# Ratios Matter

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## Frequently Asked Questions

### How do you measure C:N:P ratios of soil microorganisms?


**Robert Buchkowski**
Western Ontario University


**Measuring the stoichiometry** of soil microorganisms predates the publication of Sterner & Elser's book. Unlike many organisms where entire individuals or organs can be isolated for analysis, measuring the stoichiometric ratios of soil microorganisms is not direct or straightforward. Soil microorganisms growing outside of laboratory cultures are practically inseparable from their environment for the purpose of measuring stoichiometric ratios. The reason is that measuring stoichiometry of organisms requires relatively large amounts of material that represent millions of individual microbial cells. Except for fungal hyphae, which can be physically picked from soil or decaying wood, other soil microorganisms can only be removed from soil one-by-one making the task of collecting enough of this material for analysis almost impossible.

**The solution to measuring soil microbial stoichiometry** is to conduct paired chemical extractions of the soil that include and exclude the contents of microbial cells. The method was developed over a series of publications in the late 20$^{th}$ and early 21$^{st}$ centuries and involves shaking soil with potassium sulfate to extract C, N, and P[1]. One set of paired samples receives a dose of chloroform to lyse the microbial cells so that the difference between these paired extractions is the quantity of each element present in the microbial cells.

**An obvious limitation** of this method is that we are measuring the stoichiometry of the entire microbial community. The method would be analogous to measuring the average stoichiometry of all fish in a lake or all trees in a forest stand. Clearly, this methodological constraint limits the stoichiometric questions that can be addressed. When we interpret these data, we cannot tell the extent to which differences in the composition of the microbial community, or differences in the supply of chemical elements, explain any trends in stoichiometry across ecosystems or treatments. If you've got the resources, DNA sequencing might help sort out whether the community is changing and provide correlative data on community composition and C:N:P ratios. *Continued on page 2.*

**Frequently Asked Questions**

In this new section of *Ratios Matter*, we "answer" some **frequently asked questions** that are often posed to the ecological stoichiometry community. Our goal is to help elemental researchers who would like to measure, model, or understand something stoichiometric in an area that is new to them. Do you have a **FAQ** that you would like answered? Send it our way at: **ratiosmatter@gmail.com**

## New Associate Editors

We are pleased to announce the addition of two new Associate Editors to the *Ratios Matter* editorial team

**Angélica González** is an Associate Professor at Rutgers University where she studies how the availability, storage and transfer of energy and matter affects biodiversity, trophic interactions and ecosystem processes.

**Helena Klip** recently moved to the University of Montana where she is a Postdoctoral Research Associate examining snow algal dynamics on glaciers.

*Continued from page 1.* **Another limitation** of this method is that the chloroform extraction does not work perfectly. Laboratory tests indicate that it only removes 45% of C, 45% of N, and 40% of P in microbial biomass[2]. Furthermore, residual chloroform adds a small amount of carbon back into the fumigated sample without adding nitrogen or phosphorus. This limitation is especially relevant to work on stoichiometry, because the measured C:N:P ratio of the microbial biomass is dependent on both the measured differences between the elements in paired samples and the accuracy of these 'average' extraction efficiency parameters for the soil being studied. For stoichiometric applications, a consistent error across elements in extraction efficiency would be less problematic than a systematic difference that affects the ratios. As far as we know, data on the consistency of extraction efficiencies across elements and soil types are scarce.

**Currently, chloroform fumigation is the only method** to measure the stoichiometry of microbial biomass in soil systems (as far as we know). Other methods have been developed to measure microbial biomass carbon content, but these are not reported to measure nitrogen or phosphorus. Recent papers have reaffirmed the accuracy of chloroform fumigation as an accurate measure of microbial carbon biomass, but the validity of stoichiometric measurements has not been assessed for about a decade. We recommend that stoichiometric interpretations of microbial C:N:P ratios in soil should acknowledge these limitations and interpret differences coarsely.

[1]See **Cleveland and Liptzin. 2007**. Biogeochemistry 85:235 for a review and the papers they cite from D.S. Jenkinson and P.C. Bookes, who first reported on the method.

[2]**Jenkinson et al. 2004.** Soil Biology and Biochemistry 36: 5-7

## Conference Watch: **Ecological Society of America 2023**

**Are you attending ESA in Portland this year?** There are some 200+ presentations that include nutrients, stoichiometry, and/or both. Here is a selection of these exciting stoichiometry-centric organized sessions, symposiums, & presentations that are worth checking out.

<u>Monday, August 7</u>
**Title:** Not just nitrogen and phosphorus: other nutrients also structure a coastal tallgrass prairie plant community
**Presenter:** Chelse Prather (Univeristy of Dayton)
**Session & Time:** COS16-2 @ 13:45-14:00

**Title**: Effects of selective grazing on the response of phytoplankton stoichiometry to temperature
**Presenter**: David M. Anderson (University of British Columbia)
**Session & Time**: COS 12-3 at 14:00-14:15

**Title**: Understanding anthropogenic impacts on zoogeochemistry is essential for ecological restoration – A case study from the Kalahari
**Presenter**: Andrew J. Abraham (Aarhus University)
**Session & Time**: OOS 3-4 at 14:15-14:30

**Title**: Nitrogen addition and temperature shifts alter the chemical composition, thermodynamics, and stoichiometry of the DOM pool in Sphagnum peat
**Presenter**: Bram W. Stone (Pacific Northwest National Laboratory)
**Session & Time**: COS 5-5 at 14:30-14:45

<u>Tuesday, August 8</u>
**Symposium:** Zoogeochemistry as a Throughline in Ecology, and for a Changing World
**Session & Time:** SYMP 5 at 8:00-9:30

**Title**: Keystone molecules in biogeochemical and ecological systems
**Presenter**: Manuel T. Lerdau (University of Virginia)
**Session & Time**: COS 52-3 at 8:30-8:45

**Title**: Consistent stream seston carbon:nitrogen stoichiometry at the continental scale
**Presenter**: David. W. P. Manning (University of Nebraska)
**Session & Time**: COS 94-2 @ 13:45-14:00

<u>Wednesday, August 9</u>
**Organized Oral Session**: Approaches to Studying Zoogeochemistry
**Session & Time**: OOS 46 at 15:30-17:00

<u>Thursday, August 10</u>
**Title**: Agnostic fungi: herbaceous plant traits explain nitrogen transfer in common arbuscular mycorrhizal networks of Pacific Northwest grasslands
**Presenter**: Hilary Rose Dawson (University of Oregon)
**Session & Time**: COS 275-3 @ 14:00-14:15

Visit **https://esa.org/portland2023/** for more information on this meeting.

# Ubiquitous reactive **nitrogen** excess in human-impacted streams

**Daniel Graeber and Alexander Wachholz**

Helmholtz-Centre for Environmental Research (UFZ), Germany

**Ecological stoichiometry** provides a powerful lens through which we can understand the complex interplay between life and the environment. It's not just individual organisms that can be better understood through this lens, but entire ecosystems and landscapes. Such a large-scale perspective is at the heart of our study, Wachholz et al. 2023. We employed a **novel analytic approach** based on ternary plots of reactive C:N:P (Figure 1). This innovative method allows for clear visualization of nutrient depletion and, importantly, co-depletion. It's like having a new map that reveals previously unseen patterns and relationships. For this analysis, we used data from 574 German streams to calculate the ratios of reactive forms of dissolved organic carbon, dissolved nitrogen, and dissolved phosphorus.

**Our research found a strong imbalance,** with stream reactive C:N:P ratios tending towards nitrogen excess, largely due to nitrogen inputs from human activities. This imbalance wasn't confined to agricultural areas; it's a widespread issue affecting 94% of the assessed German streams and their catchments (Figure 1). **Excess nitrogen** overwhelms the capacity of soils and streams to self-regulate as shown by earlier laboratory and catchment studies that found high ratios of reactive nitrogen relative to organic carbon and phosphorus hindered denitrification and assimilation of nitrogen into biomass.

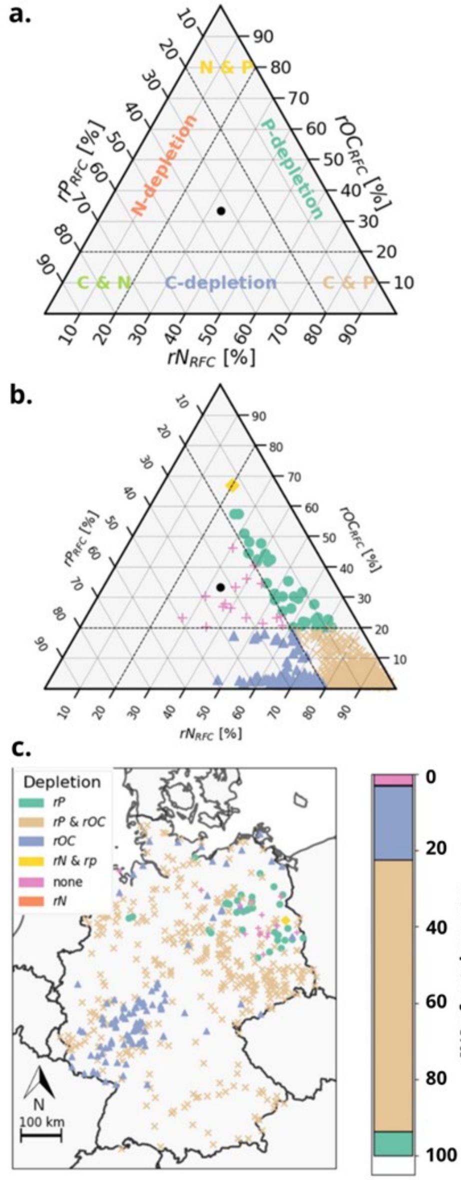



Figure 1. (a) Classification in the ternary plot. The data is normalized by the Redfield ratio (C:N:P 106:16:1), which equals the center dot. (b) The distribution of stream C:N:P is strongly skewed to towards reactive P (rP) or rP and reactive organic C (rOC) co-depletion and reactive N (rN) excess, with (c) spatial commonalities of C:N:P, mainly generated by local soil wetness (i.e., wetlands), and occurrence of sedimentary aquifers.

**Elemental imbalances extended** beyond the individual streams assessed in the study and can also impact downstream water bodies, including lakes and marine ecosystems. Recent lake research implies that excessive relative inputs of reactive N are as important as reactive P for eutrophication, linked to detrimental effects like toxic algal blooms. Moreover, the depletion of reactive organic carbon influences the competition for nutrients between heterotrophs and autotrophs, shifting ecosystems towards dominance by autotrophs (e.g., phytoplankton) with potential cascading effects on aquatic food webs and the carbon cycle.

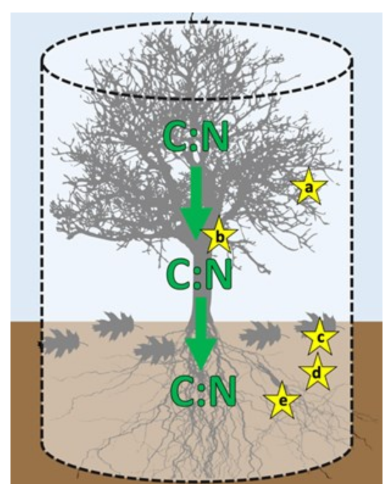
**Our work is a stark reminder** of how our actions ripple through the environment, altering the natural balance and affecting the intricate web of life in our water bodies. We hope that our work will inspire further research and, more importantly, help to develop a stoichiometric perspective on sustainable management practices to mitigate the impact of human activities on the environment and maintain the ecological integrity of aquatic systems.

**Wachholz, A., Dehaspe, J., Ebeling, P., Kumar, R., Musolff, A., Saavedra, F., Winter, C., Yang, S., Graeber, D., 2023. Stoichiometry on the edge - Humans induce strong imbalances of reactive C:N:P ratios in streams. Environmental Research Letters 18, https://doi.org/10.1088/1748-9326/acc3b1**

# INCyTE Seeking Stoichiometric Observatory Participants

**Are you a stoichiometry enthusiast** who works on terrestrial ecosystems? Would you like to be part of developing and utilizing a global stoichiometry dataset? The INCyTE (Investigating Nutrient Cycling in Terrestrial Ecosystems) Network is excited to announce plans for a coordinated distributed sampling this summer and is looking for participants. The goal of the sampling campaign is to collect paired tree core, foliar, and soil samples to examine patterns of C:N ratios in trees across ecosystems around the globe.

- Minimum participation includes collecting soil, foliar, and tree core samples from a total of 9 trees.
- INCyTE will cover analytical costs for samples collected from up to 9 trees.
- Participants will help develop a C:N database and possibly one of the largest tree core stoichiometry datasets known.
- Participants will be part of a global network of collaborators and invited to participate in related events and publications.




Additional information and sampling protocols can be found on the INCyTE website: https://www.umt.edu/incyte/

# Interview with a Stoichiometrist: Patrick Thomas

**Kimberley Lemmen**
University of Zurich

**Preface.** *Ratios Matter is pleased to feature a new type of article, Interview with a Stoichiometrist, where authors tell us about one of their recent publications. In this first installment, Kimberley Lemmen "sits down" with Patrick Thomas (*EAWAG, Switzerland*) to discuss:*

**Thomas, PK, C Kunze, DB Van de Waal, H Hillebrand, & M Striebel. 2022.** Elemental and biochemical nutrient limitation of zooplankton: A meta-analysis**. *Ecology Letters 25*: 2776-2792**

**Last fall an exciting meta-analysis** caught the eye of the stoichiometry community. This study examined the effects of elemental and biochemical limitation of primary producers on consumers. The authors drove into the complexities of resource limitation, examining the effects of individual and co-limitation as well as both their direct and indirect impacts on consumers. I sat down with the lead author of the study, **Patrick Thomas**, to discuss how he entered the world of stoichiometry and to dive deeper into the findings of this paper.

Patrick is a **BIG** fan of algae. During his undergrad, he quickly became obsessed with the idea that farming microalgae could help with some of our big climate/energy/food security problems. He pursued this infatuation by doing a MSc that dabbled in stoichiometry by testing whether algal food quality in terms of elemental ratios would influence grazing rates by zooplankton. Patrick then moved to Germany for his PhD and joined the lab of Maren Striebel and Helmut Hillebrand, where the allure of stoichiometry was inescapable. Patrick jumped to the rank of fully-fledged stoichiometry nerd at the Woodstoich IV workshop in 2019. Since 2022, he's been a postdoc at the Swiss Federal Institute of Aquatic Science and Technology (EAWAG) in the group of Anita Narwani. While his favorite real element is Si, given its role in diatom ecology, he is quick to tell me that his favorite fictional element is Dilithium, for its role in the functioning of warp drives (obviously). Below is our chat about his recent publication.

**KL: Is there a story behind the main research question?**
PT: This is a long story that goes back to the 1990's. There was a dialogue about whether limitation by elements or by other essential nutrients, like fatty acids, was more influential to the aquatic producer-consumer link (and, to what extent their effects are interactive). Fast forward a few decades, and there was plenty more data to address such questions about the determinants of food quality with a meta-analysis.

**KL: What was the biggest surprise to come out of this research project?**
PT: The biggest surprise was how few studies out there integrate both stoichiometric and biochemical food quality. The subset of studies that have a look at both, though, makes it very clear that algal food quality and its effects on food webs can only be best evaluated when you assess both potentially limiting elements and biomolecules.

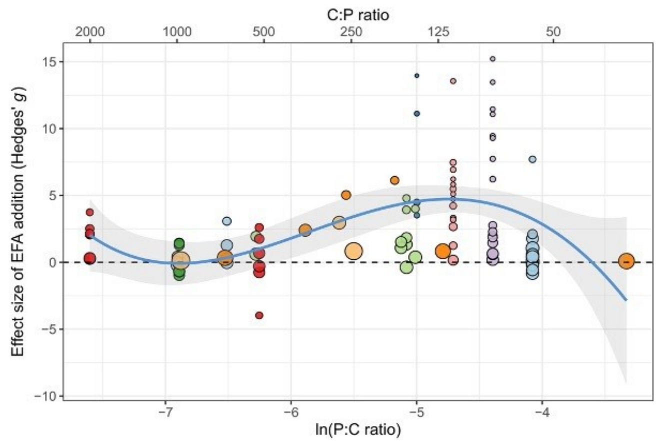



*Figure 1. Relationship between variation in ln-transformed P:C ratio and the effect size of FA addition. The size of each point indicates the reliability of each effect size (i.e. the weighting in the form of inverse variance), and colors indicate different studies. From Thomas et al. (2022)*

**KL: What would you like readers to take away after reading this paper?**
PT: We all know that extremely high seston C:P ratios cause problems for zooplankton. However, at somewhat lower C:P levels, there are a handful of molecules that can also make life hard for consumers (Figure 1), at which point stoichiometry might take a backseat. The beauty of stoichiometry is in its simple elegance, but inviting a few essential biomolecule friends to the party is a nice addition that, in my opinion, still keeps things simple and elegant while also painting a more complete picture of the world.

**KL: What is the importance of this work to ecological stoichiometry?**
PT: Our synthesis is a reminder that our favorite lab rat strains may behave very differently from diverse natural communities. Poor-quality monocultures can show the extremes of stoichiometric constraints, but there is much less work explicitly testing how zooplankton are constrained by nutrient ratios in natural communities. Beyond this, we also show where other gaps are (e.g. in taxonomy), so hopefully, this can help in guiding future research directions.

**KL: Is there anything else you would like to include?**
PT: To be clear, we chose to focus only on "controlled" studies where food quantity effects could be ruled out and thus not be a confounding factor. There is also a whole world of data with field/mesocosm studies which we did not include that could give more insights through additional synthesis work.

In his current project, Patrick is focusing on algal exudates, so although stoichiometry is less of a focus, it is always in the back of his mind. For example, he is currently thinking in terms of applied research and how we could decrease the N and P demands for algal biofuels. He tells me that certain algal species excrete hydrocarbons which can be easily harvested without killing cells (called "algae milking"); if this is successful, harvesting hydrocarbons (molecules with very high C:P ratios) should be helpful to overcome stoichiometric limitations to producing sustainable biofuels. In the future, Patrick thinks that the field of ecological stoichiometry could do a better job of integrating theory with engineering and industrial/agricultural applications and communicating this across the disciplines. There are still too many papers, news articles, and talks describing how algae grow on "only sunlight and $CO_2$". He thinks that growing microalgae for carbon capture, biodegradable polymer production, animal feed, and biofuels will help us out eventually, but we need to be lucid about the nutrient demands of each of these applications and the trade-offs involved in deciding where we allocate limited P supplies.

# Sources or Sinks? A detrital stoichiometry ***k*-onundrum**

**Clay Prater**
University of Arkansas

**Organic matter decomposition** plays a key role in the elemental dynamics of ecosystems around the world. Yet, this process has traditionally been studied using only two measurements of total mass loss (i.e., beginning and end of experiment) and modeled using the first-order decay coefficient k. This means that fine-scale temporal changes in detrital elemental stoichiometry and its sink-source elemental dynamics have received much less attention.

**A new study, Robbins et al. (2023),** addresses this issue by compiling data from published studies of mass loss and stoichiometric time-series of detrital decomposition in stream ecosystems. Detritus was a relatively steady net source of N through time, whereas detrital P dynamics were "wigglier" and decomposing leaves switched from being a P sink to a P source after the loss of ~40% of detrital mass. Perhaps the coolest revelation, though, was the finding that C:N and C:P ratios converged over time with detrital biomass breakdown coinciding with increased fungal biomass (Fig. 1). This pattern is strikingly similar to those of microbes building biomass (Hillebrand et al. 2013. Limnol. Oceanogr. 58: 2076-2088) and shows that whether they are giving or taking away, microbes rule stoichiometric cycles!

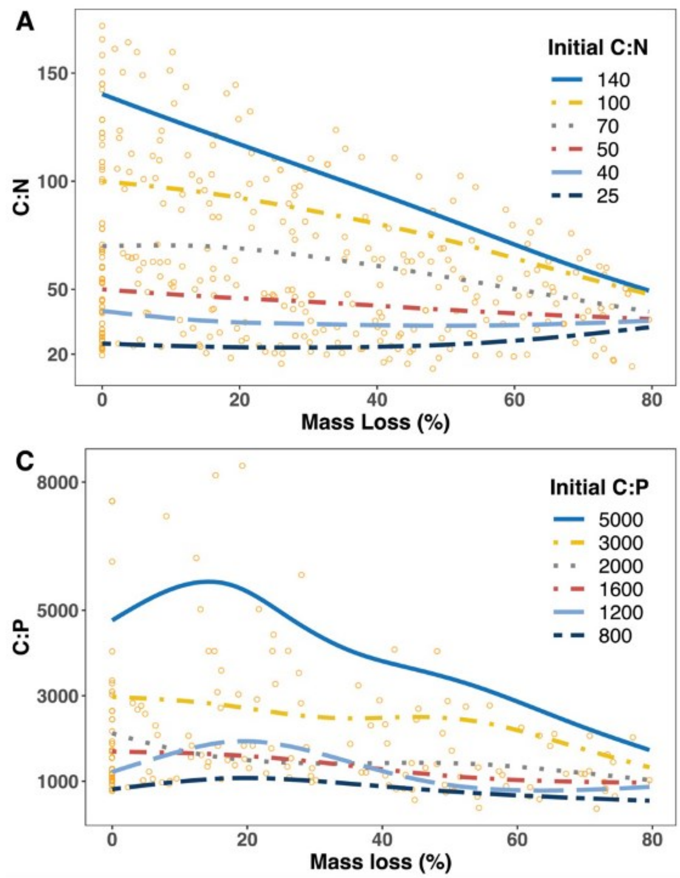



Figure 1. Predictions of detrital C:N (A) and C:P (C) as a function of mass loss for litters of different initial stoichiometric composition.

**From the paper:** *"Overall, we advocate for more temporally explicit studies of litter nutrient decomposition to provide insights into mechanisms of litter decomposition."*

**Robbins, C.J., Manning, D.W.P, Halvorson, H.M., Norman, B.C., Eckert, R.A., Pastor, A., Dodd, A.K., Jabiol, J., Bastias, E., Gossiax, A., & Mehring, A.S., Nutrient stoichiometry dynamics of decomposing litter in stream ecosystems: A global synthesis. 2023. Ecology, e4060. https://doi.org/10.1002/ecy.4060**

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# DID YOU KNOW? N:P Stoichiometry and Tomatoes

**One place you will see N:P ratios available** for public consumption are on bags of commercially available fertilizers at your local hardware store. Many of the bags have a "balanced ratio" of N:P:K of 10:10:10 by mass. This might seem balanced until you realize that this is a very low N:P molar ratio of 2.2. Interestingly, other N:P:K ratios are available to purchase either with greater amounts of P (hard to believe) or with P entirely missing.

Maybe ratios matter? The N:P ratio of your fertilizer is thought to affect the relative growth of vegetative vs. fruiting tissues in plants. This N:P story seems to hold water especially with the tomato-growing crowd. For tomatoes, high N:P ratios may result in excessive vegetative growth and reduced yield of fruit. In contrast, low N:P ratios, such as the molar N:P ratio of 1.5 seen on the bag of fertilizer in the photo, supposedly lead to greater tomato fruit production. Unsubstantiated reports from the internet back this up. For example, the commenter, **Fusion_power**, posted this comment to a discussion board found on the website, tomatoville.com:


"*Mature plants need a fairly specific nutrient balance because tomatoes can over absorb nitrogen leading to either chlorotic growing tips or excessive shoot growth without setting fruit. The ratio that works best here in my climate is 8:24:16 (N-P-K) or as close to that ratio (1:3:2) as you can get. Why this ratio? I live in a high rainfall area. Both nitrogen and potassium are fairly mobile in the soil and will leach away pretty fast...*".

In other words, add a bunch of P to your tomatoes to produce fruit.

**The N:P ratio of the mature tomato fruit** has been reported to be ~11-12 (by mol), which does make it a relatively P-rich plant tissue (see Juárez-Maldonado et al. 2017). While it is comforting that there is empirical support for the "tomato need lots of P to grow fruit" idea, there are many related stoichiometric questions about the relative supply of N and P in soil, the tomato's ability to acquire each element, and how the plant allocates these two elements to leaves vs. fruit.

So if you want to juice up your tomato yield, you might try grabbing a bag of phosphorus. However, please add fertilizers to your garden responsibly because you could end up helping grow a lot more than just tomatoes (like algae somewhere downstream)!

**Juárez-Maldonado et al. 2017. Macro-nutrient uptake dynamics in greenhouse tomato crop. Journal of Plant Nutrition 40: 1908-1919.**