Soil Biology & Biochemistry 76 (2014) 278–285

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Elemental stoichiometry of Fungi and Bacteria strains from grassland leaf litter

Céline Mouginot [a], Rika Kawamura [a], Kristin L. Matulich [b], Renaud Berlemont [a], Steven D. Allison [a, b], Anthony S. Amend [b, 1], Adam C. Martiny [a, b, *]

[a] Department of Earth System Science, University of California, Irvine, CA 92697, USA
[b] Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA

ARTICLE INFO

Article history:
Received 7 April 2014
Received in revised form
8 May 2014
Accepted 9 May 2014
Available online 23 May 2014

Keywords:
Nutrient stoichiometry
Growth rate hypothesis
Redfield
C:N:P ratios
Resource allocation

ABSTRACT

In most terrestrial environments, our knowledge of the elemental composition and stoichiometry of microorganisms stems from indirect whole community analyses. In contrast, we have little direct knowledge of the elemental composition of specific microorganisms and the variation between and within Fungi and Bacteria. To address this issue, we isolated and identified the elemental content of 87 strains of Fungi and Bacteria isolated from grassland leaf litter. The isolated strains were affiliated with a broad range of diversity including Ascomycota and Basidiomycota for Fungi, and Proteobacteria, Bacteroidetes, and Actinobacteria for Bacteria. The C:P and C:N but not N:P ratios were significantly higher in Fungi than in Bacteria. Extensive strain variation in elemental composition was partly linked to phylogeny and growth rate. Across all strains, the geometric mean C:N:P was 88:15:1. This overall ratio was significantly higher than reported for other leaf litter and terrestrial whole communities but closer to the canonical Redfield ratio characterizing marine microorganisms. This result warrants further investigation into the discrepancy between whole community and isolated strain elemental ratios.

1. Introduction

The ratios of carbon (C), nitrogen (N), and phosphorus (P) in the environment and within organisms link the biogeochemical cycles of these important elements (Sterner and Elser, 2002). Despite the importance of stoichiometric ratios, less is known about their magnitude and variation in microorganisms in terrestrial environments. In the most extensive comparison of different soil microbial communities, Cleveland and Liptzin (2007) found an average microbial C:N:P molar ratios of 60:7:1. On leaf litter, microbial communities also show low C:N:P ratios of 16:4:1 (Van Meeteren et al., 2008). These ratios are starkly lower than the averages observed in marine environments as described by the Redfield ratio (106:16:1). Such values suggest that terrestrial microorganisms generally are depleted in C and N – or enriched in P – compared to marine microorganisms.

There are several possible biological mechanisms for variations in elemental composition among communities (Sterner and Elser, 2002). First, differences in environmental conditions like temperature or nutrient availability can influence elemental composition (Woods et al., 2003; Scott et al., 2012; Xu et al., 2013). Second, the growth rate and associated allocation to growth vs. resource acquisition machinery may vary. Specifically, the growth rate hypothesis postulates negative relationships between C:P and N:P ratios and growth rate (Sterner and Elser, 2002). Third, specific lineages may have a unique elemental composition and thus changes in microbial community composition can lead to differences in elemental ratios (Quigg et al., 2003; Zimmerman et al., 2014). Some studies have suggested that Fungi in comparison to Bacteria have a higher C:N (Strickland and Rousk, 2010) but otherwise little is known about phylogenetically related differences in the elemental ratios among soil or leaf litter microorganisms.

To directly investigate the C:N:P ratios of microorganisms in leaf litter, we analyzed the elemental ratio of 87 phylogenetically-diverse fungal and bacterial strains isolated from a semi-arid Mediterranean grassland ecosystem. We asked if the average C:N:P ratios of Fungi and heterotrophic Bacteria from this habitat differed from each other, from the ratio observed in whole communities in terrestrial ecosystems (60:7:1), or from the Redfield ratio. We next

* Corresponding author. 3208 Croul Hall, Department of Earth System Science, University of California, Irvine, CA 92697, USA. Tel.: +1 949 824 9713; fax: +1 949 824 3874.
E-mail address: amartiny@uci.edu (A.C. Martiny).
[1] Current address: Department of Botany, St. John 101, University of Hawaii, 3190 Maile Way, Honolulu, HI 96822, USA.

http://dx.doi.org/10.1016/j.soilbio.2014.05.011
0038-0717/

asked if differences between microorganisms were due to phylogenetic constraints or growth rate differences. Finally, to make a direct comparison with marine microorganisms, we compared the stoichiometry of these leaf litter strains to a suite of previously analyzed marine heterotrophic Bacteria (Zimmerman et al., 2014). We hypothesized that average stoichiometric ratios of terrestrial Fungi and Bacteria would be statistically indistinguishable from Cleveland and Liptzin's ratio and significantly lower than the Redfield ratio and the average ratios of marine isolates. Furthermore, we expected that stoichiometric variation among isolates would in part be due to growth rate differences and phylogenetic history.

2. Material and methods

2.1. Fungi isolation and growth

45 fungal isolates were isolated from leaf litter samples from a Mediterranean grassland ecosystem at Loma Ridge, CA (33.4°N, 117.4°W, elevation 365 m). The site climate is characterized by hot dry summers, a mean annual temperature of 17 °C, and mean annual precipitation of 325 mm (Potts et al., 2012). Nearly all precipitation falls between October and April. Soils are a fine-loamy, mixed, thermic Typic Palexeralfs sandy loam (California Soil Resource Lab, http://casoilresource.lawr.ucdavis.edu) with a pH of 6.8. The litter layer consists of senesced plant material from primarily exotic annual grasses of the genera *Avena*, *Bromus*, and *Lolium* as well as annual forbs such as *Erodium*, *Lupinus*, and *Vicia*. Litter microbial communities are dominated by bacterial biomass, though Fungi represent up to ~20% of microbial biomass in summer (Alster et al., 2013). Fungal cultures were obtained from leaf litter using a dilution to extinction method. Briefly, leaf litter was homogenized in a blade grinder, suspended in sterile water, rinsed, and passed through a series of filters to obtain a size fraction between 106 and 212 μm. Filtrate was suspended in 30 ml of 0.6% carboxy-methyl-cellulose solution. 10 μl of filtrate was added to 500 μl solid malt extract agar (MEA) medium (agar 20 g/l, malt extract 5 g/l, yeast extract 5 g/l, C:N:P ratio w/o agar = 290:35:1) augmented with kanamycin and ampicillin (50 mg/l). Tubes were examined weekly for growth. Fungal cultures were transferred to liquid MEA medium and incubated overnight with shaking at room temperature and maintained at (22 °C). Before harvesting for elemental analysis, petri dishes containing MEA media with a pre-combusted (500 °C, 4 h) 47 mm GF/F filter (Whatman, Florham Park, NJ) were inoculated. Each isolate was propagated aseptically on a filter using a sterile cotton applicator wetted with 0.9% NaCl and placed on the top of MEA media during incubation. As a blank treatment, seven filters were humidified with NaCl. Petri dishes were incubated at room temperature for up to two months until sufficient biomass had appeared.

2.2. Fungi biomass isolation using freeze-drying

Filters with Fungi biomass were removed from the agar plates and collected into 5 ml vials. The vials were flash frozen in liquid nitrogen and stored at −80 °C until further processing. The frozen filters were freeze-dried overnight with a pressure below 30 Pa at −25 °C in a freeze-drying system (Freezone 4.5, Labconco, Kansas City, MO) and homogenized with a disperser (Ultra-Turrax T8, IKA Wilmington, NJ) until obtaining a fine powder. The culture powder was stored at −80 °C in 5 ml pre-combusted (500 °C, 4 h) glass vials until further analysis.

2.3. Bacteria isolation and growth

5–10 g of leaf litter were weighed and washed down through a sieve column of 106, 212, and 2000 μm pores with 750 ml–1000 ml of autoclaved, distilled water to separate leaf litter particles. Leaf litter particles were then collected from the 106–212 μm fraction in a sterile Falcon tube and rinsed with 5–10 ml of distilled water to create a litter suspension. The suspension was then poured over a Millipore Sterifil 47 mm Aseptic Vacuum Filter System suspension chamber set up with a sterile, nylon Millipore Sterifil 47 mm Filter O-ring with 100 μm pores. 200 ml of distilled water was poured down the suspension chamber and collected as the first wash water. The first wash was then inoculated onto LB media plates and incubated at room temperature. 42 individual colonies were picked and re-transferred onto LB plates. This process was repeated three times to ensure clonal isolation.

Each culture was then incubated in pre-filtered (0.22 μm) LB media (C:N:P ratio = 189:49:1) at 25 °C with shaking (225 rpm) and growth was monitored at OD_{600}. We first identified the full growth curve and estimated the growth rate in the logarithmic phase. On a following run, we then sampled replicate cultures in mid-log phase for particulate elemental content and flow cytometry. Specifically, technical replicate samples of cells from 250 μl samples were captured on pre-combusted (500 °C, 4 h) GF/F filter (Whatman, Florham Park, NJ). Replicate 500 μl samples were fixed at final concentration of 0.1% glutaraldehyde and stored in liquid nitrogen. Once defrosted, samples for flow cytometry were incubated in the dark for 15 min with 2 μl 10,000× diluted SybrGreen (Life Technologies, Grand Island, NY). Samples were then diluted with autoclaved and 0.2 μm filtered media and counted on an Accuri C6 Flow Cytometer (BD Biosciences, San Jose, CA).

2.4. Determination of particulate organic material

To quantify particulate organic carbon (POC) and nitrogen (PON), filter samples were thawed and allowed to dry overnight at 65 °C. We weighed three replicates of each sample (isolates and blanks) with a microbalance and then packed the material into a 30 mm tin capsule (CE Elantech, Lakewood, NJ) and analyzed C and N content on a FlashEA 1112 nitrogen and carbon analyzer (Thermo Scientific, Waltham, MA), following the protocol of Sharp (1974). POC and PON concentrations were calibrated using known quantities of atropine and peach leaves at each run. The amount of particulate organic phosphorus (POP) was determined in each sample (three replicates per isolate and blank) using a modified ash-hydrolysis method (Lomas et al., 2010). We also directly determined the media composition (without agar) for both Fungi and Bacteria using the POC, PON, and POP protocols.

2.5. PCR and phylogenetic analysis

Genomic DNA was extracted from morphologically distinct cultures using 10 μl each of the extraction and dilution solutions from the Extract-N-amp Plant kit (Sigma–Aldrich, St. Louis, MO) and incubated according to the manufacturer's directions. To identify fungal strains, we PCR amplified the ITS region as well as the adjacent ~600 bp of 28S rRNA using the ITS1F and TW13 primers (Gardes and Bruns, 1993). Approximately 0.1 μl of each DNA extract was added to a PCR cocktail containing 1.2U Taq polymerase, 1× PCR buffer containing 1.5 mM $MgCl_2$, 200 μM of each dNTP, and 0.5 μM of each primer in a final volume of 25 μl. For Bacteria, we PCR amplified the full 16S rRNA gene using the pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′) primers as designed by Edwards et al. (1989). Approximately 5 μl of each DNA extract was added to a PCR cocktail containing 0.3 μl of Taq polymerase (5 units per μl), 15.75 μl of Premix F (Epicentre, Madison, WI), and 50 μM of each primer in a final volume of 26.5 μl. Sequences were generated with Sanger paired-end sequencing and were deposited in GenBank under the