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Impact of different fertilisers on elemental content in young hybrid aspen stem wood

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Abstract. The biomass production using fast-growing tree species such as hybrid aspen (*Populus tremuloides* Michx. *x Populus tremula* L.) has been recognized as an environmentally friendly and cost-effective approach. Growing these species can reduce the negative impact of earlier land mismanagement and at the same time provide additional biomass growth. The application of fertilisers may introduce not only the necessary macro elements (N, P, K) but also significant amounts of toxic heavy metals. Therefore, the knowledge about elemental flows from fertilised soil to the different parts of hybrid aspen trees is essential and especially meaningful for the evaluation of element content in specific environmental ecosystems. The impact of different fertilisers (sewage sludge, digestate and wood ash) on the concentrations of micro- and macro elements in the wood of six-year-old hybrid aspen stands grown on former agricultural land was studied. The determination of element concentrations in different tree rings of hybrid aspen trees was accomplished by inductively coupled plasma mass spectrometry (ICP–MS). Isotope ratio mass spectrometry (IRMS) was used to determine the nitrogen and carbon content and isotope ratios in different parts of hybrid aspen trees. Stem disc samples from hybrid aspen trees were obtained from agricultural land in the central part of Latvia. Samples were taken from six-year-old hybrid aspen trees that at the moment of planting were fertilised with sewage sludge, a residue of biogas production (digestate) and wood ash. The obtained results indicated that the chemical element accumulation in hybrid aspen was affected by the applied fertiliser type. In this study, the use of wood ash, as well as digestate, affected the elemental content in hybrid aspen to a greater extent than the use of sewage sludge, relative to unfertilised (control) subplot. The analysed elements varied in the analysed stem plane (across the tree rings). The most significant changes between the rings were observed for the content of K and Ca.

Key words: hybrid aspen, heavy metals, macro elements, ICP-MS, IRMS.

INTRODUCTION

Aspens are fast-growing trees with wide distribution. European (or Eurasian) aspen (*Populus tremula* L.) and North American quaking aspen (*P. tremuloides* Michx.) are among the most common tree species in Eurasia and North America (Tullus et al., 2020). Despite the wide distribution and high biodiversity of aspen trees, their economic value was low a few decades ago. However, the use of aspen and aspen hybrids has now significantly increased. The fast-growing aspen trees and their hybrids such as hybrid aspen (*Populus tremuloides* Michx. × *P. tremula* L.) are used for the production of pulp and energy wood (Zeps et al., 2015; Tullus et al., 2020). Previous studies indicate that the wood of hybrid aspen is suitable for high-quality paper production as well as for the production of wood chips, especially from the logging residues of such plantations (Smilga et al., 2015).

Moreover, the plantations of hybrid aspen are environmentally friendly because there is no need for annual tillage nor the use of mineral fertilisers, herbicides and pesticides. Growing of these species can reduce the negative impact on lands that have suffered from intensive management (agricultural practices, quarries etc.), while at the same time enhancing additional biomass growth in comparison to set-aside lands. Biomass is an essential renewable resource that provides a basis for the climate-neutral economy. For example, plantations have significant potential to compensate for CO_2 emissions in order to meet the European Union goals for carbon sequestration (Bardule et al., 2016; Fahlvik et al., 2019).

Plants can accumulate and store biologically essential elements as well as contaminants from the soil, thus acting as passive samplers. The approach of using plants for the remediation of polluted areas is considered environmentally friendly and inexpensive. Studies have shown that many plant species have great potential for accumulating heavy metals. This property of plants can be used for the stabilisation, phytoremediation and treatment of problematic soils (Mala et al., 2007; Mandre, 2014). Literature studies indicate that some clones of hybrid aspen can grow well in reclaimed surface mines and under the impact of elevated concentrations of industrially emitted gases and fly ash (Mandre, 2014). Nikula et al., (2011) investigated the growth and leaf traits of hybrid aspen growing close to a motorway in comparison to a control location and suggested that the species would be suitable for plantations in moderately polluted areas alongside roads carrying heavy traffic. Since hybrid aspen is one of the species characterized by fast growth even under bad climatic conditions, as well as capable of growing in poor soils heavily polluted with industrial waste, it has significant potential for phytoremediation (Mala et al., 2007; Mandre, 2014).

The growth of poplars and aspens especially at a young age is fast compared to conifers. Their rotation cycle often comprises only the maturation phase, and their tree ring archive contains hardly any non–juvenile rings (Meyer et al., 2018). Furthermore, the relatively wide aspen tree rings enable the analysing of the annual element concentrations in aspen wood. The tree ring samples are suitable for the analysis by advanced techniques like LA-ICP-MS, ICP-MS, and isotope ratio mass spectrometry (IRMS).

The aim of this study was to investigate the micro- and macro element content in hybrid aspen stem wood, and to evaluate the accumulation and distribution of different elements within aspen stem plane (across the tree rings).

MATERIALS AND METHODS

Stem disc samples of six-year-old hybrid aspen trees were obtained from an experimental plantation area that was part of a large-scale multifunctional plantation with a total area of about 16 ha in the central part of Latvia (56.6919 N, 25.1370 E). The hybrid aspen seedlings (clone No. 4) were produced in a nursery of the Joint Stock Company 'Latvian State Forests' were planted in the spring of 2011. The plantation density was 2.0×2.0 m between the trees. Short rotation energy crops and other deciduous trees were also grown in the plantation. According to the Food and Agriculture Organization of the United Nations classification (2006), the type of soil was *Luvic Stagnic Phaeozem* (*Hypoalbic*) or *Mollic Stagnosol* (*Ruptic, Calcaric, Endosiltic*) with predominantly loam and sandy loam soil texture at 0–20 cm depth and sandy loam soil texture at 20–80 cm depths. Four different types of fertilisation subplots were established in the plantation, each with four replications. The subplots were fertilised with sewage sludge, residue of biogas production (digestate), wood ash, and in one control subplot no fertiliser was applied. The subplots were established in the spring of 2011 and the size of each plot was 24×30 m (720 m^2). Class I sewage sludge (according to the regulations No. 362 of the Cabinet of Ministers of the Republic of Latvia) was obtained from the municipal wastewater treatment plant of 'Aizkraukles ūdens' (Aizkraukle Water). Stabilised wood ash was obtained from a boiler house in Sigulda and digestate was obtained from the methane reactor in Vecauce. Sewage sludge and wood ash were spread mechanically before planting the hybrid aspen trees, dosed at 10 t_{DM} ha^{-1} and 6 t_{DM} ha^{-1}, respectively. The digestate was applied immediately after planting the hybrid aspen seedlings as a point source fertiliser, dosed at 30 t ha^{-1}. The major nutrient content in the applied fertilisers is shown in Table 1.

Table 1. The major nutrient content in the applied fertilisers

Fertiliser	Origin	Dose	Application form	The input of major nutrients through fertilisation, kg ha^{-1}		
				N_{tot}	P_{tot}	K_{tot}
Wood ash	Boiler house, Sigulda	6 t_{DM} ha^{-1}	Mechanically	2.6	65	190
Digestate	Methane reactor, Vecauce	30 t ha^{-1}	Point source	69	1.2	99
Sewage sludge	Municipal wastewater treatment plant, 'Aizkraukles ūdens'	10 t_{DM} ha^{-1}	Mechanically	259	163	22

Three samples were prepared from each of the subplots (fertilised with sewage sludge, digestate, wood ash, and control), from a total of 12 trees (Table 2). The hybrid aspen stem discs were sawn at a height of 20 cm above ground for the representation of all six tree rings in each of the samples. The discs had an approximate thickness of 2 cm. These sample discs were air-dried, and a trace metal free sandpaper was used for polishing the sample surfaces.

The sample amount of about 0.2 g was cut out from each of the tree rings and each sample was analysed separately. Microwave-assisted acid digestion was applied for the mineralisation of the samples. Each sample was weighed into a Teflon microwave digestion vessel and sequentially treated with 6 mL of 65% HNO_3 (Trace metal grade, Fisher Scientific) and 2 mL of concentrated 30% H_2O_2 (Trace metal grade, Fisher

Scientific). The vessels were closed, and the samples were heated in a Milestone Start E microwave oven (program: heating to 150 °C over 15 min and holding at 150 °C for 30 min). After the digestion program was completed, the vessels were cooled to room temperature. The samples were then diluted to 25 mL with deionised water (prepared by using Millipore water deionisation equipment). The reliability of the measurements was verified by using a certified reference material (CRM) BCR-060 - Aquatic plant (*Lagarosiphon major*), obtained from the Institute for Reference Materials and Measurements (IRMM), Belgium. The results of analysis for the CRM showed a good agreement with the certificate values (the differences did not exceed 10%).

Table 2. The characteristics of hybrid aspens from different subplots

Subplot	The average height of trees, m	The average mass of stem (fresh biomass), kg	The average mass of branches (fresh biomass), kg	Average diameter at 1.3 m height, mm
Control	9.1 ± 0.5	15.6 ± 1.7	4.0 ± 0.6	69.7 ± 3.5
Sewage sludge	9.3 ± 0.3	19.9 ± 2.0	6.4 ± 1.3	83.7 ± 3.9
Digestate	10.6 ± 0.5	27.8 ± 3.5	9.6 ± 1.1	90.7 ± 3.8
Wood ash	7.6 ± 0.7	12.0 ± 3.5	5.0 ± 2.0	66.0 ± 8.4

For the determination of element concentrations in the samples, standard procedures were used - an Agilent 8900 ICP-QQQ Inductively Coupled Plasma Mass Spectrometer (ICP-MS) equipped with a MicroMist nebulizer was applied for the determination of the following elements – Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Rb, Sr, and Zn. The following instrumental parameters of ICP-MS were set: RF power – 1.550 W, sampling depth - 8 mm, auxiliary gas flow - 0.90 mL min^{-1}, plasma gas flow – 15 L min^{-1}, He cell gas flow - 5 mL min^{-1}. All of the analytical standard stock solutions were TraceCert (Sigma-Aldrich) for ICP (100 mg L^{-1}). For the calibration graph, five different standard solutions in the concentration range from 0.1 μg L^{-1} to 50.0 μg L^{-1} were prepared from stock standard solutions. A calibration graph with blank correction was used for calculating the concentrations of elements in the samples. An Internal Standard Mix solution from Agilent Technologies (10 mg mL^{-1}) was used as internal standard. Stability check of the ICP-MS system was performed by using two standard solutions after every ten samples. A MassHunter workstation program with its Instrument control and Offline data analysis subprograms was used.

For the determination of $\delta^{15}N$ and $\delta^{13}C$, the samples were weighed into tin capsules (the weight of each sample was ~1 mg) and then analysed in duplicate on an EA3000 elemental analyzer (EuroVector) coupled to a Nu-horizon continuous flow isotope ratio mass spectrometer (Nu Instruments). An internal standard sample of glutamic acid was used to check the reproducibility of stable isotope ratio determination. Certified reference materials USGS-40 and USGS-41 (L-Glutamic acid) were used. The $\delta^{15}N$ values were expressed in ‰ relative to air N_2 and $\delta^{13}C$ values in ‰ relative to VPDB (Vienna Pee Dee Belemnite).

The analysis of variance (ANOVA) was used to identify significant differences in element content between wood samples from the differently fertilised subplots. The F value was used as a criterion for the significance of differences, where differences were considered significant if $F > F_{crit}$ at 95% confidence level ($p \leq 0.05$).

RESULTS AND DISCUSSION

The abundance of 17 elements in hybrid aspen wood depending on the applied fertiliser type is shown in Table 3. K, Ca and P were the main elements measured in hybrid aspen during this study, varying from 1,100 to 1,300 mg kg^{-1}, from 830 to 920 mg kg^{-1}, and from 235 to 270 mg kg^{-1}, respectively. Statistical analysis of the obtained results showed that the applied fertilisers did not affect the average content of K, Ca and P in the hybrid aspen stem wood. The obtained results for K, Ca, P, and Mg in hybrid aspen stem wood were similar to those found in previous studies (Rytter and Stener 2003). Statistical analysis also showed that there were no significant differences in the content of Cr and Ni which are considered to be heavy metals. There were also very negligible changes in the content of Na. The highest increase in element abundance occurred in the samples collected from those subplots where wood ash was applied as fertiliser. For example, the highest content of Fe, Mg, Mn, Pb, Rb, and Sr was found in these samples. The use of digestate as fertiliser produced somewhat less change in element content than observed in the case of wood ash. Fertilisation with sewage sludge gave the lowest increase in element content in hybrid aspen stem samples, with the content of some elements, for example Al, Mg, Na and Zn even lower than in the control samples.

Table 3. Element concentrations in hybrid aspen stem wood from different fertilisation subplots (mean ± SD, mg kg^{-1}) (F_{crit} = 3.24, p = 0.05, significant differences between groups denoted with letters a, b, c, d)

Element	Control			Digestate			Wood ash			Sewage sludge			*F*
Al	3.8a	±	1.3	5.8b	±	0.7	11.8c	±	3.6	2.2d	±	0.3	22.8
B	47a	±	14	7.6b	±	1.9	6.0b	±	0.7	45a	±	7	42.3
Ca	880a	±	115	840a	±	92	917a	±	68	831a	±	107	0.8
Cd	0.76a	±	0.15	0.29b	±	0.04	0.36b	±	0.07	0.58a	±	0.20	13.6
Cr	0.3a	±	0.1	0.6a	±	0.4	0.4a	±	0.1	0.3a	±	0.1	2.7
Cu	3.5a	±	0.5	1.3b	±	0.6	1.4b	±	0.7	2.5ab	±	1.0	10.9
Fe	16a	±	3	14a	±	3	30b	±	7	10c	±	2	19.7
K	1117a	±	110	1183a	±	444	1066a	±	235	1286a	±	375	0.4
Mg	307a	±	25	270b	±	36	340c	±	14	290a	±	24	6.6
Mn	6.8a	±	0.1	13.0b	±	1.4	11.6b	±	0.8	8.6a	±	1.5	32.8
Na	45a	±	21	24a	±	2	34b	±	6	23ab	±	13	3.3
Ni	0.11a	±	0.04	0.09a	±	0.03	0.13a	±	0.04	0.09a	±	0.03	1.6
P	235a	±	27	238a	±	47	270a	±	11	235a	±	40	1.3
Pb	0.15a	±	0.04	0.22b	±	0.03	0.26b	±	0.06	0.13a	±	0.03	10.0
Rb	0.41a	±	0.03	0.36a	±	0.09	0.61b	±	0.08	0.53b	±	0.13	8.2
Sr	7.1a	±	1.1	10.7b	±	1.1	12.3c	±	1.1	9.5b	±	1.0	21.1
Zn	17.4a	±	1.4	13.8b	±	2.0	11.3b	±	2.0	11.1b	±	3.9	6.9

According to the experimental results, the element content of hybrid aspen stem wood samples increased in this order: control → sewage sludge → digestate → wood ash. This can be explained with the fertiliser production process. Sewage sludge was obtained from the municipal wastewater treatment plant of Aizkraukle, a small town with no heavy industry, where mainly domestic wastewater with low abundance of trace elements is treated. Also, the produced sewage sludge was not subjected to further treatment, so it had the highest ratio of organic matter to element content. Digestate was obtained by anaerobic treatment of biological materials which degrades organic matter, thus decreasing the ratio of organic matter to element content. Wood ash is practically free of organic matter, giving the lowest ratio of organic matter to element content.

It is possible to use dendrochemistry as a tool for detecting environmental changes that influence a tree over all of its life. Conclusions about the soil properties and the available concentrations of elements during each year of tree growth can be reached by analysing the elemental composition of individual tree rings (Bardule et al., 2020).

The results obtained during this study indicated similar elemental composition of different tree rings. For example, it was shown (Fig. 1) that the concentration of Mg did not significantly change between different tree rings. The change in element content between different samples is shown as standard deviation. The same tendency was also observed for P and Ca. The obtained results can be explained by stable growth conditions – fertiliser was applied only at the time of planting, and the further growth conditions were similar.

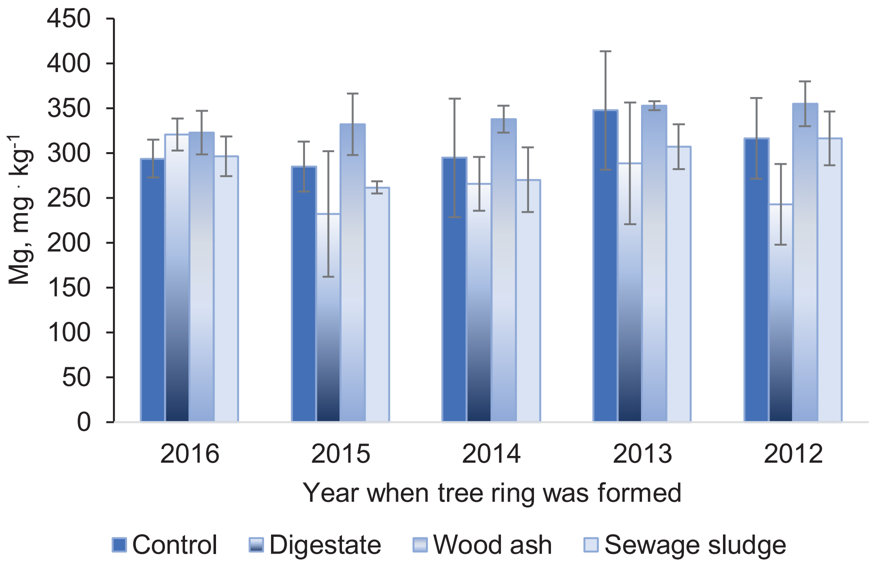

Figure 1. The magnesium content in hybrid aspen stem wood tree rings in a five year period.

The obtained results indicate that for all of the samples regardless of fertilisation type there was a higher amount of K in the more recently formed tree rings (Fig. 2). It can be explained by the fact that K is considered to be a highly mobile element and it is more effectively translocated to the growing parts of the plant (Ragel et al., 2019).

The concentration of Ca decreased in the more recently formed tree rings (Fig. 3). This trend can be explained with the low mobility of Ca, which is concentrated mostly inside cells, so it is largely immobilised in wood as the dying of old cells and formation of new cells takes place (Yang & Jie, 2005; Thor, 2019).

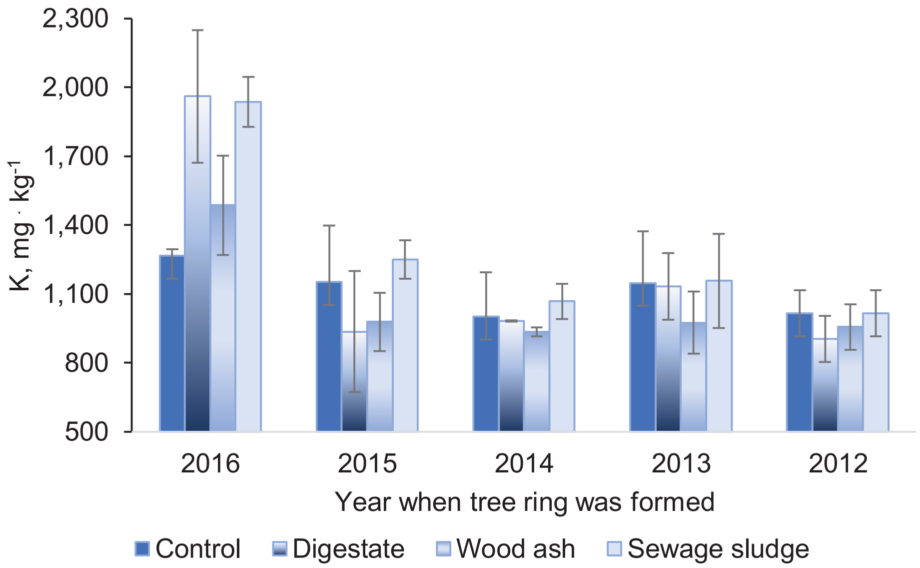

Figure 2. The potassium content in hybrid aspen stem wood tree rings in a five year period.

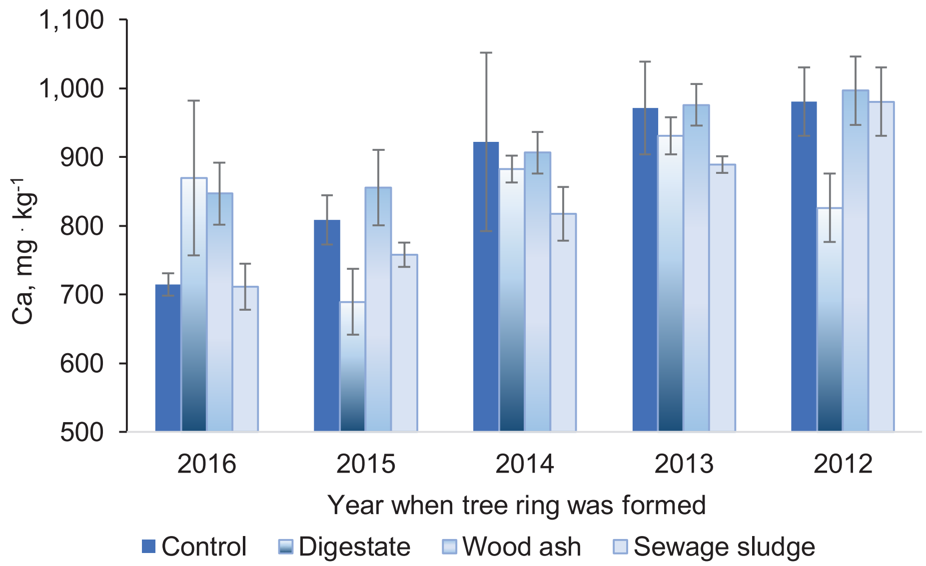

Figure 3. The calcium content in hybrid aspen stem wood tree rings in a five year period.

According to the measured N and C content (Table 4), there were no significant variations between different tree rings. The mass percentage for N was about 0.2%, and for C it was 45.5%. However, the determined isotope ratio values varied over a relatively wide range: $\delta^{13}C$ from -27.1 to -28.4 ‰ and $\delta^{15}N$ from -9.3 to -15.7 ‰.

Table 4. The $\delta^{15}N$, $\delta^{13}C$ values and mass percentages of N and C in hybrid aspen stem wood tree rings from different years (mean values ± SD)

	$\delta^{15}N$, ‰			wN, %			$\delta^{13}C$, ‰			wC, %		
Bark	-1.96	±	0.13	0.608	±	0.030	-30.54	±	0.23	46.92	±	0.08
2016	-15.70	±	0.19	0.170	±	0.003	-27.38	±	0.28	45.27	±	0.21
2015	-10.88	±	0.16	0.202	±	0.040	-26.96	±	0.08	45.76	±	0.80
2014	-11.50	±	0.26	0.171	±	0.009	-27.10	±	0.16	45.19	±	0.45
2013	-12.03	±	0.36	0.192	±	0.006	-27.91	±	0.26	45.45	±	0.21
2012	-10.20	±	0.14	0.208	±	0.019	-28.42	±	0.45	45.26	±	0.07
2011	-9.30	±	0.17	0.213	±	0.007	-28.24	±	0.06	45.56	±	0.11
Pit	-11.30	±	0.40	0.191	±	0.008	-28.27	±	0.02	44.84	±	0.71

The results of other studies show that the $\delta^{13}C$ value is mostly dependent on the type of plant and the photosynthesis mechanisms (Marshal et al., 2007). In addition, the variation in $\delta^{13}C$ values between different tree rings can be explained with the weather conditions during the particular years. However, the reasons for variations in $\delta^{15}N$ values are not as clear because that value may be affected both by the applied fertiliser and the natural nitrogen cycle (Pardo et al., 2013).

CONCLUSIONS

Although the applied fertilizers contained quite variable amounts of nutrients, the results showed that fertilisation treatments mostly did not affect concentrations of macro elements (e.g., P, K, and Ca) and some microelements (e.g., Cr, Ni) in stem wood of hybrid aspen. Thus, it seems that the soil had an adequate amount of these nutrients for the juvenile hybrid aspen trees or there is a large natural variation of nutrient and microelement content in the soil in the research object. Accumulation of the several microelements (e.g., Fe, Mg, Mn, Pb, Rb, and Sr) in hybrid aspen is affected by the type of fertiliser that has been applied. In this study, the use of wood ash or digestate affected the elemental content of hybrid aspen wood to a greater extent than the use of sewage sludge, compared to an unfertilised control plot.

When comparing the elemental concentrations between different tree rings, higher concentration of potassium was observed in the most recent tree ring, indicating the ability of plants to efficiently transport potassium to the growing parts of the plant.

The results obtained in this study indicated that the calcium content gradually increased from younger to older tree rings, thus pointing to the low mobility of Ca^{2+} ions.

The variability of elemental abundance between different tree rings was quite small for the majority of the studied elements, probably due to the stable growth conditions.

The $\delta^{15}N$ and $\delta^{13}C$ values showed greater variations that could provide data about growth conditions of processes that are not observable from the measurements of elemental abundance.

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