Volume 4 Issue 3 November 2020

Ratios Matter

Logged Ratios Matter Too

Yes, we saw it too! We are, of course, referring to the recent paper by Peter Isles, “The misuse of ratios in ecological stoichiometry” in *Ecology*. The central thesis of the article is that ratios are frequently ‘misused’ in statistical analysis and visualizations because they are assumed to have nearly-normal distributions. Isles (2020) reminds us that this is not the case and dubs the treatment of ratios as “normal” variables as erroneous.

As stoichiometrists, we should all be aware that ratios (of all types) have mathematical properties that make them difficult to interpret. See, for example, the miles per gallon (MPG) fallacy that has been demonstrated in economics (Larrick and Soll. 2008. Science) where linear increases in MPG yields progressively less fuel savings per unit distance. In stoichiometry, a similar example can be seen with N:P ratios. Decreasing the N:P from 16 to 8 (8 units) infers a doubling of P, but increasing the N:P by 8 units (to 24) translates to a P decline of only 1/3rd. This kind of non-intuitive interpretation is relatively familiar to practitioners of ecological stoichiometry.

Isles (2020) points out that use of “normal” methods to summarize, analyze and present ratio data will tend to yield biased results. In a ratio of two variables, the numerator and denominator have asymmetrical influence on the overall ratio, which creates a bias in descriptive statistics. We encourage readers to review his paper for excellent examples of this bias. For example, if you calculate the arithmetic mean N:P ratio and the mean P:N ratio for several samples, these two means will not be equivalent (i.e., mean N:P ≠ 1/mean P:N). Therefore, taking the mean TN:TP of several lakes will be biased towards P-limitation, while taking the mean TP:TN will be biased towards N-limitation (despite the underlying TN and TP data being the same). These problems extend beyond the arithmetic mean to estimates of variation and thereby affect our ability to make inferences from statistical analyses that do not otherwise account for this bias.

Methods to eliminate this bias and perform statistical analysis using more conventional methods are relatively simple. Simply log-transforming the data eliminates the bias in the arithmetic mean. For example, an 8-fold increase in N and and 8-fold increase in P have equal weight when calculating an arithmetic mean of logged N:P data. Similarly, the central tendency of a ratio is more accurately represented by a median or a geometric mean (along with quantiles). For statistical modeling, log-transformation allows for 'normal' statistical methods to be used (e.g., linear regression), but generalized linear models can also be used with ratio data provided a log-link function is used (readily available in most statistical software packages). Many non-parametric methods can also be used for analysis of ratio data, since the rank order of samples is not influenced by this ratio bias. *Continued on page 2.*

DID YOU KNOW?

Beyond his Law of the Minimum, Justus von Liebig is also widely credited with promoting the "sear, then roast" technique that most of us routinely use to cook (apparently incorrectly) our steaks and roasted meats. His idea was that searing seals in juices and makes the meat more nutritious. Despite this theory having been tested and shown to be incorrect, on both a juiciness and nutritional basis, this practice still continues largely unabated. So if you want to get "searious" about cooking your steak, maybe you shouldn't be taking advice from a mid-19th century stoichiometrist.

Source: H. McGee. *Searing the roast: An uneven history of uncertain benefits.* New York Times. December 18, 1985.

Continued from page 1. **The biggest hurdle to log transformations** is the difficulty that can arise with interpretation (Menge et al. 2018: Nature Ecol. Evol.). For many situations, it may be appropriate to convert descriptive statistics and modeling results back into the original units for interpretation or even using a combination of log-transformed and untransformed data to understand the results.

Isles (2020) highlights that the issues of statistical representation of ratio data can easily be solved using available methods. Yet, there is a larger question of interpretation. Unsurprisingly, this question has already been asked and answered many times in the annals of ecology. For example, Raubenheimer (1995, Functional Ecology 9: 21-29) noted, "One difficulty is that it [analysis of ratios] is frequently treated as a problem of statistics but is in fact equally an interpretive problem of mapping statistical outcomes on to biological explanations."

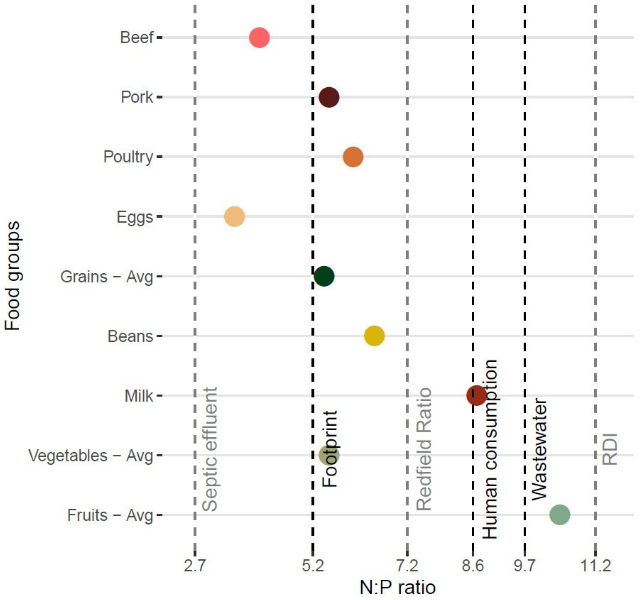
Isles (2020) touches on using geometric means to retain biological meaning whilst also addressing potential issues with using ratios. We should also continue to think carefully about what some of our commonly used ratios mean biologically. As an example, for species with weeks-long life cycles or the ability to store nutrients, the annual mean N:P doesn't necessarily provide meaningful information regardless of the way it is calculated. Remember that correctly calculating descriptive statistics does not guarantee easy biological interpretation.

Contributed by Robert Buchkowski, James Larson, Francis Q. Brearley, Catriona Jones, Clay Prater, Dedmer van de Waal, Nicole Wagner and Paul Frost

Isles, P.D.F. 2020. The misuse of ratios in ecological stoichiometry. Ecology. doi: https://doi.org/10.1002/ecy.3153

Americans tread unevenly with their nitrogen and phosphorus footprints

As environmentally conscious people, many of us are interested in reducing our impacts on the planet. Useful tools such as C footprint calculators have allowed us to quantify the effects of our daily lifestyles on green-house gas emissions and make changes towards reducing the burden we place on the environment. However, as stoichiometrists, we are aware that human perturbation of N and P cycles, and their ratios, also has important ecological consequences. To quantify these effects, Metson et al. (in press) recently estimated the P footprint of an average American using a well-established methodology for calculating N footprints (Leach et al. 2012. Env. Develop. 1:40). They also examined the consequences of human dietary preference on stoichiometric N:P released into the environment during food production and sewage treatment (see Figure). The overall N:P stoichiometry of the footprints associated with food production and consumption was ~5 (by mass), but the stoichiometry of environmental losses associated with individual food items varied considerably. While food production, and in particular meat production, was the predominant control on the size of N and P footprints, wastewater treatment also strongly affected N:P ratios in downstream loss. N:P loss ratios for all food types were much lower than human consumption ratios and recommended daily intake targets suggesting: 1) that P is less efficiently managed in food production relative to N and 2) that this is linked to preferential consumption of stoichiometrically imbalanced, high-P diets. With this in mind, how will you balance your stoichiometric footprint the next time you're at the grocery store?



Stoichiometric N:P ratios (mass) of nutrient loss into the environment. Food release ratios (circles) include nutrients released along the food production chain including fertilizer application, harvesting, processing, consumption, and recycling. Dotted vertical lines are reference values calculation in this study (black) and literature values (grey).

From the Paper: "*While strategies like reducing meat consumption will effectively reduce both N and P footprints by decreasing overall synthetic fertilizer nutrient demands, consideration of how food production and waste treatment differentially affect N and P releases to the environment can also inform eutrophication management.*"

Contributed by Clay Prater

Metson, G.S., G.K. MacDonald, A.M. Leach, J.E. Compton, J. Harrison & J.N. Galloway. In Press. The U.S. consumer phosphorus footprint: where do nitrogen and phosphorus diverge? Environmental Research Letters. DOI: https://doi.org/10.1088/1748-9326/aba781

Stoichiometry on Location: Experimental Lakes Area, Canada

Aerial view of IISD-ELA Lakes 225 (left) and 224 (right). L224 is sampled routinely as part of long-term monitoring of ELA reference ecosystems.

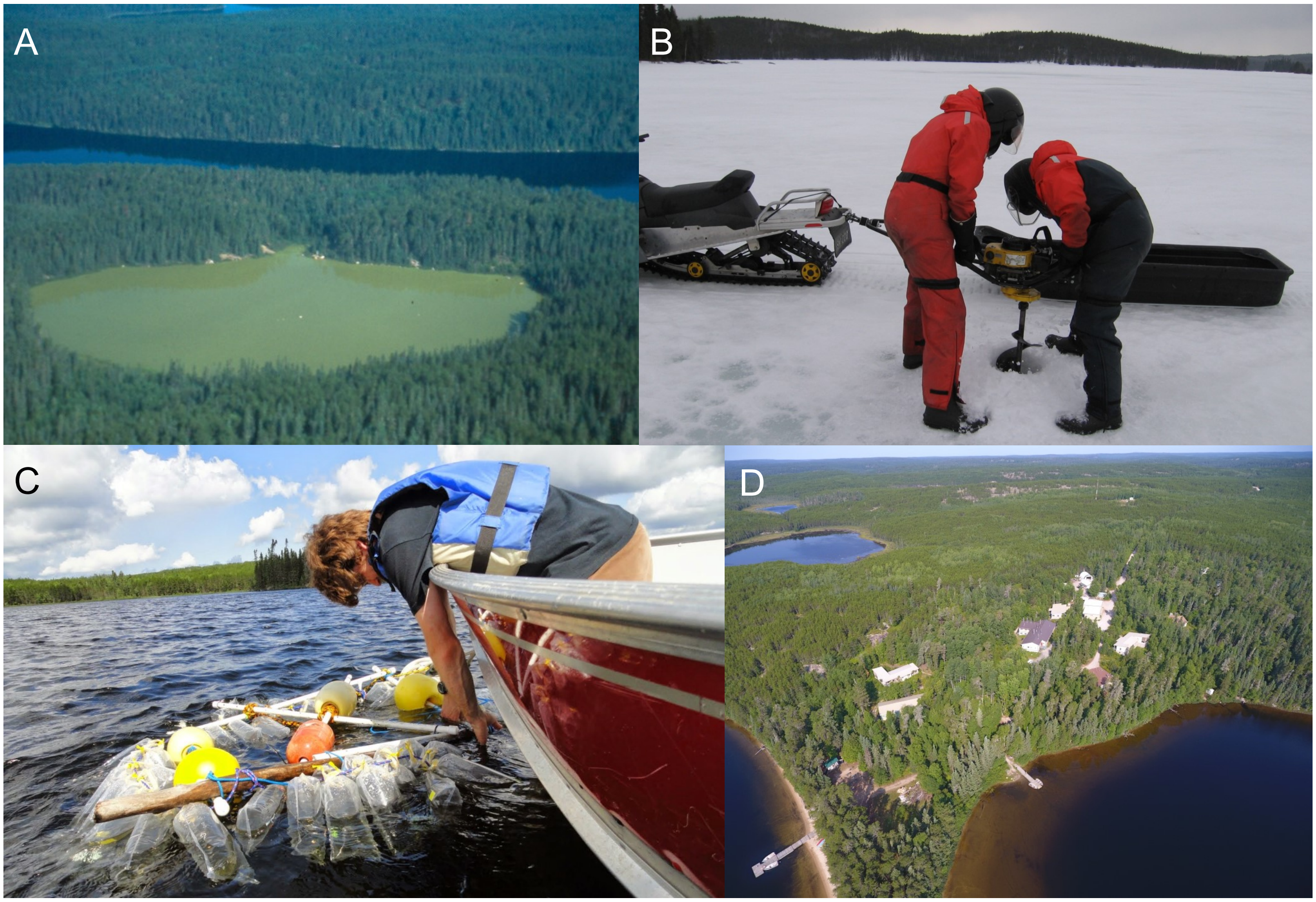
About the location. The IISD-Experimental Lakes Area (ELA) is in a remote part of northwestern Ontario, Canada that contains hundreds of small pristine lakes largely unaffected by human activity. The area is typified by classical boreal forest on the Precambrian Shield with thin soils, low nutrient exports, and naturally oligotrophic and soft water lakes. Fifty-eight of the lakes in the area have been set aside by special legislation for the purpose of whole-ecosystem experimentation to explore the effects of human activities, with the goal of providing solutions for the effects of those activities. ELA researchers also regularly monitor a series of unmanipulated lakes and streams and have accumulated more than 50 years of data on meteorology, hydrology, water chemistry, primary producers, invertebrates, and fish. Created in 1968, ELA was operated by the federal government of Canada until its transfer to the International Institute for Sustainable Development in 2014. ELA is now an independent charitable organization that continues to pursue world-class limnological research involving extensive collaborations with academic institutions, government agencies, industry, and other NGOs. Since its inception, ELA has conducted a wide range of research on ecological stoichiometry with more than 90 papers and theses produced on the topic. **Click here for a full list of IISD-ELA publications.**

Stoichiometric history and key contributions. ELA was originally created to study cultural eutrophication using whole-lake experiments, primarily through manipulations of loadings of C, N, and P. These studies were led by David Schindler and showed that algal biomass is primarily determined by P, but the algal community composition is strongly affected by N:P and, to a lesser extent, C:P availability. Manipulations of N and P using different loading ratios demonstrated that deficiencies in N could be largely overcome through increases in N-fixation. One study of artificial fertilization in Lake 227 using different N:P treatments has now continued for 53 years. Two new whole-ecosystem manipulations began in 2019 and explored the hypothesis that sediment redox conditions and ferrous iron availability are important determinants of cyanobacterial abundance in conjunction with P.

In the 1990's, Jim Elser and Bob Sterner along with a large group of collaborators undertook a series of observational, experimental, and whole-lake manipulations to better understand stoichiometric relationships in lake foodwebs. They found that the introduction of piscivorous pike to eutrophic Lake 227 led to reduced densities of minnows and increased *Daphnia* abundance, which altered nutrient cycling and subsequently contributed to decreasing algal abundance and cyanobacterial dominance. At the same time, introduction of pike to oligotrophic Lake 110 failed to generate a trophic cascade, apparently because high C:P ratios of phytoplankton prevented the proliferation of *Daphnia*.

The IISD-ELA is located in northwestern Ontario in the middle of Canada.

These experiments built on early stoichiometric studies at ELA by Patrick Healey, Len Hendzel, Bob Hecky, and others that pioneered the use of nutrient ratios to assess algal nutrient status. Using data from lakes in northwestern Ontario that included ELA, Bob Sterner and coworkers developed the light:nutrient hypothesis to explain variations in particulate C:P ratios. The light:nutrient hypothesis was further supported by mesocosm experiments led by Jim Elser and Jotaro Urabe in Lake 239 at ELA that found lower light intensities could reduce seston C:P ratios and improve food quality for zooplankton.



Clockwise from upper left: A) Lake 227– the location of a 50+ year fertilization experiment. B) Winter sampling of water under thick lake ice. C) Deploying a boassay experiment to test phytoplankton responses to nutrient additions. D) Birdseye view of ELA camp next to Lakes 239 (bottom right) and 240 (bottom left).

Continued from page 5. Stoichiometric work at ELA has also included research on littoral and benthic communities. For example, Paul Frost found especially high C:P ratios in periphyton in ELA lakes, which likely constrained the growth of littoral mayflies. Earlier work by Michael Turner found that low dissolved inorganic carbon:nutrient ratios in experimentally acidified lakes likely contributed to C-limitation of attached algae, lower periphyton C:P ratios, and the proliferation of filamentous algal mats.

The tradition of stoichiometric work continues at ELA, mostly led by graduate students. Some recent examples include Ceara Talbot who explored the effect of experimental flooding on N:P cycling, Joey Tonin, who studied the effects on zooplankton of variations in C:P ratios in deep algal peaks, and Sandra Klemet-N'guessan, who will be considering factors affecting N:P recycling by fish as part of her Ph.D. studies at ELA. To learn more about the IISD-ELA, including how to support their mission and research, visit their website at: **https://www.iisd.org/ela/**.

Contributed by Michael Paterson and Paul Frost

Selected Stoichiometric Publications from the IISD-ELA

Elser, J.J., T.H. Chrzanowski, R.W. Sterner & K.H. Mills. 1998. Stoichiometric constraints on food-web dynamics: A whole-lake experiment on the Canadian Shield. Ecosystems 1:120-136.

Elser, J.J., R.W. Sterner, et al. 2000. Pelagic C:N:P stoichiometry in a eutrophied lake: responses to a whole-lake food web manipulation. Ecosystems 3:293-307.

Frost, P.C. & J.J. Elser. 2002a. Effects of light and nutrients on the net accumulation and elemental composition of epilithon in boreral lakes. Freshwater Biology 47:173-183.

Frost, P.C. & J.J. Elser. 2002b. Growth responses of littoral mayflies to the phosphorus content of their food. Ecology Letters **5**:232-240.

Healey, F.P. & L.L. Hendzel. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Canadian Journal of Fisheries and Aquatic Sciences 37:442-453.

Hecky, R.E., P. Campbell & L.L. Hendzel. 1993. The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnology and Oceanography 38:709-724.

Molot, L.A., S.B. Watson, et. al. 2014. A novel model for cyanobacteria bloom formation: the critical role of anoxia and ferrous iron. Freshwater Biology 59:1323-1340.

Schindler, D.W. 1977. Evolution of phosphorus limitation in lakes. Science 195:260-262.

Schindler, D.W., R. E. Hecky, et. al. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proceedings of the National Academy of Sciences USA 105:11254-11258.

Schindler, D.W. 2012. The dilemma of controlling cultural eutrophication of lakes. Proceedings of the Royal Society B: Biological Sciences 279:4322-4333.

Sterner, R.W., J.J. Elser, E.J. Fee, S.J. Guildford & T.H. Chrzanowski. 1997. The light:nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. American Naturalist 150:663-684

Turner, M.A., E.T. Howell, et al. 1994. Roles of nutrients in controlling growth of epilithon in oligotrophic lakes of low alkalinity. Canadian Journal of Fisheries and Aquatic Sciences 51:2784-2793.

Urabe, J., M. Kyle, W. Makino, T. Yoshido, T. Andersen & J.J. Elser. 2002. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology 83:619-627.

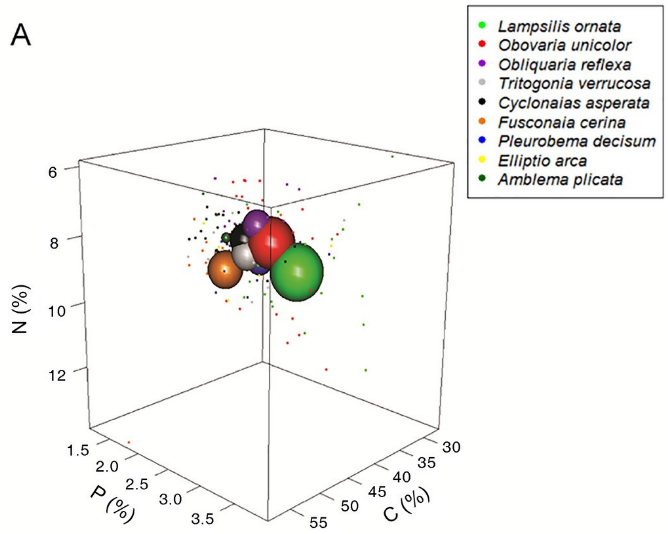


Fig. 3a from Atkinson et al. (2020). The stoichiometric niches of the nine species used in the stoichiometric niche analysis. Sphere size indicates the relative stoichiometric niche volume and is centered around the average C, N, and P contents of each species.

Which came first: the ratio or the niche?

When it comes to determining ecosystem function, functional diversity has been demonstrated to be a more robust measure than species richness alone. Used in conjunction with phylogenetic measures of diversity, this approach conceptually links evolutionary and ecological processes by looking at the evolution of functional traits within a guild. Not all functional traits are born equal, however, and thus far, trait-based approaches that consider ecosystem functions relevant to both community members and ecological processes are few and far between.

Enter the stoichiometrists! For years we have been quantifying traits that directly affect community members through shifts in nutrient availability, have predictable impacts on the key ecosystem process of nutrient cycling, and explicitly link the flow of nutrients through a system with the species present within that system.

Although stoichiometric traits have previously been linked to taxonomy at a family level, Atkinson et al. take this further by looking at stoichiometric niches (body C:N:P variation) within a single, closely-related feeding guild of filter-feeding freshwater mussels from the subfamily Ambleminae. Despite occupying very similar feeding niches, the authors found that the nine species each had clearly defined stoichiometric niches (Fig. 1). Furthermore, evolutionary history played a key role in niche variation, demonstrating a potential causal link between evolutionary history and niche partitioning.

As well as casting an interesting light on the link between evolutionary and ecosystem processes, these findings highlight the importance of understanding stoichiometric variation in closely-related species.

From the Paper: "*Species within the same feeding guild or phylogenetic grouping may differ dramatically in their traits and ecological functions, and ignoring these nuances may lead to poor evaluations of the ecosystem services and functions provided by organisms occupying similar functional guilds.*"

Contributed by Catriona Jones

Atkinson, C.L., B.C. van Ee and J.M. Pfeiffer. 2020. Evolutionary history drives aspects of stoichiometric niche variation and functional effects within a guild. Ecology 101(9):e03100. 10.1002/ecy.3100

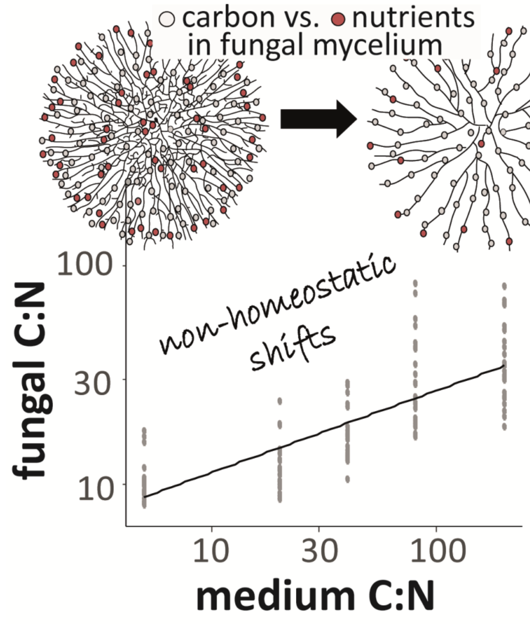
Profiles in Stoichiometry

Nine Questions for Tessa Camenzind

Tell us about your background and scientific journey. How did this lead to your interest in ecological stoichiometry? While I worked my entire “scientific life” on nutrient deposition, limitations, and its effects on fungal communities and activity in different ecosystems, at some point the question arose: what amounts and proportions of elements do fungi actually need? I realized that our ecosystem scale analyses do not provide a satisfactory answer and surprisingly we also lack physiological studies. So after a former collaborator, Alexander Tischer (University of Jena), explained to me every possible process in soil by this magic but unpronounceable concept of stoichiometry, I started reading into it and realized that this is the perfect tool. And here I am, working on fungal stoichiometry as a postdoc at the Plant Ecology lab at Freie Universität Berlin.

What challenges have you faced in your work and how did you overcome them? Fungi have their own will and have never followed my expectations, neither in the field nor in the lab. But when I opened my mind, I realized that the unexpected responses were often the most exciting scientific findings. The variable C:N ratio was in fact not a flaw of the experimental design, but a very exciting sign of non-homeostasis. Also, growth traits never nicely correlated as expected, but turned out to have explanatory power when observed in trait-space. There were also many practical hurdles to overcome including microbial contamination, the difficulty to quantify fungi precisely in soils (not yet overcome), and the struggle to create control media without unwanted element traces (this challenge is quite difficult as pure media are very time- and money-consuming).

What is your favorite element and why? I told my son (age 12) what a funny question that is, but he said, “Easy, aurum (Au)”. Good point - scientifically, though, I would answer my favorite is really C, which is not only making ~45% of fungi but also combines ecosystem processes at so many levels from the atmosphere to deep soil storage. My least favorite is Na, which kept me busy several years. It turns out that the microbes I looked at couldn´t care less about this element.



Relationship between fungal C:N and medium C:N ratios grown under different medium N concentrations. For the full story, see Camenzind (in press) Ecology Letters.

Please tell us a little about your most recent research/manuscripts. Following the question of what exactly are the nutritional demands of fungi, we conducted laboratory experiments with saprobic fungi along C, N, and P gradients. Using a special growth medium based on Liebig´s Law and stoichiometric principles, the results showed that fungal nutrient demands are tightly coupled to the special strategy of mycelial growth. Under nutrient limitation, most isolates showed high extension rates, which we interpret as a switch to exploration under unfavorable conditions. In parallel, mycelia vary C:nutrient ratios much more than expected; a mechanism (amongst others) related to internal nutrient translocation and recycling mechanisms that characterize fungal growth.

What are the advantages of using fungi to study ecological stoichiometry? I would rather say I see ES as an advantage to study fungi and am still pushing forward to establish fungal C:N:P as a trait in fungal ecology. The resulting knowledge, however, may be used to better understand ES and dynamics observed in soil. Saprobic fungi act like catalysts of litter decomposition with their enzymatic capacity and an extremely efficient uptake and exploration strategy. Thus, I hope better insights into fungal stoichiometry and nutrient foraging strategies will also improve our understanding of principles of ES.

Do you have any suggestions on how we can diversify current stoichiometric/ecological research? I am relatively new to this field, but clearly the strength of ecological stoichiometry is to break down complex processes into simple formulas, which then can be implemented in carbon and nutrient cycling models. In my opinion, especially in soil more research is needed to validate and refine these simple formulas experimentally. From the perspective of a microbial ecologist, the biggest challenge is not only to generate new data using microbial isolates, but also to converge existing knowledge at the organismic and ecosystem level by interdisciplinary collaborations.

What are your plans for stoichiometric research in the future? As mentioned, the big challenge now is to transfer the gained knowledge from the organismal level to a soil system. Analyses in more complex media show that simple findings under controlled conditions do not hold in complex “real world situations”. Thus, the next step will be continuing these analyses with more complex C sources, heterogeneous resource environments, fungal communities and also other elements. Apart from these necessary next steps, I am open to suggestions and offers.

Interview by Francis Q. Brearley

Contact us by email:
ratiosmatter@gmail.com

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Selected Recent Stoichiometry Publications

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