MICROBIAL METABOLIC FLUX ANALYSIS USING POSITION-SPECIFIC 13C-LABELLED GLUCOSE AND FRAGMENT 13C ANALYSIS OF PLFA

W. Wu¹, P. Dijkstra², M. A. Dippold¹

¹Georg-August-University of Goettingen, Germany, ²Northern Arizona University, USA

Isotope labelling experiments with position-specific 13C labelled substrates (e.g., sugar and amino acids) can reveal the dominant utilization pathway of organic matter by microbes (Dippold et al., 2013; Apostel et al., 2015) while microbial 13CO2 production can be used to estimate intercellular metabolic flux patterns (Dijkstra et al., 2011). Additionally, the 13C incorporation into microbial components of living cells e.g., biomarkers such as phospholipid derived fatty acids (PLFA) can provide insights into carbon utilization pathways and biomass formation for individual groups of microbes (Bore et al., 2017; Bore et al., 2019). 13C-PLFA data can also be utilized for metabolic flux modelling: the precursor of PLFAs, acetyl-CoA, can be linked to the flux pattern of the central C metabolic network (glycolysis, pentose phosphate pathway (PPP), tricarboxylic acid cycle (TCA)). Therefore, we tested, for the first time, whether modelling of metabolic fluxes is possible based on the 13C incorporation from substrates in the carboxylic group of the PLFA fragment, assuming that this group is directly derived from acetyl-CoA.

We developed a method to analyze the 13C abundance of three fragments of PLFA including the carboxyl, the propionate fragments and the molecule ions by conventional electron impact-gas chromatography-mass spectrometry (EI-GC-MS) by selective ion mode to analyze mass isotopomer distribution pattern. The artificial position-specific 13C labelled palmitate standards showed good agreement between theoretical and experimental estimates with a deviation of < 0.05 mol % 13C. This analysis method was utilized to estimate the fragment 13C abundance of PLFA produced by pure bacteria strains (Bacillus licheniformis and Pseudomonas fluorescens) and a microbial community in soil grown on different position-specific 13C glucose (six single and one uniformly 13C labelled glucose -99 at%). It shows that the 13C incorporation from different positions varied significantly between the microbial species (Figure. 1).

To interpret the different labelling patterns in the PLFAs, a 13C-MFA model was modified. The model was a stochiometric network consisting of 18 reactions and 8 biomass biosynthesis pathways which cover glycolysis, PPP, TCA, gluconeogenesis and anaplerotic reactions. The measured 13C enrichment of the carboxyl group (i.e., fragment of m/z 74; Figure 1) in PLFA was utilized to calculate metabolic fluxes. Results indicate that Gram-positive bacterium B. licheniformis maintains equal C flux through the glycolysis and PPP, whereas Gram-negative bacterium P. fluorescens mainly utilizes PPP to produce acetyl-CoA for PFLA synthesis. In contrast, the diverse microbial community in soil directs 80% of the C flux from glucose via PPP.

Our study highlights that the fragment-specific 13C analysis of PLFA after incubation with position-specific 13C labelled metabolic tracers can be used to track the intramolecular carbon heterogeneity and the diverse metabolic adaption of individual species in complex soil community. The 13C-MFA model can further provide a quantitative estimate to understand the intercellular process during organic matter degradation in soil or sedimentary biogeochemistry.
Figure 1. 13C fractional labelling (13C-FL) estimates of PLFAs grown on different 13C labelled glucose. (A) B. licheniformis, (B) P. fluorescens and (C) soil microbial community. C1, C2, C3, C4, C5, C6 and U refer to the position-specific 13C labelled glucose (GLC), which are GLC–13C, GLC–213C, GLC–313C, GLC–413C, GLC–513C, GLC–613C and GLC–U, respectively. 74, 87 and M refer to the dominant peaks in the mass spectra of fatty acid methyl esters with fragments C3H6O2+ (m/z 74), C4H7O2+ (m/z 87), and molecular ion (M), respectively. Red crosses indicate outliers, which are mainly contributed from unsaturated fatty acids.

References: