



Tracing photosynthetic carbon in leaves with nanoSIMS after $^{13}\text{C}\text{O}_2$ labelling

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To understand the carbon allocation of the tree and forest ecosystem, it is important to consider the residence time of carbon in different pools at suitable time scales. For example the carbon used for respiration will stay a few minutes to a few days in the tree, the carbon used for storage or structure of leaves will stay months to years, and the carbon used for wood structure, it will stay over the whole lifespan of the tree. The leaves are the entrance of carbon in trees where it can be used for foliage growth and maintenance or exported to the other organs or the other forest ecosystem compartments.

Tracing carbon isotope using NanoSIMS technique is one of useful methods to estimate where and how long the carbon stay in the tree organs. In this study, $^{13}\text{C}\text{O}_2$ pulse labelling were conducted and ^{13}C was measured by IRMS to see the amount of C remaining in the leaves with time. NanoSIMS was used to localize where the labelled C remained within the leaf tissue.

Twice labelling were done on branches of *Quercus serrata* at FFPRI (Forest and Forest Products research Institute) in Kyoto, Japan. The first labelling was in 30 April 2012 when the leaves start flushing and the second one was in 29 May 2012 when the leaves were completely deployed. For both labelling experiment, one branch was selected and covered with transparent plastic bag. CO_2 concentration was recorded with IRGA and air temperature inside the chamber was monitored. Then $^{13}\text{C}\text{O}_2$ was injected into the bag, and after 1 hour, the bag was removed and the branch was again exposed to ambient air. Leaves were collected before and 10-12 times after labelling and their isotope compositions were measured by IRMS. The leaf collected just after labelling and 6 days after labelling were used for NanoSIMS observation. Samples for nanoSIMS were preserved in glutaraldehyde and then embed in epoxy resin. The sliced sample were placed on the silicon wafer and observed by NanoSIMS 50L (Cameca, France).

The ^{13}C was highest just after labelling and decreased with the time. Half life time of $\delta^{13}\text{C}$ was longer in April-labelling than May-labelling that indicates that more carbon was retained in structural growth and less exported non-assimilatory organs in April than in May. The NanoSIMS observation showed ^{13}C in starch grains was shown only in May in leaves sampled 6 days after -labelling. This suggests that the usage of carbon differ depending on the stage of leaf formation.