Characterization of methodological variability in compound specific isotope analysis of amino acids (CSIA-AA)

PhD Seminar

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Compound specific stable isotope analysis of amino acids (CSIA-AA) has paved the way for detailed quantification of macronutrient flow within individuals, among species, and throughout ecosystems. The approach has opened doors for the study of complex environmental relationships by aiding in the identification and characterization of organismal physiology, consumer-resource linkages, movement ecology, and more. Such revelations sparked a rapid increase in CSIA-AA research publications in the last few decades. This has naturally been accompanied by innovations to its underlying methods. Since its conception, several distinct analytical methods have been used to obtain amino acid isotope data, yet to date, the extent to which methodological differences impact CSIA-AA data has not been extensively studied. Following a meta-analysis of application-based CSIA-AA literature from 1990-2022, we discovered that the largest source of variance is the derivatization procedure, with the method employed seeming to be rooted in the location of the laboratory performing the study rather than the subject of the study. Thus, there exists a potential mismatch between the laboratory methods employed and those optimal for a given analysis. We also found that trifluoroacetic anhydride (TFAA) derivatization procedures were most common, though its use is highly localized to the Americas. Using the meta-analysis, we are developing a framework to identify areas that have the largest impact on data quality.

Given that TFAA is the most common derivatization method, we looked at variance within this procedure in depth, finding that as much as a 100% difference in experimental parameters existed within published variants of the method. To determine the extent to which these differences impact isotope data, we orthogonally tested ranges of esterification reaction times (60-120 min), acylation reaction times (10-20 min), and temperatures of each reaction (90-110°C) seen in literature, and their δ 13 C values were measured via GC-C-IRMS in quintuplicate. We found that different experimental parameters resulted in different δ 13 C results even post data correction. Notably, as the esterification reaction time increased, the range of δ 13 C values obtained increased, while as esterification temperature increased, the range of δ 13 C values decreased. With differences within a single technique causing significant changes in the isotope value, the overall lack of standard technique in the field as a whole could have even further consequences. As such, further studies into the impact of methodological variance on isotope data should be conducted.