



## Tracing the allocation of recently assimilated C into key metabolites in Norway spruce (*Picea abies*) shortly after bud break

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Plants allocate carbon (C) to sink tissues depending on phenological, physiological or environmental factors. We still have little knowledge on C partitioning into various cellular compounds and metabolic pathways, especially during tree growth after bud break. Here we investigated C partitioning of freshly assimilated C in Norway spruce by in-situ  $^{13}\text{C}$  short-term pulse labeling 15 days after bud break. We quantified  $^{13}\text{C}$  incorporation into tree compartments (needles, branches, stem) and into water soluble organic carbon (WSOC) by elemental analyzer-isotope ratio mass spectrometry (EA-IRMS). In addition, we determined  $^{13}\text{C}$  allocation into key metabolites of amino acids, hemicellulose sugars, fatty acids and alkanes by compound-specific  $^{13}\text{C}$  analysis via gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).

The  $^{13}\text{C}$  allocation within the trees reflected the needles as major C sink accounting for 86% of the freshly assimilated C. After 6 h  $^{13}\text{C}$  was distributed over a broad spectrum of plant metabolites but not homogeneously. Highest allocation was observed into structurally relevant compound classes of hemicellulose-derived sugars and proteinogenic amino acids (0.6% and 10% of needle  $^{13}\text{C}$ , respectively). However, needle growth also caused high C allocation into pathways not involved in formation of structural compounds like pathways in secondary metabolism, C-1 metabolism or amino acid synthesis from photorespiratory activity. C allocation into such pathways could be identified due to the high enrichment of key metabolites within the amino acids. In addition, high  $^{13}\text{C}$  allocation was found into the n-alkyl lipid biosynthesis (0.2 % of needle  $^{13}\text{C}$ ) with 1) higher allocation into intracellular than cuticular fatty acids, presumably for thylakoid membrane formation and 2) decreasing  $^{13}\text{C}$  allocation along the lipid transformation and translocation pathways (precursor fatty acids (C16 & C18) > elongated long chain fatty acids > decarbonylated n-alkanes).

Consequently, the combination of  $^{13}\text{C}$  pulse labeling with compound-specific  $^{13}\text{C}$  analysis of key metabolites enabled identification of relevant C allocation pathways during needle growth after bud break. Besides primary metabolism, synthesizing structural cell compounds, a complex network of various pathways consumed the freshly assimilated  $^{13}\text{C}$  and kept the majority of the assimilated C in the growing needles.