

## Can $^{13}\text{C}$ stable isotope record of Norway spruce tree rings display the effect of environmental conditions?

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**ABSTRACT:** The Bohemian Forest Mts. (Šumava) were exposed to heavy atmospheric pollution in the last century. A possible negative effect of atmospheric pollution on tree physiology has been studied using the isotopic composition,  $\Delta^{13}\text{C}$ , of tree rings. The wood is depleted of  $^{13}\text{C}$  relative to the air due to isotopic discrimination against  $^{13}\text{C}$  and preferred uptake of  $^{12}\text{C}$  during photosynthesis. The ratio  $^{13}\text{C}/^{12}\text{C}$  ( $\Delta^{13}\text{C}$ ) reflects the relative magnitudes of net assimilation and stomatal conductance that relate to demand and supply of  $\text{CO}_2$ . Carbon-13 data are thus a useful index for assessing intrinsic water use efficiency (mark period of drought or root system damages) and also could indicate assimilation organ injury (needle damage caused due to acid rain etc.). A decrease in  $\Delta^{13}\text{C}$  implies a negative effect of environmental conditions on tree physiology. Presumably, changes in soil pH and aluminium content as an indirect effect of atmospheric pollution could have an impact on tree physiology. Our results showed that the isotopic signal varied around the average, but the  $\Delta^{13}\text{C}$  signal was decreasing from the 1950s to 1980s and then increasing again starting in the 1990s. This trend is in accordance with the monitored atmospheric pollution and soil solution pH changes.

**Keywords:**  $\Delta^{13}\text{C}$ ; air pollution; tree physiology; Bohemian Forest Mts.; soil pH; aluminium; soil acidification

In the past, the nutrient balance and development of trees in many areas of Central Europe were affected by acidification that occurred in this region (MATZNER, MURACH 1995). The acidification of soils caused the loss of base cations and an increase in the content of acid cations in the cation exchange complex of the soils (SÉGUIN et al. 2004). Global climate changes connected with increasing temperature and decreasing precipitation can also constitute another interference of spruces which are rooting in the upper soil layers (PUHE 2003). However, there is still an uncertainty to what degree and how long the trees have been affected.

A parameter is needed to assess the effect of environmental conditions on spruce tree development in the past and to improve a decision-making of for-

est management in the future. The  $^{13}\text{C}$  stable isotope record in tree rings has been shown to provide a valuable insight into the history of environmental effects (moisture, temperature, atmospheric and soil pollution) on the tree physiological activity (WEST et al. 2006). The carbon stable isotope ratio ( $^{13}\text{C}/^{12}\text{C}$ ) of plant biomass is a widely used indicator because of the integrative response of the isotopic ratio to multiple eco-physiological constraints during the time of biomass development (DAWSON et al. 2002). Carbon dioxide in the atmosphere is composed of molecules with a light atom of C ( $^{12}\text{C}$ , 98.89%) and molecules with a heavy atom of C ( $^{13}\text{C}$ , 1.11%). There is an isotope discrimination against carbon dioxide with  $^{13}\text{C}$  during carbon dioxide fixation

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Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 600 766 5801, the Grant Agency of the Czech Republic, Project No. 206/07/1200, the Grant Agency of the Czech University of Life Sciences, Project No. 20074003, and the Grant Agency of Faculty of Forestry and Wood Sciences, Project No. 23/2007.

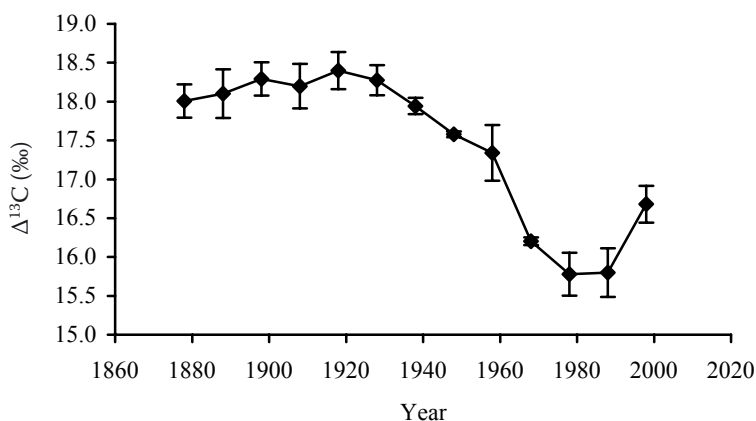


Fig. 1. Long-term trends of  $\Delta^{13}\text{C}$  average values of tree rings from research conducted by ŠANTRŮČKOVÁ et al. (2007)

in photosynthesis, resulting in depletion of  $^{13}\text{C}$  in plant biomass. The rate of discrimination is affected by environmental conditions such as temperature, water availability, atmospheric pollution, nutrient availability etc., which affect stomatal conductance and photosynthesis rate (FARQUHAR et al. 1982; MARTIN, SUTHERLAND 1990; MCCARROLL, LOADER 2004; HELLE, SCHLESER 2004). For trees, the effects of environmental changes on the tree physiological activity in the past can be recorded from the fluctuation of the carbon stable isotope ratio in tree rings and assigned to the exact year or time period (GUYETTE, CUTTER 1994).

Norway spruce (*Picea abies* [L.] Karst.) is a dominant tree species in commercial forests in the Czech Republic. The forests have been affected very much by increasing sulphur and nitrogen deposition and subsequent soil acidification over more than one century (PSENNER, CATALAN 1994). Spruce forests in acid sensitive areas, usually those with the crystalline bedrock and naturally low base saturation of soils, have remained exposed to the effect of soil acidification after the decline of atmospheric deposition, which can result in the growth depression of trees. Growth depression of Norway spruce was

detected in the Bavarian Forest (WILSON, ELLING 2004) and the northern part of the Czech Republic (KROUPOVÁ 2002). In the Bohemian Forest Mts., the negative effects of atmospheric depositions and soil acidification on isotopic composition and chemistry of tree rings and, therefore, on the tree physiological activity have been indicated (ŠANTRŮČKOVÁ et al. 2007; Fig. 1). However, the preliminary study by ŠANTRŮČKOVÁ et al. (2007) was performed using the  $^{13}\text{C}$  stable isotope record from three trees only. The main objective of our study was to enlarge the data set and to validate the finding that the tree physiological activity was negatively affected by atmospheric depositions and soil acidification in this area.

The research was carried out in a forest stand located in the catchment of the Čertovo Lake in the Bohemian Forest Protected Landscape Area. This area was exposed to heavy atmospheric pollution in the last century (VESELÝ 1994; Fig. 2), which was followed by significant soil acidification (KOPÁČEK et al. 2001, 2002a). The MAGIC7 model suggested that soil pH did not vary significantly until the late 1950s, then it began to decrease at the same time with increasing Al concentration. Acid deposition and also Al content in the soil solution decreased in the 1980s (MAJER et al. 2003; Fig. 3).

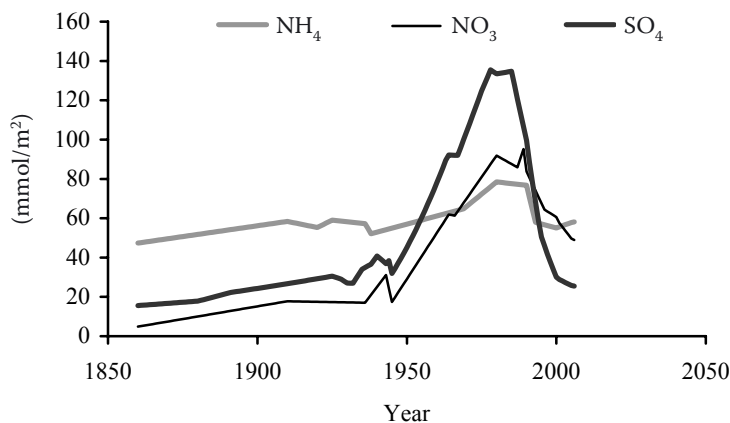


Fig. 2. Long-term trends of atmospheric deposition. Data were derived from KOPÁČEK et al. (2001)

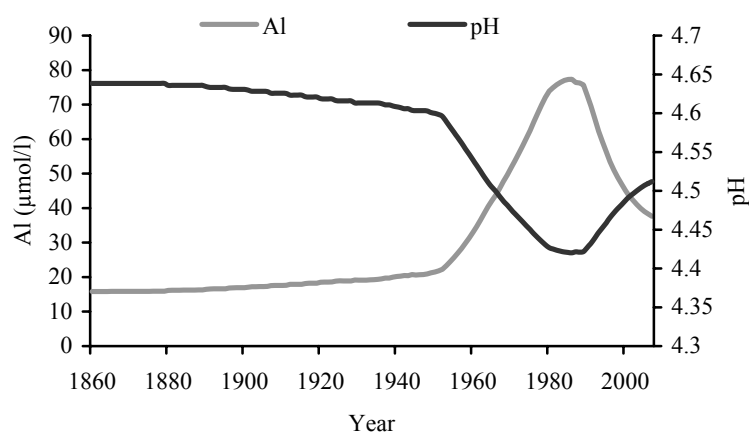


Fig. 3. Long-term trends of pH and Al concentration of soil solution (adapted according to MAJER et al. 2003)

## MATERIAL AND METHODS

### Site description

The area has a humid climate with wet cold winters and wet mild summers. A trend of increasing temperatures has been detected in this area since the 1960s (KETTLE et al. 2003). The mean annual temperature is 3.4°C and the mean annual precipitation is 1,228 mm. The bedrock of the catchment is composed of mica-schist (muscovitic gneiss), quartzite, and small amounts of pegmatite (VESELÝ 1994). Soil types mostly belong to Cambisols, Podzols and Lithosols on steep slopes in the watersheds. Some information about soil properties is in Table 1; for a more detailed description see KOPÁČEK et al. (2002b).

The Čertovo Lake catchment is covered with 90 to 150 years-old Norway spruce (*Picea abies* [L.] Karst.) forest of at least secondary origin, with scarce European beech (*Fagus sylvatica* L.). The land use history of the catchment suggests important timber harvesting

and charcoal and potash production from the Middle Ages to the late 19<sup>th</sup> century (VESELÝ 1994).

### Sampling and analyses

We randomly selected three Norway spruce trees older than 150 years in the Čertovo Lake catchment in the area of Jezerní hora Mt. The selected trees were without apparent defects such as putrefaction or crown damage. Trees were sampled from near breast height (cores from two opposite exposures). Rings were sectioned by decades and analyzed for the isotopic composition. Only those rings were evaluated that were formed after the juvenile effect (LEAVITT, LONG 1985; LIU et al. 2004) ceased (40 years). Samples were dried and homogenized in a ball mill (MM200 Retsch, Haan, Germany). Isotopic analyses were carried on an elemental analyzer (EA1110, ThermoQuest Italia s.p.a.) linked to DeltaXLplus (ThermoFinnigan, Bremen, Germany). The ratio of <sup>13</sup>C to <sup>12</sup>C was expressed in delta (δ) notation with reference

Table 1. Average composition of individual soil horizons in the Čertovo Lake watershed adapted according to KOPÁČEK et al. (2002b)

Soil properties	C <sub>O</sub>	C <sub>A</sub>	C <sub>Ae</sub>	C <sub>E</sub>	C <sub>B</sub>	C <sub>C</sub>
Depth	5	10	24	17	20	22
Soil (kg/m <sup>2</sup> )	4	24	159	81	80	184
pH <sub>H<sub>2</sub>O</sub>	3.6	3.6	4.3	4.1	4.2	4.7
C (mol/kg)	41	26	12	3.3	3.6	3.1
N (mmol/kg)	1,603	1,028	450	143	151	54
P (mmol/kg)	34	34	31	13	28	16
Ca (mmol/kg)	50	30	33	32	22	18
Mg (mmol/kg)	28	56	146	67	108	172
K (mmol/kg)	61	242	386	542	474	477
Al (mmol/kg)	0.3	1.5	2.7	2.7	2.6	2.6
Fe (mmol/kg)	102	241	596	206	595	637
Mn (mmol/kg)	3.2	3.6	4.0	5.5	4.4	5.1

to standard material ( $\delta^{13}\text{C} = R_{\text{sample}}/R_{\text{standard}} - 1$ ), which was fossil belemnite in this case (Vienna-PDB, VPDB, MCCARROLL, LOADER 2004).

There is a discrimination against  $^{13}\text{C}$  in C3 plants by the carboxylating enzyme Rubisco ( $\sim 27\%$ ) and during diffusion through the stomata ( $\sim 4.4\%$ ), which is linked to photosynthesis through the ratio of intercellular to atmospheric  $\text{CO}_2$  concentrations ( $c_i/c_a$ ). Discrimination in C3 plant can be expressed as:

$$\Delta (\text{‰}) = a + (b-a)(c_i/c_a)$$

where:

- a* – discrimination against  $^{13}\text{CO}_2$  during diffusion through the stomata,
- b* – net discrimination due to carboxylation,
- $c_i/c_a$  – intercellular and ambient  $\text{CO}_2$  concentrations (FARQUHAR et al. 1982; MCCARROLL, LOADER 2004).

The highest  $\Delta^{13}\text{C}$  values show plants at optimum environmental conditions (optimum growth and mostly largest isotope discrimination). The sensitivity of  $\Delta^{13}\text{C}$  is weak around the optimum of plant growth. The sensitivity of  $\Delta^{13}\text{C}$  to environmental changes increases progressively below and above the optimum (MARTIN, SUTHERLAND 1990; HELLE, SCHLESER 2004).

In this study, isotope ratios were expressed in terms of discrimination against  $^{13}\text{C}$  in the atmosphere ( $\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{ATM}} - \delta^{13}\text{C}_{\text{PLANT}})/(1 + \delta^{13}\text{C}_{\text{PLANT}}) \approx (\delta^{13}\text{C}_{\text{ATM}} - \delta^{13}\text{C}_{\text{PLANT}})$ ; FARQUHAR et al. 1989) to remove the effect of atmospheric  $\delta^{13}\text{C}$  decline. The atmospheric  $\delta^{13}\text{C}$  signal was corrected using estimates based on the Antarctic ice core record (MCCARROLL, LOADER 2004).

## RESULTS

The pattern of changes in an isotopic signal displayed the same trend for all trees (Fig. 4), though

average  $\Delta^{13}\text{C}$  was shifted approximately by one ‰ (17.5‰, 17.2‰ and 16.2‰, respectively). The  $\Delta^{13}\text{C}$  increased from the late 1850s till the end of the 19<sup>th</sup> century. Then it slowly decreased until the 1980s and the decrease becomes faster from 1950s till 1980s. The past decrease corresponds to the period of heavy atmospheric and soil pollution of the area (Figs. 2 and 3).  $\Delta^{13}\text{C}$  has been increasing since the early 1990s, indicating biological recovery.

## DISCUSSION

Variation in the  $^{13}\text{C}$  isotopic signal at the end of the 19<sup>th</sup> century and at the beginning of the 20<sup>th</sup> century might be a reaction to the closure of pasturing and timber harvesting (VESELÝ 1994) in conjunction with the long-term effect of spruce monocultures, with their natural acidifying influences (HERBAUTS, DE BUYL 1981). The rapid decrease in  $\Delta^{13}\text{C}$  in tree I began in 1920, for the other two trees (tree II and III) in 1940. The rapid decrease between the 1950s and 1980s is in accordance with the period of heavy atmospheric pollution which accelerated soil acidification followed by decreased base cation availability and increased aluminium toxicity (KOPÁČEK et al. 2002a; SÉGUIN et al. 2004). ŠANTRŮČKOVÁ et al. (2004) noted that the greatest changes in soil chemistry and biochemistry took place in the litter and humus horizons where spruces had most of their roots (PUHE 2003; OSTONEN et al. 2005). Higher aluminium concentrations induce a shift of roots into the upper soil layers, because aluminium of even less than micromolar concentrations inhibits root elongation (MA 2005). The results indicate tree abionosis, i.e. the harmful effect of soil acidification on the trees.

Also the acid rain which fell in the 1970s and 1980s could have impacted the isotopic signal. Sulphur

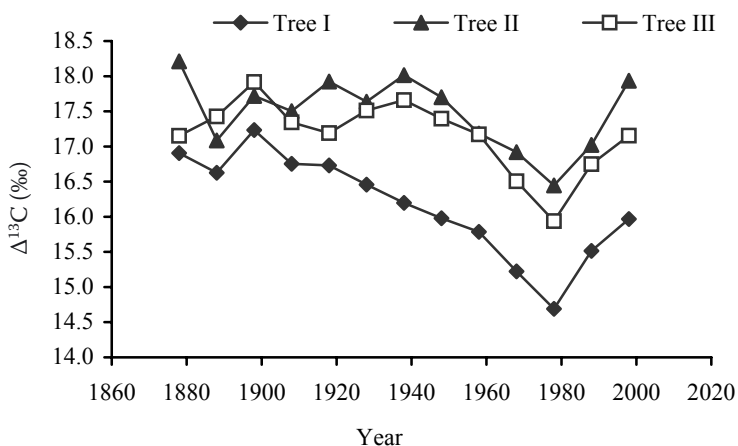


Fig. 4. Long-term trends of  $\Delta^{13}\text{C}$  signal in 10-year tree samples from Jezerní Mt.

emissions may have caused especially foliage damage (SUTINEN et al. 1998), thereby affecting carbon fixation. The relatively quick recovery can be due to the assimilatory apparatus regeneration. An increase in temperature of about 1.5°C (KETTLE et al. 2003) has not probably yet had any impact on the biological recovery that started in the 1980s.

The differences in  $\Delta^{13}\text{C}$  between the trees may reflect their different genetic dispositions or social and ecological positions. The same trend in the time change of  $\Delta^{13}\text{C}$ , however, shows that all three trees were exposed to the same effect of environmental conditions; this is more important than the absolute values. The isotopic signal changes appreciated relative to the average of the whole trees. The presented results correspond to the analyses previously performed on other four trees (ŠANTRŮČKOVÁ et al. 2007) from a nearby area.

There is no consensus in terms of what type of material to use for isotopic analyses. SCHWEINGRUBER (1996) reported that the most reliable values of  $\Delta^{13}\text{C}$  were given by measuring isotopes in cellulose because only the cell-wall component contains non-mobile organic elements. But LOADER et al. (2003) and ELHANI et al. (2005) suggested that the climate signal in the  $\Delta^{13}\text{C}$  values of whole wood may be stronger than the one in cellulose or lignin. HARLOW et al. (2006) stated that holocellulose extraction was unnecessary for most analyses of tree-ring  $\Delta^{13}\text{C}$ . BORELLA et al. (1998) argued that wood is as good a climate proxy as cellulose. It is also recommended to use only late wood (MCCARROLL, LOADER 2004). However, the tree growth is extremely slow in many areas and separation of latewood has proved to be almost impossible when the rings are really narrow. HILL et al. (1995) noted that the  $\delta^{13}\text{C}$  value of early wood correlates best with the late wood formed in the previous year because early wood cells are manufactured partly using stored photosynthates and smaller cells of latewood formed during the summer (SWITSUR et al. 1995). The whole ring can only give an integrated carbon isotope value which is frequently taken as an annual record of environmental conditions. Often, it may merely be information about a very specific part of the year. In many cases, wood is laid down during a short period of the year (frequently in Central European trees) and the isotopic signal primarily corresponds to the conditions of this time interval (SCHLESSER et al. 1999). Whole rings (late wood and early wood) were used for isotope analyses in this study. 10-year averages are used for  $\Delta^{13}\text{C}$  interpretation, thus the  $\Delta^{13}\text{C}$  interference of early wood performed in the previous year is extrinsic. Cellulose extraction was not made.

As compared to needle analyses, the analysis of  $\Delta^{13}\text{C}$  of tree rings provides a long term record of the effect of environmental conditions. Needle analyses might provide information only about the effect of environmental conditions in the current year (SOLBERG, TORSETH 1997; SUTINEN et al. 1998). This would also be true of analyses of soil changes induced by air pollution and interpreting these changes in connection with tree physiology (MATZNER, MURACH 1995; SOLBERG et al. 2004).

## CONCLUSIONS

Stable isotope dendroecology is a relatively young field with advances in sample preparation technique, clear physiological background and understanding how environmental factors influence the isotope fractionation. Stable isotope methods have recently emerged as one of the most powerful tools for understanding the relationship between plants and their environment. The applied method seems to be good and is worth testing in other regions. Our results confirm the negative effect of atmospheric and soil pollution on tree physiology.

## Acknowledgement

Special thanks to Jiří Květoň and Martina Vašková for their work with the mass-spec, and Keith Edwards for language corrections.

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Received for publication November 7, 2007

Accepted after corrections March 21, 2008

## Mohou stabilní izotopy uhlíku $^{13}\text{C}$ v letokruzích smrku ztepilého indikovat změny v podmínkách prostředí?

**ABSTRAKT:** V minulém století byly lesy v oblasti Šumavy vystaveny silnému znečištění ovzduší. Možný negativní efekt znečištění ovzduší na fyziologii smrku ztepilého byl studován pomocí stabilních izotopů  $\Delta^{13}\text{C}$  v letokruzích stromů. Rostliny během fotosyntetické fixace uhlíku preferují  $^{12}\text{C}$  před  $^{13}\text{C}$ , a proto dřevo stromů obsahuje méně  $^{13}\text{C}$  v porovnání se vzduchem. Poměr lehkého a těžkého izotopu uhlíku v rostlinách je závislý na rychlosti fotosyntézy a otevřenosti průduchů. V izotopovém signálu jsou proto zachycena období sucha, případně poškození kořenového systému stejně jako asimilačního aparátu (např. poškození jehlic způsobené kyselými dešti). Pokles v  $\Delta^{13}\text{C}$  v rostlině indikuje negativní efekt podmínek prostředí na fyziologii stromu. Podle předpokladu, že změny pH půdy a obsahu hliníku jako nepřímého efektu znečištění ovzduší mohou ovlivňovat fyziologii smrku, by se tyto změny mohly studovat pomocí skladby izotopů v letokruzích smrku. Izotopový signál v letokruzích stromů se během analyzovaného období pohyboval kolem průměrné hodnoty, zatímco signál  $\Delta^{13}\text{C}$  klesal mezi roky 1950 až 1980 a opět stoupal po roce 1990. Tento trend je v souladu se zaznamenanými změnami v pH půdy a v atmosférické depozici.

**Klíčová slova:**  $\Delta^{13}\text{C}$ ; znečištění ovzduší; fyziologie stromu; Šumava; pH půdy; hliník; acidifikace půdy

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