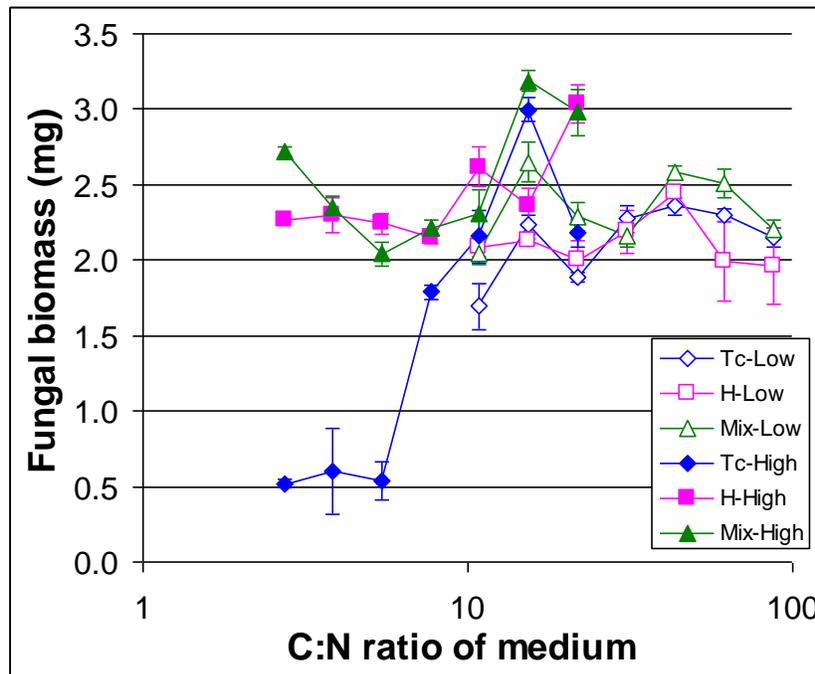


Fig. 4. Fungal biomass accrual of aquatic hyphomycetes at day 24 in relation to C:N ratio of liquid medium. Means  $\pm 1$  SE are shown.

Fig. 5 shows the fungal biomass accrual of aquatic fungi in relation to N:P ratio in the medium. We observed a slight trend of lower fungal biomass with higher N:P ratio of the medium for most treatments. Since C:N of the medium did not have a clear affect on fungal biomass (Fig. 4), we attribute the pattern seen in Fig. 5 to P limitation at higher N:P ratios of the medium.

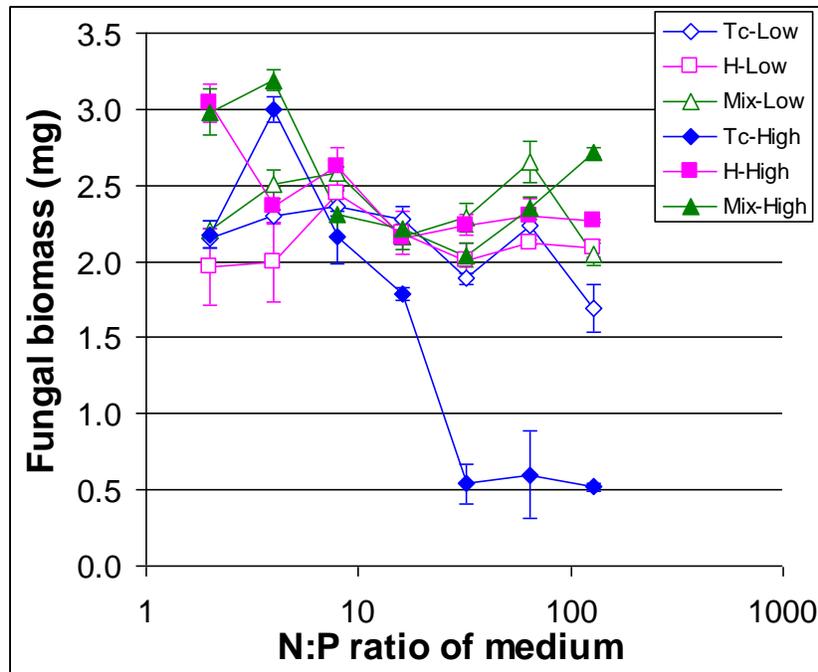


Fig. 5. Fungal biomass accrual of aquatic hyphomycetes at day 24 in relation to N:P ratio of liquid medium. Means  $\pm 1$  SE are shown.

Figure 6 depicts the C:P ratio of fungal biomass in relation to the C:P ratio of the medium. The 1:1 line represents non-homeostasis (i.e.  $1/H=1$ ) while the horizontal line with a slope of 0 (i.e.  $1/H=0$ ) would be typical for an organism or community with strict homeostasis, i.e. the ability to maintain the same nutrient ratio of the biomass regardless of the nutrient ratio of the resource (medium). None of the species showed an  $1/H$  value of 0 or 1 to classify them as strictly homeostatic or non-homeostatic, respectively. The following  $1/H$  values were obtained based on regressions: 0.261 (*T. chaetocladium*;  $R^2=0.53$ ,  $p=0.008$ ), 0.335 (*H. lugdunensis*;  $R^2=0.69$ ,  $p<0.001$ ) and 0.292 (fungal community of 7 species;  $R^2=0.73$ ,  $p<0.001$ ). Each treatment had  $1/H$  values within the  $0.25 < 1/H < 0.5$  range, classifying all of them as ‘weakly homeostatic’ with respect to

C:P. Overall, under the varying C:P ratios and concentrations in the medium, fungal biomass C:P ratio ranged from of 40 to 203 depending on fungal species.

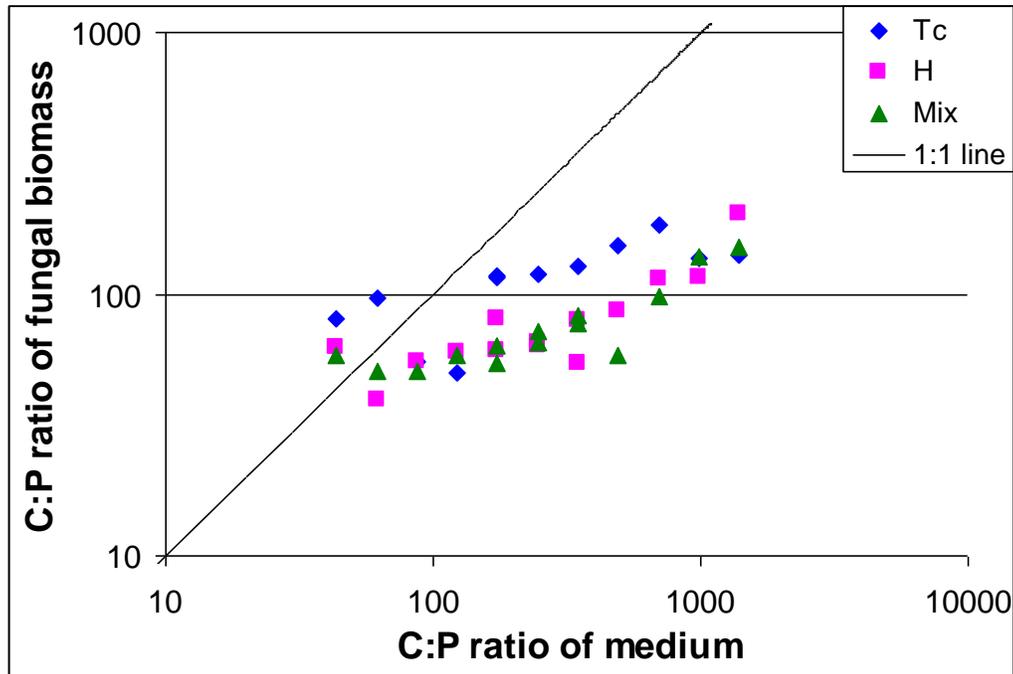


Fig. 6. Relationship between C:P ratio of fungal biomass and C:P ratio of liquid medium.

Figure 7 shows the C:N ratio of fungal biomass in relation to the C:N ratio of the medium. The following  $1/H$  values were obtained based on regressions: 0.022 (*T. chaetocladium*;  $R^2=0.02$ ,  $p=0.70$ ), 0.162 (*H. lugdunensis*;  $R^2=0.53$ ,  $p=0.003$ ) and 0.112 (fungal community of 7 species;  $R^2=0.41$ ,  $p=0.013$ ). The regression for *T. chaetocladium* was not significant (slope did not differ from 0). Each treatment had  $1/H$  values within the  $0 < 1/H < 0.25$  range, classifying all of them as ‘homeostatic’ with respect to C:N. Overall, under the varying C:N ratios and concentrations in the medium, fungal biomass C:N ratio ranged from 7 to 16 depending on fungal species.

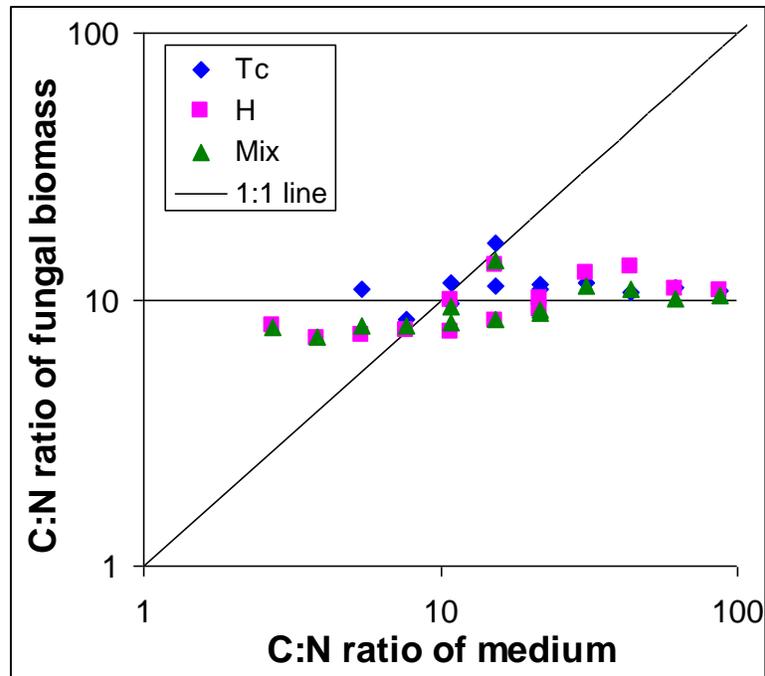


Fig. 7. Relationship between C:N ratio of fungal biomass and C:N ratio of liquid medium.

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Data showing the relationship between N:P ratios of the medium and N:P ratios of fungal biomass is shown in Figure 8. The following  $1/H$  values were obtained based on regressions: 0.090 (*T. chaetocladium*;  $R^2=0.11$ ,  $p=0.29$ ), 0.159 (*H. lugdunensis*;  $R^2=0.42$ ,  $p=0.013$ ) and 0.157 (fungal community of 7 species;  $R^2=0.56$ ,  $p=0.002$ ). As with C:N ratios (above), the regression for *T. chaetocladium* was not significant (slope did not differ from 0). Each treatment had  $1/H$  values within the  $0 < 1/H < 0.25$  range, classifying all of them as ‘homeostatic’ with respect to N:P. Overall, under the varying N:P ratios and concentrations in the medium, fungal biomass N:P ratio ranged from 5 to 20 depending on fungal species.

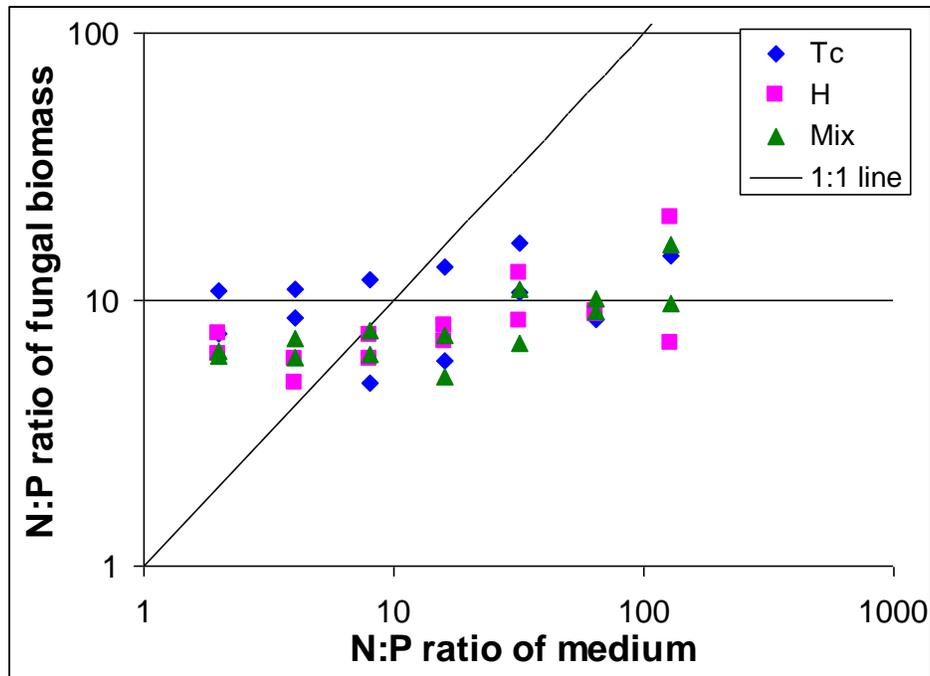


Fig. 8. Relationship between N:P ratio of fungal biomass and N:P ratio of liquid medium.

## Discussion

There was a much greater variability in fungal biomass C:P ratio, 40-203, than observed for C:N and N:P ratios. Fungal C:N ratio showed the lowest variability and ranged from 7 to 16. This indicates that despite the varying concentrations of nutrients in the medium fungi were homeostatic with respect to their C:N ratio or N content. On the other hand, aquatic fungi were only weakly homeostatic with respect to their C:P ratio, suggesting severe P limitation at higher medium C:P ratios and a possibility of P storage (in the form of polyphosphate) when P was plentiful (low C:P of the medium).

As noted previously, the Redfield ratio is relatively unknown for heterotrophs, especially fungi, and the degree to which heterotrophic bacteria and fungi are homeostatic under varying concentrations and ratios of N and P is also unknown. The

Redfield ratio for autotrophic microbes such as algae is  $C_{106}N_{16}P_1$ . Based on data from this experiment, average fungal C:P ratio was around 90 (range 40-203), fungal C:N ratio averaged around 10 (range 7-16) and fungal N:P ratio averaged about 9 (range 5-20). So, fungal nutrient ratios or fungal “Redfield ratio” can be summarized as  $C_{90}N_9P_1$ . This suggests that fungi in general are more N and P rich than algae, but less “nutrient dense” than bacteria (e.g. Makino and Cotner, 2004; Sterner and Elser, 2002).

The homeostasis in fungi is somewhat similar to that of bacteria as indicated by Makino and Cotner (2004) who found bacterial communities to be homeostatic in terms of biomass N:P. Makino and Cotner (2004) also observed their bacterial communities to be homeostatic with respect to C:P ratio; however, the 1/H values obtained for fungal biomass C:P in this experiment indicate that aquatic fungi are weakly homeostatic. Evidence also suggests non-homeostasis in some bacterial communities. Lake water was collected across a range of 50 to 1200 for C:P supply and 7 to 60 for N:P supply and biomass nutrient ratios of 31 to 464 and 7 to 41 were found for C:P and N:P, respectively (Tezuka, 1990 as cited by Makino and Cotner, 2004). Large variations for C:P and relatively small variations for N:P were also found in our study, both for single species treatments and for mixed treatment (7 species fungal community). Interestingly, the goodness of fit of regressions ( $R^2$ ) in our experiments was generally higher for 7 species fungal assemblage vs. single species treatments indicating that nutrient ratios and responses of microbial community may be more stable than ratios or responses of its components.

Our studies revealed that aquatic fungi have N and P content, C:N, C:P and N:P ratios or “Redfield ratio” intermediate of those of algae and bacteria. It can be related to

the overall metabolic activities or growth rates typical for these groups of organisms, being the fastest in case of bacteria and the slowest for algae. Overall, aquatic fungi were capable of maintaining nearly constant stoichiometric ratio of C:N regardless of changes in their resource stoichiometry or concentrations, while fungal C:P ratio varied to some degree depending on medium C:P ratio and P concentration. The  $1/H$  values obtained in this study indicate that fungal biomass is weakly homeostatic with respect to C:P and homeostatic with respect to C:N and N:P

## References

- Benstead JP, Rosemond AD, Cross WF, Wallace JB, Eggert SL, Suberkropp K, Gulis V, Greenwood JL and Tant CJ. 2009. Nutrient enrichment alters storage and fluxes of detritus in headwater stream ecosystem. *Ecology* **90**: 2556-2566.
- Dodds WK and Cole J. 2007. Expanding the concept of trophic state in aquatic ecosystems: It's not just the autotrophs. *Aquatic Sciences* **69**:427-439.
- Dodds WK. 2007. Trophic state, eutrophication and nutrient criteria in streams. *Trends in Ecology and Evolution* **22**:669-676.
- Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology letters* **10**: 1135-1142.
- Makino W and Cotner JB. 2004. Elemental stoichiometry of heterotrophic bacterial community in a freshwater lake: implications for growth- and resource-dependent variations. *Aquatic Microbial Ecology* **34**: 33-41.
- Persson J, Fink P, Goto A, Hood JM, Jonas J and Kato S. 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* (in press).
- Sterner RW and Elser JJ. 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton, NJ.