^{13}C Measurements of Alpha-cellulose from Tree-ring Samples Using the IRMS Spectrometry

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Abstract. Carbon stable isotope analyses were done on the α-cellulose extracted from 18 tree-ring samples of the 35 year old tree of the genus Populus using continuous flow system, Flash HT 2000 connected with Delta V Advantage isotope ratio mass spectrometer (IRMS). This technique provides a fast analysis of a very small amount of the sample (about 400 μg). The values of δ13C were identified in the range from –26.57 ‰ to –24.51 ‰ vs. PDB standard, with averaged standard deviation of about 0.22 ‰, and covered the time span from 1970 to 2001.

Introduction

Trees are widespread and sensitive to the environmental changes. They record the palaeoenvironmental information for potential reconstructing of the past climate with annual resolution [*Hall et al.*, 2008]. The tree-ring width records have been supplemented with tree-ring stable isotope analyses (carbon $\delta^{13}C$, oxygen $\delta^{18}O$ and hydrogen $\delta^{2}H$) that provide useful information for the reconstruction of palaeoclimate [*Csank et al.*, 2011], wood growth [*Schifman et al.*, 2012] or the determination of past environment regeneration [*West et al.*, 2001]. The carbon isotope discrimination can also be considered a good indicator of water availability to the plant [*Pita et al.*, 2001].

Stable isotope analyses of wood samples have been mostly carried out on the α-cellulose extracted from the wood sample as it is relative immobile in tree rings, and conserves the information about climate conditions in each year [*McCarroll et al.*, 2006; *Rinne et al.*, 2005]. Several studies compared $\delta^{13}C$ results obtained from α-cellulose and bulk wood material. For example *Verheyden et al.* [2005] found a significant linear correlation between the $\delta^{13}C$ of α-cellulose and bulk material of the mangrove tree ($\delta^{13}C_{bulk\ material} = 0.92\ (\pm 0.08)^{*}\ \delta^{13}C_{\alpha\text{-cellulose}} - 2.91\ (\pm 2.04)$; $p < 0.001$); for $\delta^{13}C$ values of α-cellulose between –24 ‰ and –27 ‰).

The goal of the present paper was to test the suitability of the continuous flow IRMS technique for stable carbon isotope analysis of α-cellulose extracted from tree-ring samples.

Materials and methods

Samples

Stable carbon isotope study was carried out on the tree of the genus *Populus* tree-ring samples from a tree located at the university campus located about 3 km from the downtown of Bratislava.

The α-cellulose was extracted from the examined tree-ring samples as it is relatively immobile and thus can keep isotopic information from the time of its formation. In the process of α-cellulose extraction it was first necessary to remove the resins as they represent a mobile fraction in the wood. It was performed in the continuous reflux of a benzene and ethanol mixture. In the next step the lignin was removed using an acidified sodium chlorite solution, and this step was followed by removal of hemicellulose in sodium hydroxide. This process resulted in the extraction of pure α-cellulose from the wood sample.

We have analysed 18 samples from different rings of a 35 year old poplar. The sample numbering started from the centre of the tree to the tree bark and some of tree-rings were omitted in the process of

extraction. The tree-ring samples covered the time span from 1970 to 2001. The amount of 400 µg of α-cellulose from each sample was wrapped into a tin capsule and placed in the sequence between IAEA sucrose standards. The samples were combusted in the sequence together with the IAEA and internal standards.

Analytical technique

In our experiment the isotope ratio mass spectrometer (IRMS) Delta V Advantage from Thermo Fisher was used. For sample combustion the peripheral unit Flash HT 2000 was connected to IRMS spectrometer. A small amount of the sample (about 400 µg) was wrapped into a tin capsule, which was dropped through the furnace tube heated to 1020 °C. The sample was flash-combusted with assistance of oxygen pulse and the resulting gases were flown through a silica column packed with chromium oxide, reduced copper and silver cobaltous-cobaltic oxide, which served as further oxygen donors to ensure complete sample combustion. In this way all carbon was fully oxidised to CO_2 and $\delta^{13}C$ in this form was measured by the mass spectrometer. The principle of the Thermo Scientific Flash HT is depicted in Figure 1.

The isotope ratio is expressed in delta notification (in per mil—‰) relative against the reference standard, in our case it is the reference gas CO_2:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{reference}} - 1 \right) \times 1000$$

where R_{sample} a $R_{reference}$ represent the ratio of the heavier to the lighter isotope of the same element in the sample and in the reference standard, respectively, for carbon it is the ratio $^{13}C/^{12}C$. This ratio was calculated relative to PDB using IAEA sucrose (–10.40 ‰) and our internal sucrose (–26.50 ‰) standard.

Results

The Flash HT 2000 peripheral unit connected with the IRMS spectrometer Delta V advantage was used for the carbon isotope analyses. The CO_2 resulting from the combustion of samples was diluted with 80% of the carrying gas (helium) to obtain a sample peak comparable to the reference peaks. First three and then five replicates of chosen single samples were measured and $\delta^{13}C$ values were averaged. Figure 2 shows the results of analysed $\delta^{13}C$ values in 18 samples of extracted α-cellulose that cover the time range from 1970 to 2001. The measured values range from –26.57 ‰ to –24.51 ‰ vs. PDB standard, with averaged standard deviation of about 0.22 ‰.

We have also performed thirteen control measurements of $\delta^{13}C$, where the results were averaged from five replicates. The comparison of the first and second series of measurements showed that there

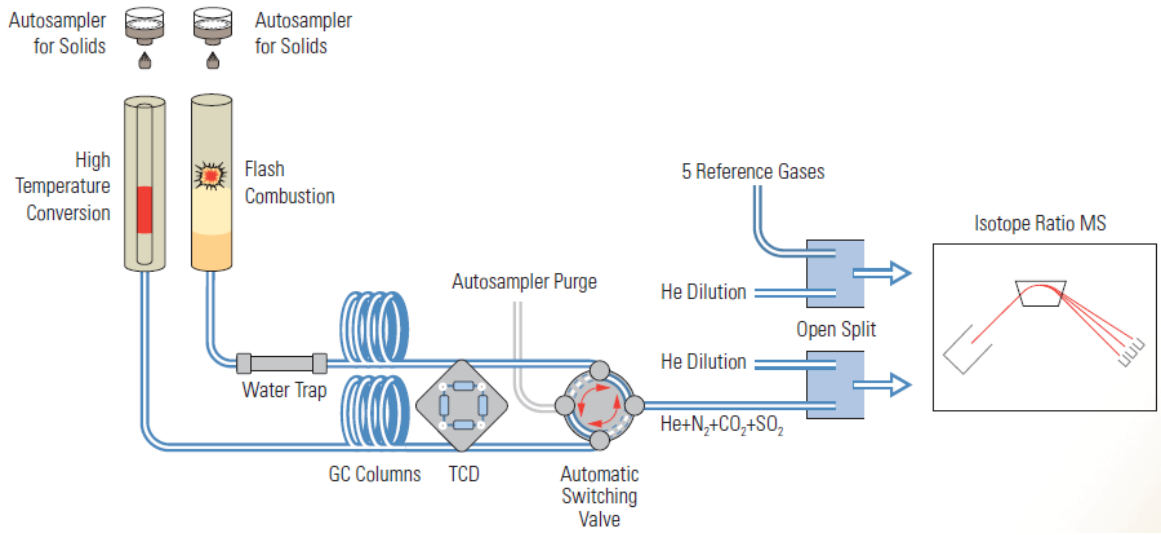

Figure 1. Principle of the Thermo Scientific Flash HT 2000 elemental analyser. [*Thermo Scientific*, 2012].

was only one sample having a bigger difference than 0.5 ‰. The very possible source of this mismatching is the inhomogeneity of the samples or natural variability as it is mentioned by *Leavitt* [2010]. According to this work circumferential variability generally falls into the range of differences of 0.5–1.5 ‰ for $\delta^{13}C$. While the range of isotopic composition of the same ring of different trees at a site (the inter-tree variability) is 1–3 ‰ for $\delta^{13}C$, what of course depends on the tree species. According to this our repetition results are in very good agreement (Figure 3).

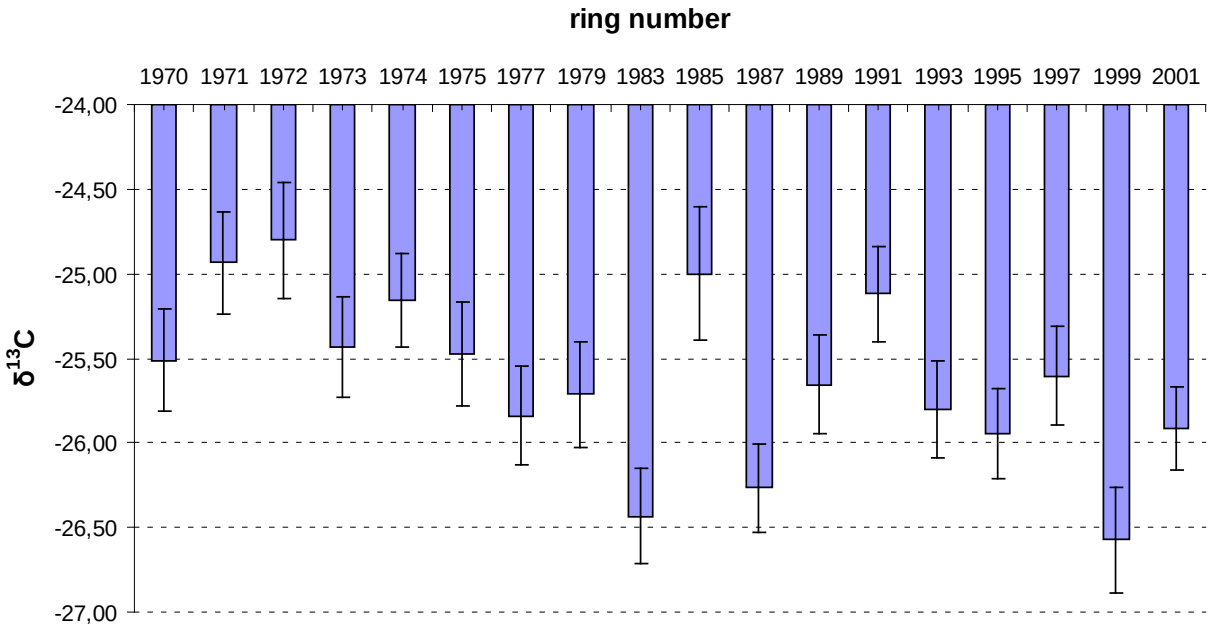

Figure 2. $\delta^{13}C$ values from α-cellulose extracted from individual poplar tree-rings. These results were obtained from triplicate sample measurements and cover the time range from 1970 to 2001.

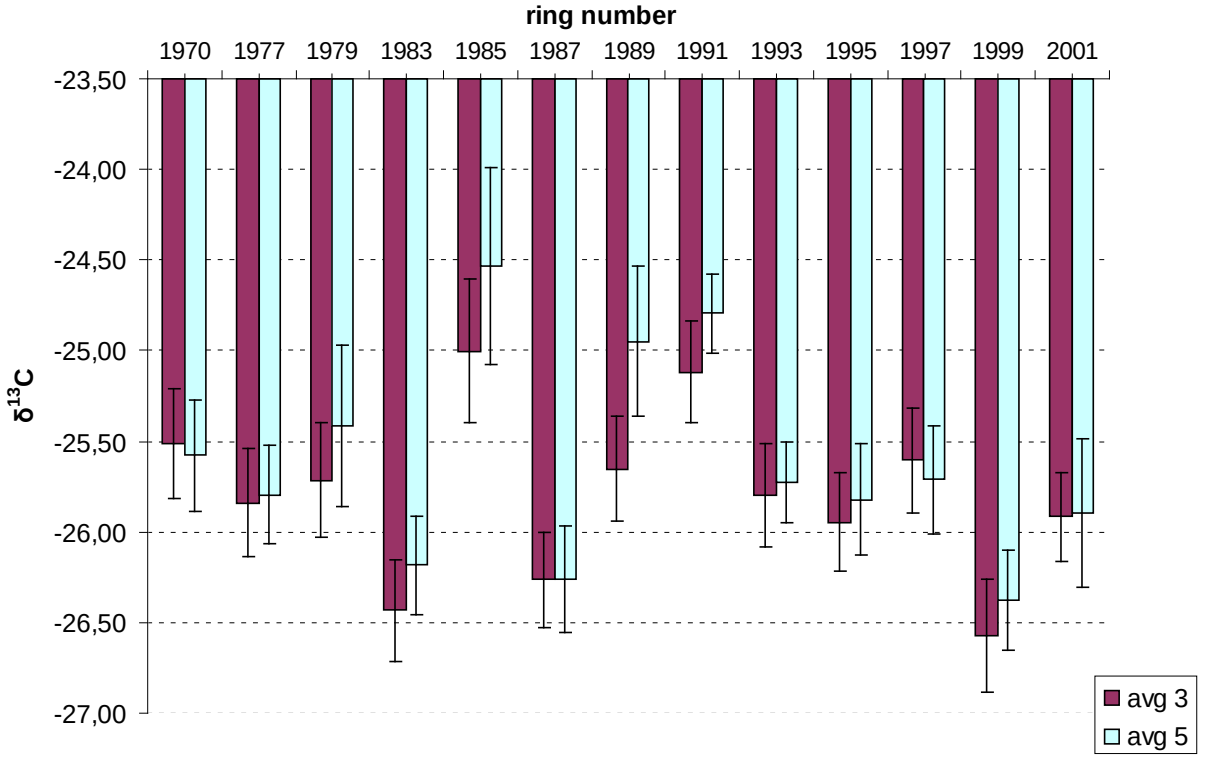

Figure 3. Comparison of the $\delta^{13}C$ values of the three and five repetition measurements of the chosen tree-ring samples.

Conclusion

On-line analysis using elementar analyser connected with isotope ratio mass spectrometer could speed up the analysis in comparison to the old techniques. We have measured $\delta^{13}C$ in α-cellulose from the tree-rings of 35 year old poplar. Our $\delta^{13}C$ values from 18 samples were identified in the range from –26.57 ‰ to –24.51 ‰ vs. PDB and covered the time span from 1970 to 2001. We have also performed the control measurements with five repetitions of a single sample and found out good agreement with the previous series of measurements. Advantages of this on-line (continuous flow) analysis are its rapidity and a fact that a very small amount of sample is sufficient. However, the sample should be homogeneous enough.

The presented $\delta^{13}C$ data will be used for the assessment of long term variations of carbon isotopes in the atmosphere and biosphere, specifically for the corrections of ^{14}C data during the evaluation of the impact of fossil fuel CO_2 on the atmosphere that is important for better understanding of recent changes in the climate of the Central Europe, as well as for the estimation of ^{14}C produced by the nuclear industry.

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References

Csank, A. Z. et al. Climate variability in the Early Pliocene Arctic : Annually resolved evidence from stable isotope… In Palaeogeography, Palaeoclimatology, Palaeoecology 308 (2011). 339–349, 2011.

Hall, G. et al. Stable carbon isotope ratios from archaeological charcoal as palaeoenvironmental indicators, In Chemical Geology 247 (2008), 384–400, 2008.

Leavitt, S. W. Tree-ring C-H-O isotope variability and sampling. In Science of The Total Environment 408 (2010), 5244–5253, 2010.

McCarroll, D., et al. Isotopes in tree rings. In Isotopes in Palaeoenvironmental Research 10 (2006), 67–116, 2006.

Pita, P. et al. Carbon isotope discrimination and its relationship to drought resistance under field conditions in genotypes of Eucalyptus globules Labill. In Forest Ecology and Management 141 (2001), 211–221, 2001.

Rinne, K. T., et al. On the purification of α-cellulose from resinous wood for stable isotope (H,C and O) analysis. In Chemical Geology 222 (2005), 75–82, 2005.

Schifman, L. A. et al. Carbon isotope variation in shrub willow (Salix spp.) ring-wood as an indicator ot long-term water status, growth and survival. In Biomass and Bioenergy 36 (2012), 316–326, 2012.

Thermo Scientific FLASH HT Plus Elemental Analyzer for IRMS, 2012 Thermo Fisher Scientific Inc. BR30189_E04/10C, 2012.

Verheyden, A. et al. Comparison between d13C of a-cellulose and bulk woodin the mangrove tree Rhizophora mucronata: Implications for dendrochemistry. In Chemical Geology 219 (2005), 275–282, 2005.

West, A. G. et al. The evaluation of $\delta^{13}C$ isotopes of trees to determine past regeneration environments. In Forest Ecology and management 147 (2001), 139–149, 2001.

Yin, Ch. et al. Early growth, dry matter allocation and water use efficiency of two sympatric Populus species as affected by water stress. In *Environmental and Experimental Botany* 53 (2005), 315–322, 2005.