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**Analysis of isotope labeled amino acids by UPLC/ESI/TOFMS to determine nitrogen fluxes**

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Understanding nitrogen uptake and internal nitrogen fluxes in a tree is economically important, since nitrogen (N) is the key determinant in tree growth. By studying the uptake and assimilation of $^{15}$N-labeled ammonium and nitrate in poplar trees the uptake routes and the continuous flux in the amino acid metabolism can be understood.

In this study we show a feeding experiment, where poplar plants were fed with $^{15}$N-labelled ammonium and nitrate. For the analysis of this experiment we have developed a protocol for analysis of AccQ-Tag™ derivatised amino acids by UPLC/ESI/TOFMS.

*Populus* trees were grown in liquid culture, and after a short starvation period the trees were fed with $^{15}$N-labelled ammonium and nitrate. Samples (roots and leaves) were taken after 0, 0.5, 1, 3 hours and a long effect sample after 7 days. The amino acids were extracted using a methanol:water:chloroform (3:1:1) mixture. The dried extract was dissolved in 10mM HCl and derivatised using the AccQ-Tag™ (Waters). The derivatized amino acids were analyzed on UPLC/ESI/TOFMS. The accumulated data were integrated and isotope ratios calculated to follow the incorporation of $^{15}$N.

By derivatizing the amino acids with AccQ-Tag™ the chromatography and sensitivity of the amino acids increased dramatically compared to its non-derivatized form. The AccQ-Tag™ derivatization was developed for HPLC/UV-analysis in the beginning of the 1990’s. The method has proven to be a robust and sensitive method for amino acid analysis. Interestingly we found that the sensitivity of the amino acid derivates is even better on our ESI/TOFMS. Another advantage by using mass spectrometry is a more reliable identification of the analytes and this is especially useful when unclear peaks are found in the chromatogram. By using mass spectrometry we are able to follow the incorporation of $^{15}$N by studying the isotopic pattern of the derivatised amino acids.

Preliminary results from our feeding study shows incorporation of $^{15}$N in most of the studied amino acids in both roots and leaves. The incorporation seems to be faster in the roots compared to the leaves. The rate of incorporation also differs between the different amino acids. Using this data our aim is to present a “road map” of nitrogen uptake and internal nitrogen fluxes in *Populus*. 