Norway Spruce as an Environmental Archive for Sulphur Dioxide

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

Timothée Barrelet

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Leiter der Arbeit:
Prof. Dr. Urs Krähenbühl
Departement für Chemie und Biochemie der Universität Bern
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Von der Philosophisch-naturwissenschaftlichen Fakultät angenommen.

Bern, 26.01.2006   Der Dekan:
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La nature est un temple où de vivants piliers
Laissent parfois sortir de confuses paroles
L'homme y passe à travers des forêts de symboles
Qui l'observent avec des regards familiers.

Charles Baudelaire

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1. Introduction

1.1 Aim of the thesis

Since the 1750ies, human activity is increasingly altering the natural carbon, nitrogen and sulphur cycles. Worldwide sulphur dioxide emissions are essentially anthropogenic and likely to increase during this century. Immission changes in Switzerland are monitored using environmental archives on one hand, and measuring networks on the other hand. One type of archive, peat bogs, was studied in our research group before: Sulphur content profiles found in the peat bogs of Düdingen and St Moritz all reflected the local sulphur dioxide immissions. Peat moss takes up atmospheric sulphur dioxide, and higher plants are known to do this as well. Thus, the presence of forests at both sites led to the idea of comparing the results with a second type of environmental archive: trees. Aim of the present thesis was to investigate the suitability of trees as archives for sulphur dioxide.

Compared to peat bogs, trees are easier to sample, can be found over a wide range of altitudes and even in city centres. Furthermore, dating is facilitated by the presence of tree rings. Unlike lifeless environmental archives such as ice cores, sediments and speleothems, a tree is a living being. Thus, knowledge about its physiology is essential. This is particularly important for sulphur, the element investigated in this work to retrace the pollutant sulphur dioxide. Notwithstanding its low concentration compared to nitrogen, sulphur nutrition plays an important role in plants and is a macronutrient as well. Because it has been the subject of numerous metabolism studies, and because it is the dominant tree in Switzerland, Norway spruce was chosen for the present study.

Data obtained by the National Air Pollution Monitoring Network (NABEL) show that there are huge differences between the SO_2 immissions in alpine, rural and urban areas. This is why three distinct areas were chosen for our work. Norway spruce was the main tree species in each of the sampled forests. Sampling had to be optimised in order to avoid contamination, and precise dating of the drill cores was achieved using radiodensitometry at WSL.

Microwave acid digestion had been used previously for analysing sulphur in peat bogs. The method was therefore adapted to wood. At the University of Bern and at EMPA, microwave acid digestion of the collected drill cores and subsequent analysis by atomic spectrometry allowed assessing the total sulphur content, although with a relatively coarse time resolution.

Since high time resolution is preferable for environmental archives, the next step was the achievement of annual resolution using LASER ablation. For that purpose, coupling of the LASER with a high resolution inductively coupled mass spectrometer had to be developed at EMPA. A further step was the measurement with seasonal resolution, which permitted an insight into distribution of elements within single year rings.

Plant physiological findings were complemented by speciation of sulphur compounds using X-ray absorption spectroscopy. Measurements were performed in collaboration with the PSI and the University of Wisconsin, with the aim to identify organic sulphur compounds in wood. Additionally, x-ray fluorescence spectrometry of soil samples at EMPA allowed studying the influence of soil composition on element content in the stem.

Norway spruce was hence investigated using a palette of state-of-the-art analytical methods and a multidisciplinary approach on the theoretical level, thus revealing interesting aspects in environmental chemistry and plant physiology.

1.2   Sulphur dioxide

1.2.1   Global SO₂ emissions

In 1987, 88% (150 Mt) of the total emissions of sulphur dioxide on the globe were anthropogenic, thus topping emanations from volcanoes [*Parlar and Angerhöfer, 1995*]. Sulfur dioxide is a by-product of coal and fuel combustion, ore reduction, numerous industrial processes, and vehicle exhaust. In industrialised countries, emissions have decreased by a factor of 3 since they reached a maximum in the late 70ies and early 80ies of the last century. Measures such as the replacement of coal and the desulphurisation of mineral oil led to this progress.

However, in emerging countries, SO_2 emissions still increase, due to the continuing use of coal power plants. For example, China covers 76% of its energy demand with coal, leading to the combustion of 980 Mio t in 1995. Back then, SO_2 emissions amounted to 21 Mt. They are expected to reach 32 Mt by 2020. Globally, according to the most probable scenarios published in the latest report of the international panel on climate change (IPCC 2001, Fig. 1.), emissions will increase again and are likely to exceed 150 Mt again.
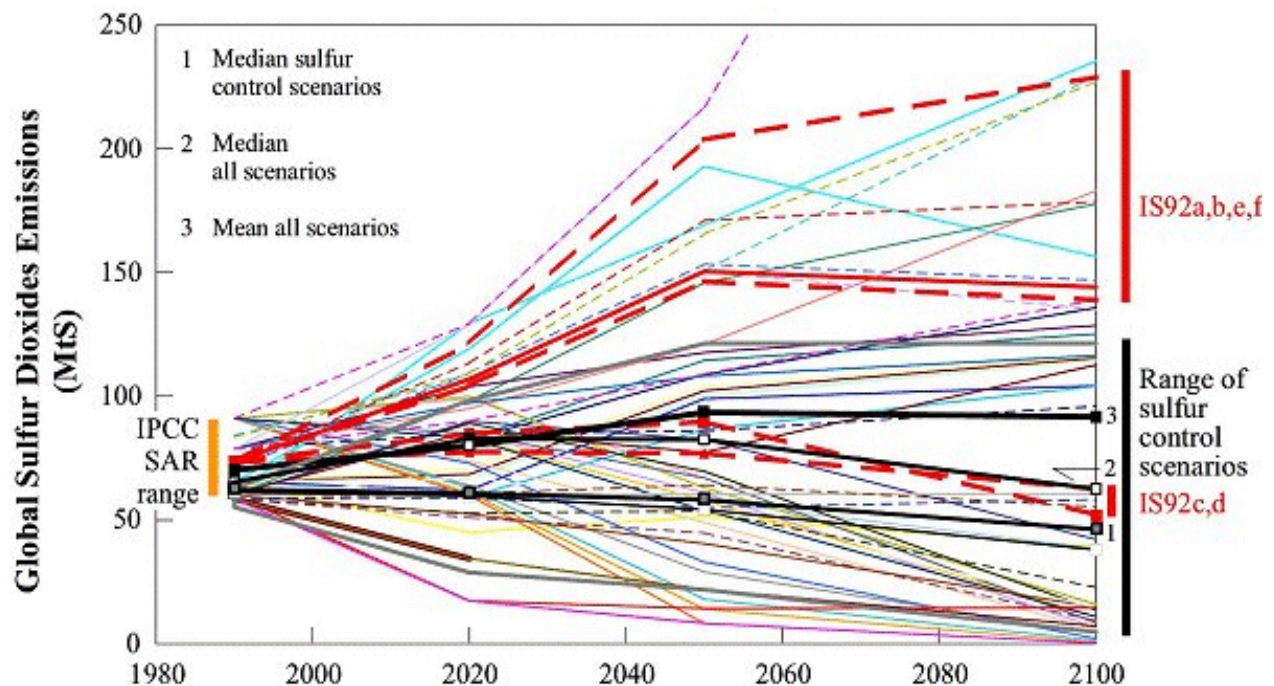
Fig.1.: Emission scenarios for SO₂ as evaluated by the International Panel on Climate Change in the IPCC report 2001 (Penner et al., 2001)

Generally, natural background concentration of SO_2 is 0.28 to 2.8 mg/m³, but values can be as high as 200 mg/m³ near pollution sources [*WHO, 2000*].

1.2.2   SO₂ emissions in Switzerland

Concerning the immission situation in Switzerland, atmospheric concentrations (3.1.2005) determined by the NABEL (National Air Pollution Monitoring Network) show that there are significant differences between the different measuring sites (e.g. the Alps and the city of Zurich). NABEL is operated by the Swiss Federal Laboratory for Materials Testing and Research (EMPA) on behalf of the Swiss Agency for the Environment, Forests and Landscapes (SAEFL). It monitors air pollution at 16 locations throughout the country. One of

them, Härkingen, is situated near a motorway. As of 3.1.2005, a SO_2 concentration of 0.00315 $\mu l/m^3$ was measured. In the city of Zurich, it amounted to 0.001 $\mu l/m^3$, and in the foothills of the Alps 0.00034 $\mu l/m^3$. Similarly to other industrialised countries, SO_2 emissions have been significantly reduced in Switzerland. Back in 1988, they were up to 10 times above these values.

1.2.3 SO_2 in the atmosphere

In the atmosphere, the lifetime of this trace gas amounts to 9 days [*Bliefert, 2002*]. Sulphur dioxide is the atmospheric sulphur compound with the second longest lifespan (longest lifetime: sulphur carbonyl, shortest lifetime: sulphur trioxide). It is oxidised to sulphate by oxygen and ultraviolet radiation. This occurs at an accelerated rate in the presence of NO_x (tenfold over that of clean air), O_3 or HO_2. [*Howarth and Stewart, 1992*]. Sulphate aerosols operate a negative radiative forcing in the atmosphere, thus leading to a cooling [*Penner et al., 1994*]. There are both a direct and an indirect effect. On one hand, incoming solar radiation is scattered or reflected. On the other hand SO_4^{2-} aerosols act as cloud condensation nuclei (CCN), increase the number of droplets and hence the amount of clouds, which leads to a greater albedo. Forcing is maximal near main sulphate aerosol source regions. The diminution of SO_2 in industrialised countries could unmask the real extent of global warming and lead to an amplified temperature rise in these regions.

1.2.4. Uptake of SO_2 by plants

Both sulphur excess and deficiency are a source of stress for plants. In developing countries, sulphur excess is predominant, while in industrialised countries, sulphur deficiency dominates. Since sulphur is not growth limiting in forests, deficiency is only a problem for crops, which give the decisive impulse to research in plant sulphur nutrition.

In case of heavy pollution (chronic atmospheric levels of 0.05 $\mu l/m^3$), SO_2 becomes an additional sulphur source for plants. For instance, sphagnum mosses, which are the main constituent of ombrotrophic peat bogs, take up SO_2 readily, since they lack the protection by a cuticle [*WHO, 2000*]. In contrast to sphagnum mosses, the pathway via the cuticle is limited for trees like Norway spruce. The gas enters the needles via stomata, and is then removed from the substomatal cavity. This occurs by solubilisation in the aqueous phase of the apoplastic space (solubility of SO_2 at 20°C: 39.374 l or 106.6 g per litre water), and is followed by the uptake into the cytoplasm of surrounding cells. In the cell, after enzymatical or non-enzymatical oxidation to sulphate, either vacuole storage or assimilation into the amino acid cysteine takes place, which is an important detoxification step [*Noji et al., 2000, Rennenberg, 1984*].

Although uptake of SO_2 by the needles reduces the uptake via roots substantially [*Giesemann et al., 2000*], the latter remains the principal uptake mechanism for incorporation of sulphur into wood. Allocation of sulphur from source needles to the stem is low [*Schupp et al., 1992*]: instead, the following needle generation receives most of the sulphur [*Schneider et al., 1994*] and fallen needles then contribute to raise the soil sulphur content.

For forest trees, uptake via roots after wet deposition is the principal mechanism of sulphur acquisition. The huge canopy surface of a Norway spruce tree amplifies this phenomenon: important amounts of aerosols and fog are deposited, because crown interception is three times higher than for a deciduous tree like beech (*Fagus sylvatica*) [*Ulrich et al., 1979*]. Accordingly, sulphur accumulation in the soil under spruce is four times higher than under beech. A third uptake mechanism through the bark takes place when sulphate-containing water runs along the stem.

1.2.5. Toxicity of SO$_2$ for plants

Sulphur dioxide is phytotoxic at concentrations above 0.03 µl/m^3 in air [*WHO, 2000*]. The limit values set in the World Health Organisation (WHO) guidelines are 0.01 µl/m^3. Chronic damage of plants by SO$_2$ is of cumulative nature and more important than acute exposure [*WHO, 2000*]. It modifies the physiological response to biotic and abiotic stress. For instance, Silver fir (abies alba) reacts to it with a slowdown of its growth [*Elling et al., 1999*]. Norway spruce is less sensitive, but low-temperature stress augments the sensitivity of the tree to SO$_2$ [*WHO, 2000*]. Thus, the effects of the pollutant can be enhanced by biotic and abiotic stresses. Conversely, SO$_2$ can modify the plant's stress response, often impairing its resistance. Furthermore, synergetic effects of SO$_2$ can occur with other gases such as O$_3$ [*Csintalan and Tuba, 1992*] and NO$_x$ [*Kasana and Lea, 1994*].

Indirect effects of SO$_2$ on trees are due to a lowering of the pH value of precipitations (acid rain). This acidification is particularly pronounced in mist, where solute concentration can be tenfold superior to those in rain [*WHO, 2000*]. Under such conditions, it is likely that cations are leached out of Spruce needles [*Burkhardt and Drechsel, 1997*]. Soil acidification is buffered on four levels. Soil composition is of great importance, since the buffering capacity depends on carbonate, silicate and exchangeable base cations content. On the fourth buffer range (pH 3.8-4.2), Al^{3+} cations are released [*WHO, 2000*]. They are toxic to the root system, and if the pH value reaches 3.8, heavy metals are released in turn.

1.3 Sulphur metabolism

Sulphur is one of the six macronutrients for plants, together with nitrogen, phosphorus, potassium, calcium, and magnesium. Although it is only 3% to 5% as abundant as nitrogen [*Leustek and Saito, 1999*], it plays a crucial role for protein biosynthesis (cysteine and methionine) and defence mechanisms (glutathione, metallothioneins). Cysteine is the final product of the reductive pathways. Its biosynthesis is the reaction connecting N, C and S assimilation [*Koprivova et al., 2000*]. This underlines the impact of global change on vegetation: massive N immissions, rising CO$_2$ emissions and changing SO$_2$ emissions, depending on the continent.

Since the presence of S in wood is not solely due to anthropogenic SO$_2$ emissions, knowledge about sulphur nutrition is important. This is why not only total S content, but also speciation of S compounds in Norway spruce wood is particularly interesting. All investigated trees have witnessed a constantly changing N, C and S input during their entire lifetime, with still augmenting atmospheric concentrations of NO$_x$ and CO$_2$, and a sharply rising and falling SO$_2$ concentration in the late 1970ies, early 1980ies. Thus a closer look on their metabolic reaction to global change is insightful, especially because Norway spruce represents 48% of all trees in Switzerland.

Plants cover their demand for sulphur by reduction of inorganic sulphate. Chloroplasts are the primary site of SO$_4^{2-}$ reduction. Sulphate is activated by ATP sulphurylase to adenosine 5'phosphosulphate (APS) [*Kopriva and Rennenberg, 2004*]. APS is then reduced by APS reductase to sulphite in a reaction which depends on glutathione (GSH). Sulphite is further reduced by a ferredoxin-dependent sulphite reductase. Sulphide is then incorporated into the amino acid skeleton of O-acetyl-L-serine (OAS) by the enzyme OAS (thiol) lyase, thus forming cysteine. It can be further metabolised to methionine or directly incorporated into proteins or glutathione. This tripeptide has important functions as storage and transport form for reduced sulphur. It also acts in defence against oxidative stress (in presence of reactive oxygen species, ROS) and regulates S assimilation. However, Norway spruce is not particularly sensitive for photo-oxidative stress or ozone [*von Ballmoos, 2003*].
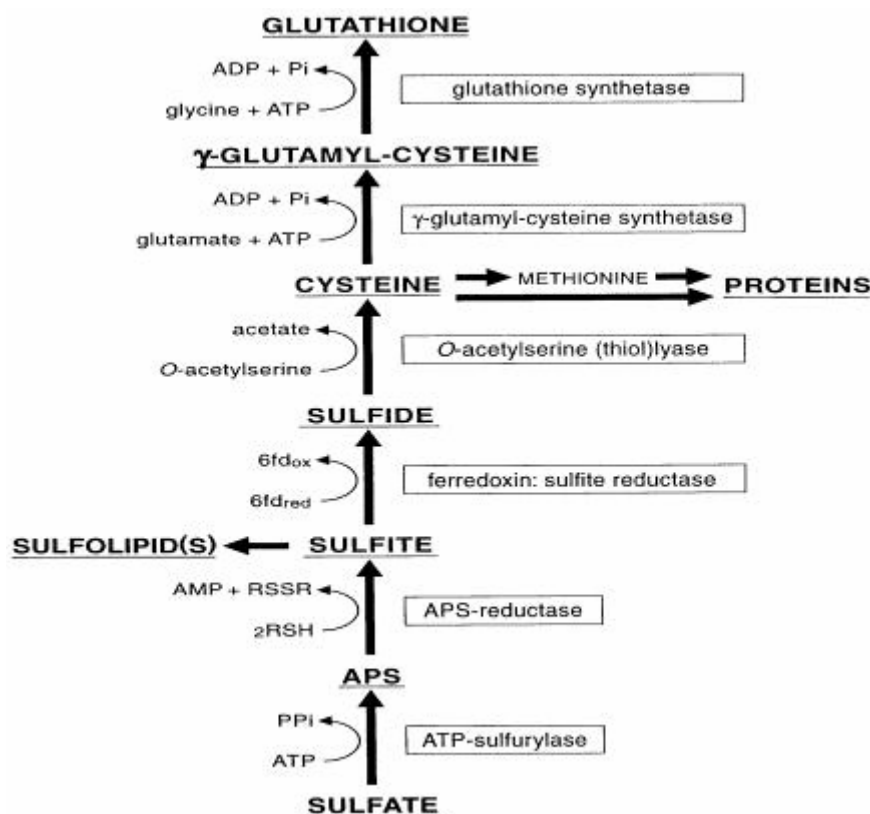
Fig. 2. : Assimilation of S in higher plants [De Kok, 1990]

The regulatory interactions between sulphate and nitrate are known to be well coordinated: deficiency for one element represses the other pathway. Nitrate assimilation depends on the supply of carbon skeletons produced during CO_2 assimilation. Less is known about interactions of sulphur and carbon assimilation, but most occur in the context of photosynthesis. Thus, sulphate assimilation is dependent on photosynthesis as a direct or indirect source of reduction equivalents. In the dark, the flux through sulphate assimilation is lower than in the light. S limitation reduces both growth and photosynthesis. Carbohydrates themselves are known to regulate SO_4^{2-}: for the synthesis of cysteine SO_4^{2-} requires the carbon skeletons of OAS as acceptors of reduced sulphide.

1.4 Norway spruce

1.4.1 Occurence

For an environmental study, it is important to have a representative tree, which is found in several places with different immission situations. This is why Picea abies (L.) Karst. (Norway spruce) was chosen within the scope of this study. The genus Picea is very common in the northern continents, Africa excepted. Norway spruce is Europe's dominant conifer and located in the northern, central and eastern parts of the continent, outside permafrost areas. In the south, it extends to northern Greece and in the West to the Massif Central, France [*Vidakovic, 1991*]. Norway spruce is also established in places outside native range such as Britain or the Pyrenees Mountains (France). Furthermore, it is naturalised in the north-central United States, as well as in Canada [*Taylor, 1993*] In Switzerland, Norway spruce represents 48% of all trees, according to the Swiss Federal Statistical Office 2004. It occurs in places as different as the Jura, the Swiss Plateau, the Alps and even in cities.

1.4.2 Dependence on temperature, precipitations and environment

Norway spruce can bear temperatures as low as -50 to -60°C (Siberia 75°25' N) [*Schmid-Vogt et al., 1977*]. In contrast to deciduous trees, the tree still assimilates in winter, even at a temperature of -6°C. Below this point, photosynthesis (at 18°C, 5 mg CO_2 per hour and g dry needles) is interrupted. In this case, losses of chlorophyll during the winter slow the rise of photosynthetic capacity in spring somewhat. The shoot is very sensitive to frost, thus being the Achilles heel. Only the needs for warmth of larch (Larix decidua) are lower, which shows that Norway spruce is perfectly adapted for coldness [*Schmid-Vogt et al., 1977*].
Drought situations are more critical. Water demand of spruce strongly depends on local temperatures. If average annual temperatures are between -1 and 2°C, 400-450 mm precipitations are sufficient. For warmer places, 600-800 mm are optimal. Compared to other tree species, Norway spruce consumes little water. Transpiration amounts to 1.4 g water per g fresh needle weight. For comparison, birch (betula pubescens) transpires 9.5 g water per g of fresh leaves [*Schmid-Vogt et al., 1977*].
For trees, alpine environment is stressful, although pollution level is much lower compared to the Plateau. At high altitudes, spruce trees grow slowly and have a higher root percentage. Thus, their diameter is smaller and tree rings are narrower compared to trees from the Swiss Plateau, for instance. Usually, spruce trees from the Swiss Plateau reach a diameter of 60 to 80 cm and a wood volume of 8 m^3, according to the WSL (Swiss Federal Institute for Forest, Snow and Landscape Research). The tallest Norway spruce tree is growing in the Calfeisen valley (canton St Gallen, Switzerland). Its diameter amounts to 1.84 m and the wood volume is of 22 m^3. The tree has a cambial age of 350 years.

1.4.3 Wood

Lignification, which begins in the first tree rings after the cambium, and is completed with heartwood formation, is also accompanied by changes of the element content of wood. Identically to other trees, the thickening of the Norway spruce stem is due to cell division in two directions: the first cells issued from the cambium mother cells are all morphologically identical xylem and phloem mother cells [*Schoch et al., 2004*]. Their daughter cells later have distinct functions. Xylem consists of two types of cells: prosenchymatous and parenchymatous cells. The prosenchymatous cells are thick-walled fibres serving almost exclusively as support. Parenchymatous cells are classified into vessels with wide lumen (trachea) and tracheids, both adapted for water conduction. Spruce wood is made of 93 to 96.5% tracheids [*Schmid-Vogt et al., 1977*]. These elongated cells are 3.4 mm long and 30-40 μm broad, and thus several times greater than the original initial cells [*Schoch et al., 2004*]. Earlywood tracheids, which are especially large, live for 2-3 weeks. Tracheids in the latewood have a longer lifespan of 2-3 months. Long living parenchyma (storage cells) can last several years; they die off when heartwood formation occurs. The individual cell elements mentioned previously are organised in elongated, axial cell bands and are connected to a horizontal cell system, the rays (transport and storage).
The process of heartwood formation begins a few cell rows after the cambium. Sporadic degeneration of the protoplast occurs and gradual disintegration of the cytoblasts and mitochondria augments with increasing distance from the cambium. In contrast to sapwood, heartwood does not contain living parenchyma cells anymore. It contains 5 times less water and 25% less resin. Its carbohydrate content is less diversified (mannose instead of glucose, saccharose, fructose and galactose), because most of them are transformed into phenols during transition. Due to the apoptosis, which is accompanied by enzymatical decomposition of cell components, protein content falls from 0.5% near the cambium to 0.25 in direction of the pith [*Sandermann et al., 1967*]. For heartwood formation, the wooden tissue must have a minimal age and a minimal distance from the cambium.

In response to growth stresses, gymnosperms form compression wood on the stressed side of the tree (e.g. in case of mechanical pressure by wind, snow, slope or competition). This reaction wood has wider, denser and darker tree rings, compared to the opposite wood. Compression wood contains up to 25 % less cellulose than normal wood and its lignin content is augmented by up to 40% [*Timell, 1986*]. It thus provides more hydroxyl binding sites, thus leading to higher cation content [*Prohaska et al., 1998*].

1.5.   Sampling sites

Three sites with different SO_2 immission levels were selected (see fig. 3): two on the Swiss Plateau (Düdingen, 580 m a.s.l., Frieswil, 740 m a.s.l.), and one in the Alps (St Moritz, Engadine, 1900 m a.s.l.). The forests of Düdingen and St Moritz are located near peat bogs, which are ideal as environmental archives for comparison.
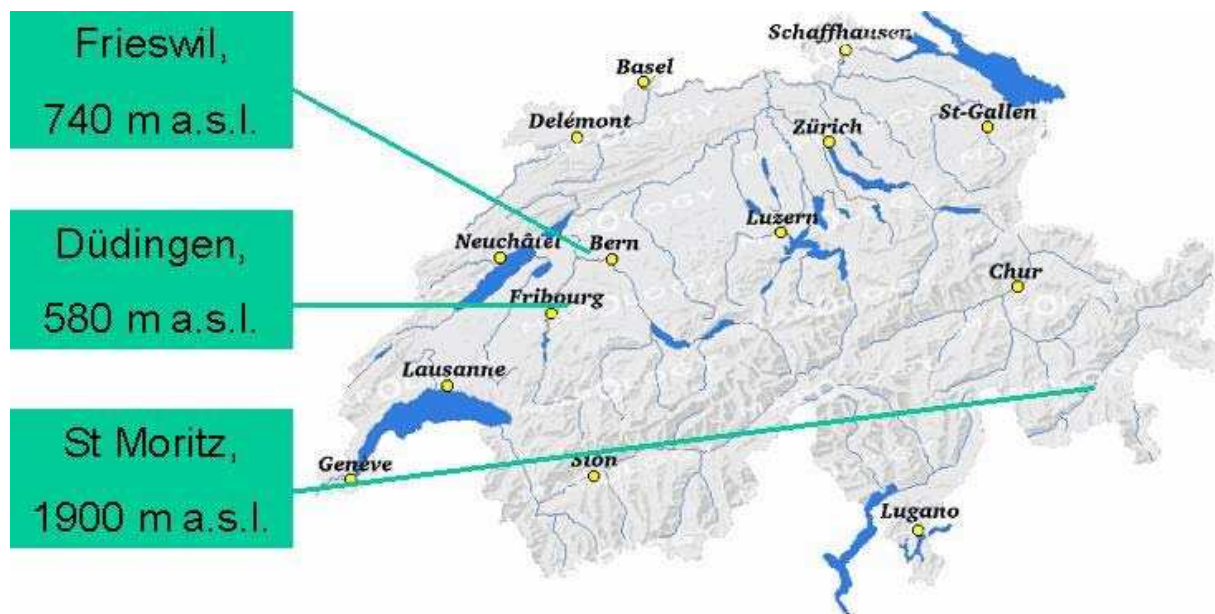

Fig.3.: Location of the sampling sites (Map: © Swissgeo, Mapology SA)

The three sampling sites represent regions in Switzerland with a distinct pattern of the SO_2 concentrations, similarly to the measuring stations monitored by the NABEL network (see chapters 1.2 and 3.2).
In St Moritz, the tree drill cores were collected 100 m above and 500 m away from the peat bog. The Engadine is secluded from SO_2 emissions coming from the industrialised Swiss Plateau, and local immissions are small.
In Düdingen, the peat bog is in 1 km distance, and the forest is located just 500 m away from the motorway A12 (Bern-Freiburg). The motorway was inaugurated in parts between 1971 and 1973. Thus, the main sulphur dioxide source in the Düdingen area is likely the traffic (diesel fuel). Interestingly, tyre abrasion did not seem to augment the soil sulphur content in the Düdingen forest, since it is the lowest of all three sample sites.
At the rural site of Frieswil there is neither a direct SO_2 source nor an additional environmental archive. The site was chosen for comparison and, as seen in the sulphur content profiles, its immission situation is typical for the Swiss Plateau.

2.   Methods

2.1.   Sampling

At each of the three sites, six trees were sampled. 6 drill cores were taken at breast height, at right angles with the stem and using a 5 mm increment borer (Suunto) and the corresponding metal tongue for sample extraction. The drill cores were taken and used as shown on fig 4.:
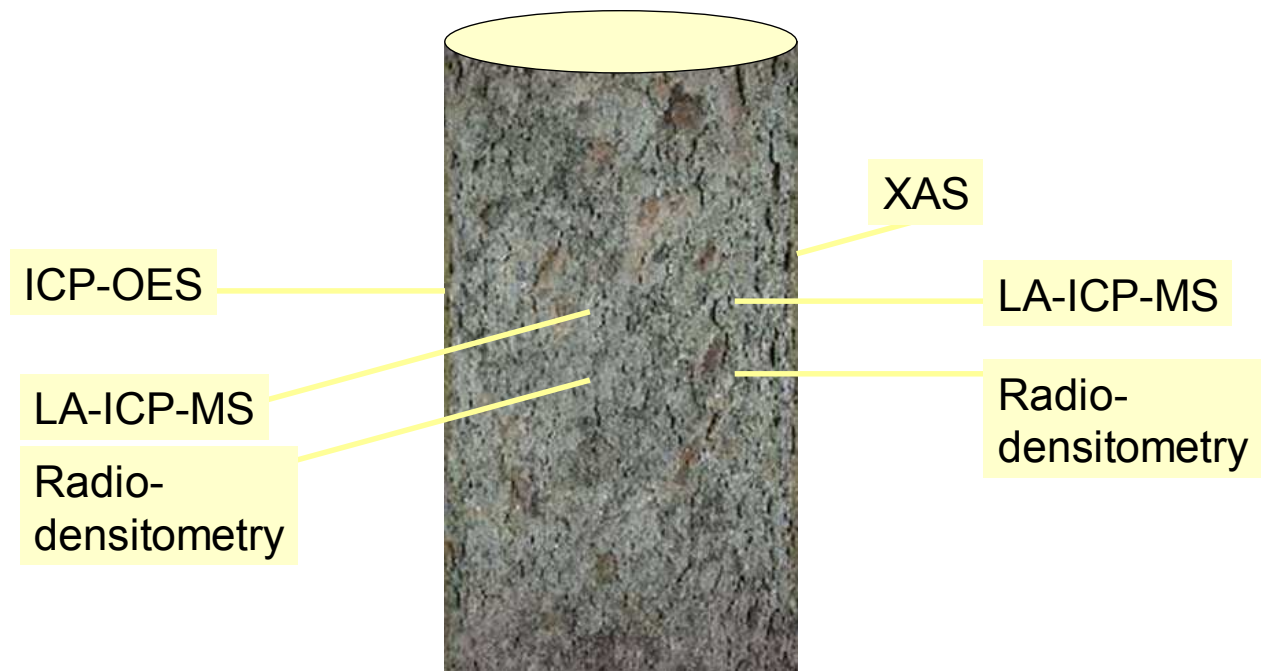

Fig. 4.: Sampling scheme for each tree

2 drill cores (southern and western side of the stem) were destined for LASER ablation ICP-MS (see chapter 2.6.), and two additional cores were taken 5 cm underneath for radiodensitometry (see chapter 2.5.). A further drill core (northern side) was taken for ICP-OES (see chapter 2.2.), and the last (eastern side) was destined for X-ray absorption spectroscopy

First, the extent of the humid sapwood has to be determined with a tape-measure. In Norway spruce, this physiologically important zone is only visible when wet. The retroactive use of a reagent (e.g. Ninhydrine [*Sandermann et al., 1967*]) is not very efficient and contaminates the sample.

The drill cores are then pressed into previously cleaned and weighted plastic rails and wrapped in saran foil. At the laboratory, the sample rails were weighted again and heated at 40°C until achievement of weight constancy.

The boreholes were neither sealed nor treated, since such measures are ineffective and sometimes even counterproductive [*Dujesiefken et al., 1999*]. Natural resin flow helps sealing off the wall of the borehole. In the worst case, 7% (Swiss Plateau) to 21% (Alps) of the trees suffer medium damage, mostly due to fungal hyphae (e.g. heart rot fungi) [*Eckstein and Dujesiefken, 1998/99*].

In order to avoid contamination of the samples, all manipulations had to be performed with gloves. Furthermore, the lubricating and cleaning agent for the increment borer had to be sulphur free. Measurements performed by ICP-OES ensured that the PhEur grade paraffin (Hänseler) used for this purpose had a very low S content compared to WD-40™.

In each forest, soil samples were taken as well. Using a garden corer, drill cores with a length of 20 cm were taken after removal of the loose foliage. After freeze-drying and grinding, the samples were measured by wavelength-dispersive x-ray fluorescence spectrometry.

2.2. Inductively coupled plasma optical emission spectrometry (ICP-OES)

The optical emission spectrometers used for this study were a sequential Varian Liberty 150 AX Turbo with axial torch (University of Bern) and a simultaneous Varian VistaPro with a radial torch (EMPA). Both were operated under standard hot plasma conditions. For sulphur determination, the optical chamber had to be rinsed with inert gas (N and Ar, respectively) because oxygen absorbs the sulphur emission line. Analysis was performed at the 180.734 nm line for sulphur, and at the 769.896 nm, 315.887 nm and 257.610 nm lines for K, Ca and Mn, respectively. For analysis of the alkaline (0.4 M NaOH) solutions from the oxygen bomb combustion on the Varian VistaPro, the Varian HF kit had to be mounted. Sulphur measurements were performed using the 181.972 nm and 180.669 nm lines. For further details see chapter 3.2.

2.3. Microwave acid digestion

The drill cores were cut into segments of 5 or 10 tree rings, depending on the weight (minimal weight 40 mg). This relatively coarse time resolution is sufficient, but not optimal for an environmental study.

Previous optimisation studies showed that the use of quartz vial inlays is counterproductive and leads to sulphur losses. Thus, the samples were directly put into the Teflon® bombs. Microwave digestion was performed in a 1:1 of nitric acid and hydrogen peroxide. A MLS ETHOS 1600 microwave oven was used for this purpose (software: easyWAVE3). The method "Holz", especially developed for sulphur analysis in wood, was applied: gentle heating during 17 minutes until the temperature of 150°C, maintained during 10 minutes.

It was verified if the addition of MgO, as recommended by some authors [*Hafez et al., 1991, Jeker et al., 2001*], was helpful for preventing sulphur losses. As seen on the figures 5 and 6, evaluation by adding increasing amounts of MgO to the nitric acid showed that there is no effect on sulphur concentration of the solution (see fig. 4 and 5). This is likely due to the high magnesium content (100-600 mg/kg) in wood compared to the low sulphur content of 20-100 mg/kg (Mg:S ratio about 5-6, see chapter 3.3). In comparison, total Mg content in a peat bog is 900-3000 mg/kg [*Schreier, 2005*], total S content is 1000-5000 mg/kg [*Jeker et al., 2001*] (Mg:S ratio about 0.6-0.9).

Recovery rate was calculated by measuring the BCR 101 standard reference material (pine needles, S content: 1700 mg/kg). It amounted to 97.3% (1654 mg/kg).

ICP-OES being a multielemental method, other elements were measured in addition to S: Mg, K, Ca, Zn, Mn, Cu, Cr, Ba, Al.
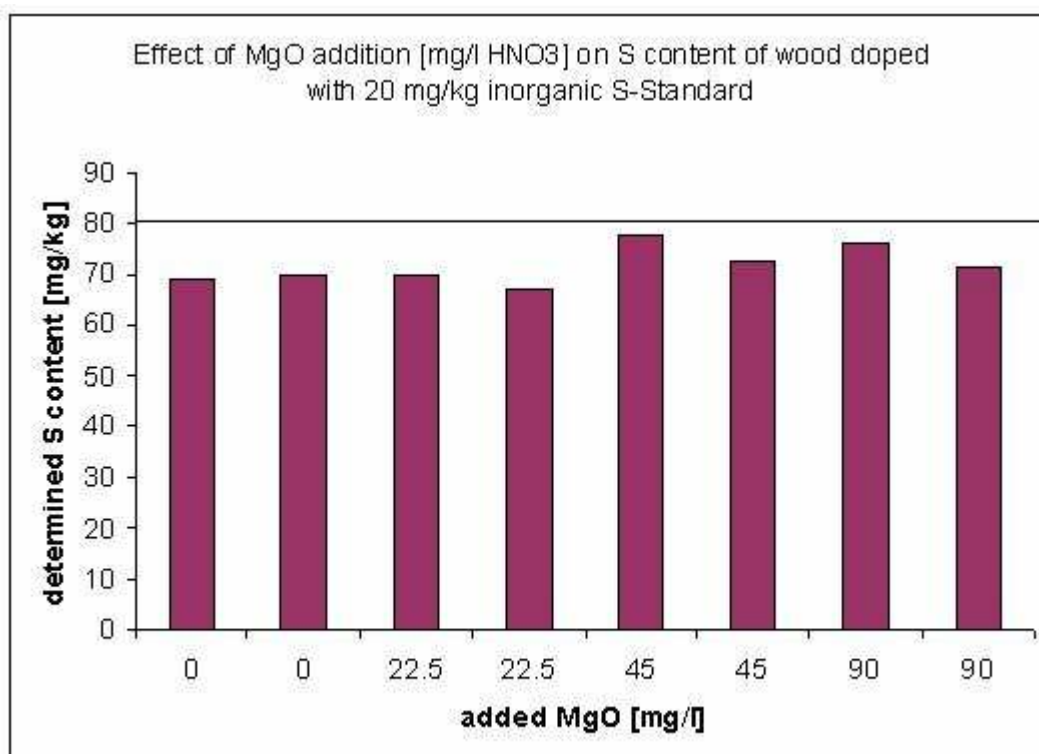
Fig. 5.: Evaluation of MgO addition using homogenised wood (average S content: 60.5 ± 4.7 mg/kg) doped with 20 mg/kg SO$_4^{2-}$. Total S content: 80.5 ± 4.7 mg/kg (horizontal black line)
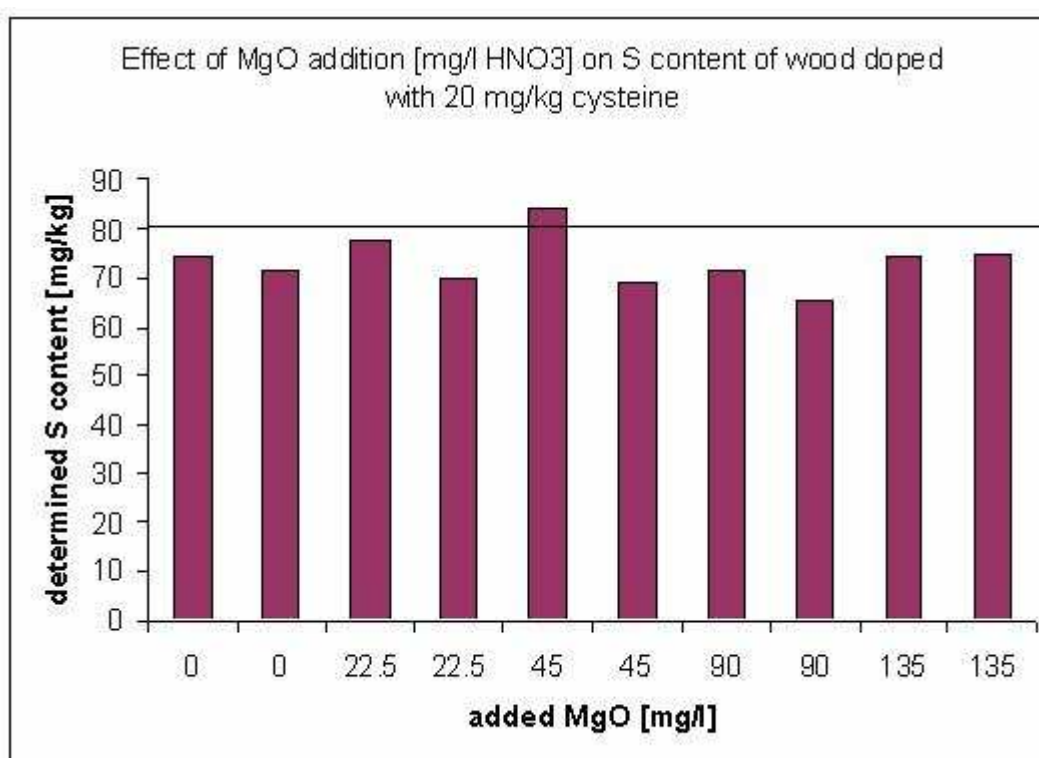

Fig. 6.: Evaluation of MgO addition using homogenised wood (average S content: 60.5 ± 4.7 mg/kg) doped with 20 mg/kg cysteine. Total S content: 80.5 ± 4.7 mg/kg (horizontal black line)

2.4   Oxygen bomb combustion

In order to have an independent analytical technique for comparison, oxygen bomb combustion was also evaluated. This method is routinely applied for determining the sulphur content of coal and petroleum products but was never used for wood before. 70 mg wood sample were put into a quartz glass crucible in its holder. A cotton thread moistened with ethanol and leading to the crucible was fixed to the ignition wire. The decomposition vessel was filled with 30 bar. The acid gasses inclusive SO_2 were absorbed in 10 ml 1M NaOH.
Oxygen bomb combustion is not advantageous for low sulphur concentrations. Another disadvantage is that it is time consuming, and chemical yield is in the order of 80%, even for samples with high S content (1700 mg/kg), such as the BCR certified reference material 101 (Spruce needles).

Fig. 7.: Total S content profiles of two drill cores issued from one single tree from St. Moritz, measured by ICP-OES after acid digestion (solid black line) and oxygen bomb combustion (dashed line).

Average bark sulphur content of the three trees (217 mg/kg) was also lower than the average bark sulphur content obtained by acid digestion (579 mg/kg). Two of three drill cores measured had values below the detection limit of 36 µg/g S (as determined for samples with a weight between 70 and 200 mg). With exception of the third core, seen on figure 7, oxygen bomb digestion profiles did not resemble the acid digestion profiles. Nevertheless, the values obtained by oxygen bomb combustion are surprising for this drill core as well, since the peak visible from 1964-83 was neither found in other St Moritz trees, nor in peat bog drill cores taken nearby. The St Moritz profile obtained by oxygen bomb combustion (dashed line) is similar to the acid digestion combustion profile (solid black line). It runs parallel to the acid digestion profile over a long period. But this similarity gets lost for the segments corresponding to the years 1944-63. The deviating profile cannot be explained by fungal decay or compression wood (see chapter 3.3), since the drill core was exempt of such spots. Since both compared drill cores come from a different direction of the tree, the deviation could be explained by different radial distribution of sulphur. However, comparison of drill

cores issued from one single tree by LA-ICP-MS and ICP-OES showed matching profiles. This was repeated for all three locations, Düdingen, Frieswil and St Moritz.

2.5. Radiodensitometry

Density and tree ring width of the drill cores was measured at the WSL in Birmensdorf by radiodensitometry. After gluing 3 cm long sample pieces on wooden support rods, 1.2 mm thick splinters were sawn out using a paired circular saw, at right angle to the fibre direction. After conditioning at a temperature of 20°C and a relative air humidity of 50%, the samples were x-rayed. The optical density of the resulting negative films corresponds to the volumetric-gravimetric wood density [*Schweingruber, 1990*]. It was measured with a Dendro 2003 microdensitometer (Walesch Electronic).

This technique not only allows exact dating of the drill cores, but also recognising growth anomalies (i.e. reaction wood) and/or fungal attack. Since such spots can lead to shifts in elemental content which are not of anthropogenic origin, it is important to detect and locate them.

Furthermore, the main analysis technique used in this study is LASER ablation ICP-MS. The quantity of material ablated from a sample depends on its density. Since wood density varies strongly (typically from 0.3 to 1 g/cm^3), it can be helpful to track the changes along the profile. This is why additional drill cores were taken 5 cm under the cores used for LA-ICP-MS.

2.6. LASER ablation high resolution inductively coupled mass spectrometry (LA-HR-ICP-MS)

Trees offer the possibility of exact dating, simplified by the tree rings. This advantage is augmented by the high spatial resolution of LASER ablation. Many parts of the Nd:YAG LASER used for this purpose are modified: crystals, observation optics, focussing and sample chamber are described in the chapters 3.1 and 3.2.

Craters with an average diameter of 100 μm result from the action of the LASER on the sample (ablation). This is an ideal compromise between high spatial resolution and optimal sample quantity. Due to the very small amount of ablated material transported by a carrier gas and the very low concentration of sulphur in wood (50 mg/kg), a very sensitive detection method is required. Thus, due to its excellent detection limits, high resolution ICP-MS is ideal. Furthermore, it is a fast multielemental method and does not request the use of chemicals, hence reducing contamination risk. Together with S, on which this work is focussed, the following elements were measured as well: C, Mg, Al, P, K, Ca, Mn, Zn, Rb, Sr, Ba, Pb.

Coupling between both instruments was achieved using Teflon tubing and a glass adapter especially designed for that purpose. The use of such tubing facilitates cleaning before sampling in order to lower contamination. Inside the sample cell, only metal holders are allowed, since the use of plasticine or tape leads to high sulphur content. For each measurement, the LASER shutter was opened for 20 s (beyond this limit, the LASER pierces the drill core and hits the plastic rail), leading to 200 shots onto the samples and to a characteristic transient signal seen on figure 8.

Thanks to the high spatial resolution, narrow rings such as in alpine trees can be analysed, as well as chemical differences between earlywood and latewood. The high resolution magnetic sector field ICP-MS resolves the problem of polyatomic interferences for the $^{32}S^+$ isotope (95.02% relative abundance), which is mainly overlaid by $^{16}O^{16}O^+$. The other natural sulphur isotopes ^{33}S (0.75%) and ^{34}S (4.21%) are rare compared to ^{32}S. Due to the low sulphur content in wood, their determination was not possible in drill cores, unfortunately.

In order to obtain signals which are independent from the density of the ablated material, the stable carbon isotope ^{13}C was used as an internal standard. Its natural variation in wood is negligible. The ICP was operated under hot plasma conditions.
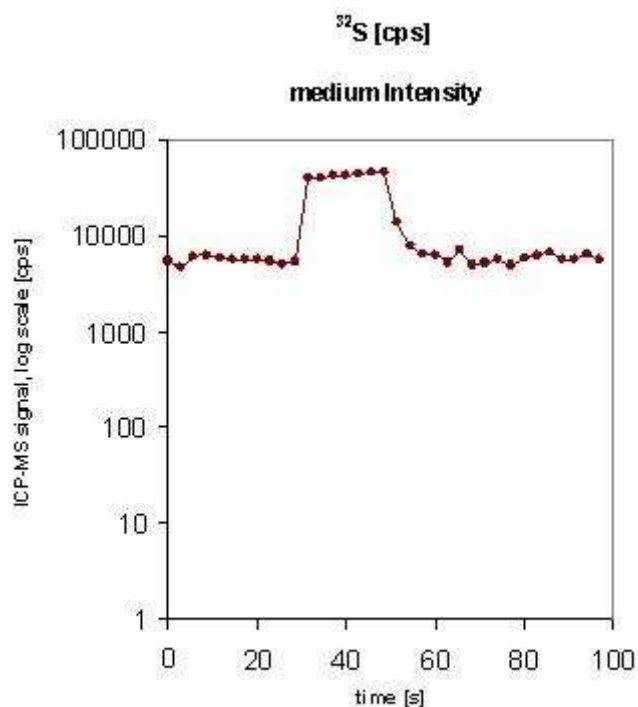

Fig. 8.: typical transient signal of a LA-ICP-MS measurement of sulphur in wood. Ablation duration is 20 s.

2.7. Preparation of doped reference pellets

Quantification by LA-ICP-MS requires suitable solid standards such as commercially available reference materials. Since no appropriate LA-ICP-MS calibration standards are procurable for sulphur in wood, self-made pellets were fabricated following the method indicated by Hoffmann [*Hoffmann and Stephanowitz, 1995*]. Two different base materials were tested for pellet confection: cellulose powder (20 microns, Aldrich) mixed with 5 % activated charcoal (p.A., Merck) and homogenised wood powder with a S content of 58 mg/kg ± 8%. Both standards were prepared as follows: 2 g powder portions were put into six different Teflon® vials and doped with 0, 40, 200, 500, 1000 and 2000 µl of a solution containing 200 µg/ml sulphur (1:5 dilution of S ICP standard solution, 1000 µg/ml S in water, Alfa Aesar). 650 µl of a 100 ppm Scandium solution (1:5 dilution of Sc ICP standard solution, 1000 µg/ml Sc in 5 % HNO3, Alfa Aesar) was added as an internal standard. 6 ml of MQ water (bidest purity grade) were poured into the vials while mixing with a plastic stick until a homogeneous paste was obtained. The paste was dried at 80°C until obtainment of weight constancy, upon which the dry content of the vials was crushed in an agate mortar. The homogeneity of the powder obtained was verified by analysis of two aliquots with ICP-OES after acid digestion. Afterwards, 300 mg of powder were used for pressing the pellets (4 min. at 300 kg/cm^2. The accuracy of the dilution series (4, 20, 50, 100 and 200 mg/kg) was verified.
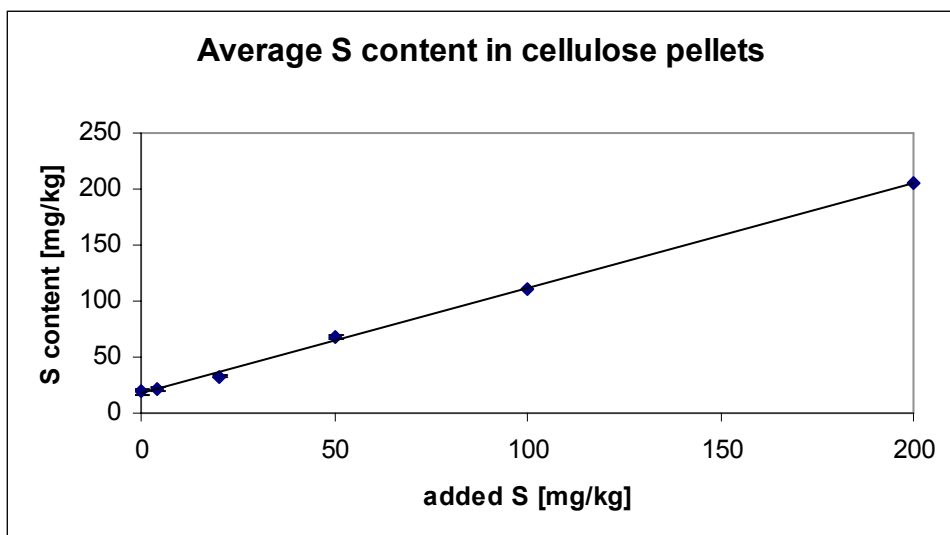
Fig. 9.: Verification of the S content of the cellulose standards with microwave acid digestion/ICP-OES. The S content of the cellulose without doping is 12.5 mg/kg.
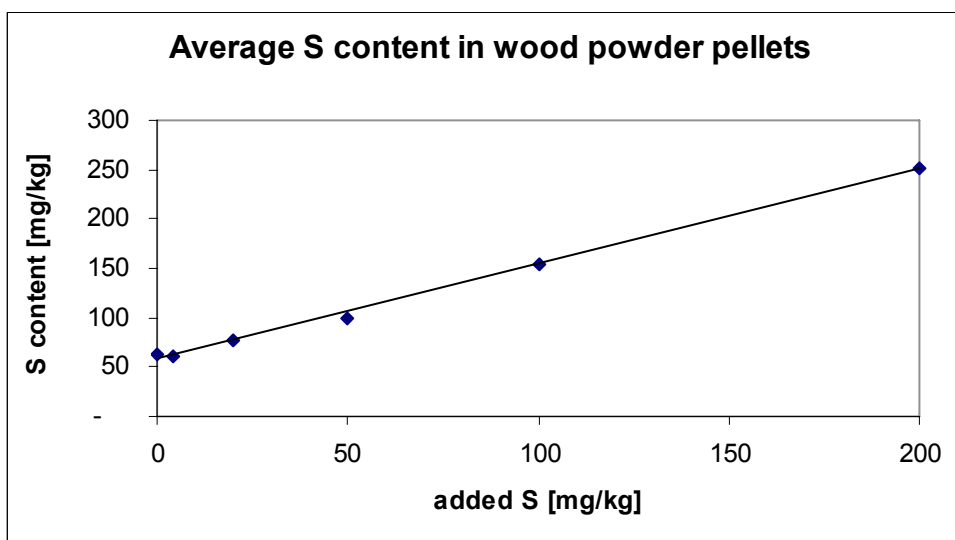
Fig. 10.: Verification of the S content of the wood standards with microwave acid digestion/ICP-OES. The average S content of the wood without doping is 60.5 ± 4.7 mg/kg.

The use of homogenised wood was deemed ideal because it is very similar to the sample matrix. Unfortunately, pills made of wood powder tend to crumble during handling and to sputter when hit by the LASER, which leads to a higher background. The addition of cellulose powder to homogenised wood might lessen the brittleness of pills composed of wood only. Cellulose pills are indeed far more resistant and practical.

2.8.   X-ray absorption spectroscopy (XAS)

The physiological processes involved in sulphur metabolism are complex. It is therefore no enough to have information about total sulphur content only. The spectrometric methods described above (ICP-OES, ICP-MS) all atomise and ionise the sample. But a virtually non-destructive method such as X-ray absorption spectroscopy, the different organic sulphur compounds are kept in their original state.

In archaeology, XAS helped to recognise the reason for the deterioration of an ancient, sunken warship in a museum. While the ship was under anoxic water, elemental sulphur and reduced sulphur had been accumulated by bacteria in the wood to a content of 0.2 – 4%. In the oxidative environment of the exhibition hall, formation of sulphuric acid led to rapid decomposition of the wood.

This method was also applied for speciation of sulphur compounds in coal [*Kasrai et al., 1996*]. However S is the most abundant heteroatom in coal, while the sulphur content of wood is about 60 times lower than in coal. Thus, application of this novel technique, which requires a huge infrastructure, including numerous ultra high vacuum pumps ($4 \cdot 10^{-10}$ – $2 \cdot 10^{-9}$ Torr), is particularly challenging for Norway spruce samples.

In the Synchrotron Radiation Center (SRC) in Stoughton (Wisconsin, USA), photons are produced by accelerating electrons until they almost reach the speed of light. The synchrotron has a diameter of 89 m. At the end of several branch connexions, research stations (beam lines) capture the photos at the wavelength required for the experiment.

The sulphur L-edge total electron yield (TEY) spectra for this work were acquired on the 6 m toroidal grating monochromator (TGM) beam line. It is annexed to the 1 GeV Aladdin storage ring of the SRC. The X-ray beam spot used in this study is narrow, which allowed measuring single tree rings.

Sulphur-specific soft X-rays (E~160-180 eV) permit larger photon energy resolution (ΔE~0.1-0.2 eV). Their detection power is superior to X-ray photoelectron spectroscopy (XPS) or XAS K–edge measurements of sulphur, penetration depth is 5 nm. C, N, and O spectra, being at higher energy than the S edge, do not interfere.

Using a microtome, 0.1 mm-thick wood slices were cut from drill core pieces of 3 cm length. Each slice was coated with 50 nm Au using a metal sputter, in order to minimise surface charge effects during X-ray irradiation [*Gilbert et al., 2000*]. After coating, the slice was fixed onto a metal holder using double-sided conducting carbon tape and additionally taped at both ends to its support.

Powdered sulphur reference compounds were also put on double-sided carbon tape for measurement. They included pyrite, elemental sulphur, $Na_2S_2O_3$, Na_2SO_4, reduced glutathione (GSH), oxidised glutathione (GSSG), cysteine, cystine, methionine and methionine sulphoxide.
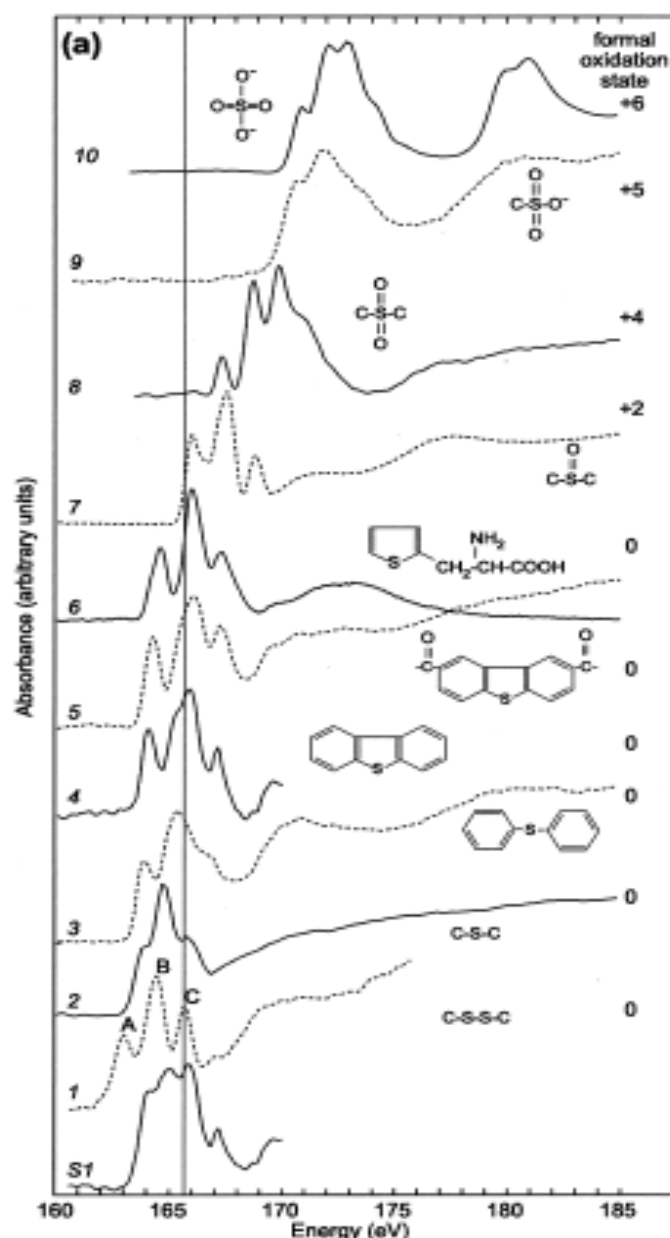
Fig. 11.: L-edge spectra of typical sulphur binding forms (Sarret et al., 1999)

When X-rays hit the Au coated drill core slice, their oscillating electric field interacts with the electron bound in an atom [*Jalilehvand, 2000*]. Radiation can then be scattered or absorbed and thus exciting the e^-. An x-ray beam passing through the sample will have its intensity reduced. The absorption coefficient depends on the type of atom. An absorption edge occurs when the energy of incident photons is sufficient to excite a core e^- to a continuum state. The energy of the absorbed radiation corresponds to the binding energy in the K, L and M shells. Sulphur L-edge spectra are very sensitive to local geometry and show how the absorbing atom is bonded. As seen on figure 11, S compounds generally display more than one peak, often showing doublets and triplets at low energy and oscillating in the region following the peaks.

2.9. Wavelength-dispersive x-ray fluorescence spectrometry (WD-XRF)

For investigation of the total elemental content, wavelength-dispersive x-ray fluorescence spectrometry (WD-XRF) was applied on pulverised forest soil samples. A Philips PW 2400 XRF spectrometer was operated with 3000 W maximum power, 60 kV maximum voltage and

125 mA maximum current. It uses types of detectors (scintillation, gas proportional and sealed proportional detector) and is equipped with an end-window Rh x-ray tube, seven crystals, as well as three collimators. The program UniQuant (4.51) was used to analyse the samples and gives quantitative results in the range 0.001 – 100%. 90 element lines were analysed with a measuring time of 4-8 s per element, which lead to a measurement duration of approximately 500 s. WD-XRF is a surface-specific method with a penetration depth of few μm, depending on analyte and matrix.

The powdered soil samples had a grain size of about 50 μm, and remaining differences in particle size lead to relative uncertainties for major and minor elements in the order of 10-20%. 30 elements were analysed as loose powders in helium at atmospheric pressure. Due to the oxidic nature of the sample material, the major elements were calculated as oxides (Na_2O, MgO, Al_2O_3, SiO_2, K_2O, CaO, TiO_2, MnO, Fe_2O_3, P_2O_5, SO_3) whereas the traces are given as elements (Cl, Cr, Co, Ni, Cu, Zn, Ga, As, Br, Rb, Sr, Y, Zr, Nb, Cd, Sn, Sb, Ba, W, Pb). Light elements such as hydrogen, carbon and oxygen, which are not detected, must be included in the calculation, which is why the loss on ignition (LOI) was determined.

3. Results

3.1 Seasonal Profiles of Sulphur, Phosphorus and Potassium in Norway Spruce Wood: New Light shed on Tree Physiology

Seasonal profiles of sulphur, phosphorus and potassium in Norway spruce wood: new light shed on tree physiology.

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Abstract: **Seasonal profiles of sulphur, phosphorus and potassium content in the wood of trees have been established for the first time. This became possible by a novel use of LASER ablation ICP-MS for measuring both elements in drill cores. This technique combines huge spatial resolution with superior detection power, and makes it possible to measure low element concentrations in relatively narrow year rings. Despite its low quantity in wood, sulphur is an important macronutrient for plants and clearly displays seasonal variations of its concentration, which corresponds to actual knowledge of sulphur metabolism in plants.**
A similar seasonal pattern was found, but not expected from phosphorus, another crucial element in tree nutrition. It was previously assumed that the distribution of phosphorus remains constant throughout the year.
Potassium, the third element measured, is accumulated in the latewood. The profiles presented in this article clearly show a seasonal variation, thus uncovering a new aspect of Norway spruce (Picea abies) metabolism.

Key words: sulphur metabolism, phosphorus metabolism, potassium metabolism, Picea abies, LASER ablation ICP-MS

Introduction

Sulphur and phosphorus have more in common than being neighbours in the periodic system: both elements are indispensable macronutrients for plants. The importance of phosphorus for the plant kingdom is known since the 19th century, and its key role in biochemical processes has been recognised during the last century. On the other hand, knowledge about sulphur metabolism in plants is far more recent. Numerous studies showed how sulphur is linked to carbon and nitrogen metabolism for cysteine and methionine biosynthesis (Kopriva and Rennenberg 2004). Another important sulphur containing amino acid is methionine, which is synthesised from cysteine by transsulphuration (Kopriva and Rennenberg 2004). Both amino acids, cysteine and methionine, are used for protein synthesis, thus playing a crucial role in cellular metabolism (Gakière et al. 2000).

In contrast to nitrogen and phosphorus, sulphur usually is not growth-limiting in forests. Together with phosphorus, its acquisition and assimilation in the rhizosphere is facilitated by mycorrhisal symbiosis, thus enhancing the tree's growth (Rennenberg 1999). Augmented biomass accumulation is tantamount to an increase of protein biosynthesis, since young trees mainly produce living, protein containing tissue. In contrast, mature trees chiefly build wood. However, for production of lignin and cellulose precursors in the cell, a high amount of

proteins is required as well. As a consequence, phosphorus demands augments, too. The metabolic link between both elements begins before sulphur reduction in the needles, where sulphate is transported into the chloroplasts with a triose-P translocator in exchange for phosphate (Leustek and Saito 1999). Inside the chloroplasts, sulphate reacts with adenosine triphosphate (ATP) to give adenosine phosphosulphate (APS). Furthermore, ATP plays its role as an energy furnisher during synthesis of cysteine. In turn, methionine is synthesised from cysteine and phosphohomoserine (PHS).

A further very important element is potassium, which is present in all parts of the plants. It is very mobile and soluble, principally being used in young tissues. Potassium plays important roles for the formation of colloids, for the activation of enzymes and for cell osmosis. A recent study about "red decline", revealed that Norway spruce react strongly in case of potassium and phosphorus deficiency, thus underlining the importance of both elements for its physiology (Engvild 2005).

In this work, seasonal profiles of the macronutrients sulphur, phosphorus and potassium were established using LA-ICP-MS. This novel use of this technique in dendrochemistry permits to study the distribution of important elements in single tree rings. Thus, an insight in the role of S, P and K for the physiology of trees is provided.

Materials and methods

Sampling

Mature trees from the Swiss Plateau were sampled using a 5 mm increment borer (Suunto). Sampling procedure was optimised in order to minimise contamination. The use of gloves was indispensable and the oil used for the preservation of the borer had to be sulphur free. PhEur grade paraffin (Hänseler) was chosen for cleaning of corer and metal tongue because of lowest contamination risk.

Determination of tree characteristics

The drill cores destined for LASER ablation were taken at breast height. For radiodensitometry, additional drill cores were taken 5 cm under the drill holes. The samples were put into weighted and previously cleaned plastic rails. Afterwards, they were dried in an oven at 40°C until weight constancy. The density and tree ring width of the supplementary drill cores was measured at WSL (Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf) by radiodensitometry. For this purpose, 1.2 mm thick splinters were sawn out of the drill cores using a paired circular saw, at right angle to the direction of fibre. After having been conditioned (20°C and 50% relative air humidity), the samples were x-rayed. The optical density of the resulting negative films, which corresponds to the volumetric-gravimetric wood density, was measured with a Dendro 2003 microdensitometer (Walesch Electronic).

Sample preparation

Drill core pieces of 3 cm length were cut with a scalpel and inserted into plastic rail pieces of corresponding size. After counting of the tree rings, a mark was applied onto the rail every 5 year rings. Two samples in rails fixed in a metal holder were placed onto an aluminium support in the cavity of the sampling cell designed by the EMPA (Bleiner and Altorfer, 2005).

LA-ICP-MS

A Nd:YAG LASER Perkin Elmer/Sciex sampler 320 EMPA modified at EMPA (Wanner et al. 1999, Bleiner et al., 2004, Bleiner and Altorfer, 2005) was coupled to an ELEMENT 2 high resolution magnetic sector field ICP-MS (ThermoFinnigan). The LASER was operated at a wavelength of 266 nm in Q-switch mode using a Q-switch delay of 300 μs. With a pulse repetition rate of 10 Hz and the shutter opened for 20 s, 200 shots were fired onto the wood sample per measuring spot. While tree ring length varies from year to year, the craters formed by the LASER show constant diameters of about 100 μm. Thus, the possible number of LASER shots per year is dependent on tree ring width.

The ablated particles were transported by 5.0 grade helium. An additional argon gas flow was added after sampling cell to keep the plasma stable. The ICP was operated under hot plasma conditions using RF power 1250 W, 1.3 l/min sample gas, 15 l/min cool gas and 0.8 l/min auxiliary gas.

^{32}S was chosen as the main natural sulphur isotope, (95.02% relative abundance). The signals of the other possible S-isotopes are much lower due to the lower relative abundance ^{33}S (0.75%) and ^{34}S (4.21%). For the monoisotopic phosphorus, the isotope ^{31}P (100%) had to be selected, for potassium, ^{39}K (93.26%) was chosen. Statistical errors of the LASER measurements were determined to: 1.2 % for ^{32}S, 4.2 % for ^{31}P, 0.8 % for ^{39}K and 0.3 % for ^{13}C.

Compared to e.g. steel samples, wood is a relatively soft material. The huge variations (changes of 85%) in wood density have an effect on the quantity of ablated aerosols and hence on the signal intensity in the ICP-MS. Therefore, an internal standardisation is necessary to obtain density independent signals. Usually C isotopes are used for this task: the use of ^{13}C as an internal standard in dendroanalysis by LA-ICP-MS is indicated by several authors (Hoffmann et al., 1996, Garbe-Schönberg et al., 1997, Watmouth et al., 1998, Kyser et al., 2003). The correlation between the wood density and the ^{13}C signal is almost linear (statistical correlation $R^2 = 0.818$) as shown in Figure 1, which displays the dependence of the ^{13}C signal on the wood density. Natural variations of ^{13}C in wood can be neglected because they are in the per mille range (Duquesnay et al., 1998), whereas the LASER ablation ^{13}C signal variations depending on sample density and thus on the amount of ablated material changes by 41%. By using ^{13}C as an internal standard, the ^{32}S, ^{31}P and ^{39}K signals are rendered independent from the quantity of ablated material.

ICP-OES measurements

Microwave digestion of 5-years wood segments in a 50:50 nitric acid and hydrogen peroxide mixture (HNO_3 65%, H_2O_2 30 %, both suprapure grade) was followed by ICP-OES (inductively coupled plasma optical emission spectrometry) analysis using a Varian Liberty 150 AX Turbo and Varian VistaPro.

Results

The conducted measurements of wood sulphur and phosphorus contents reveal seasonal variations for both elements, hereby underscoring the biochemical link between them. Figures 2., 3. and 4. show the seasonal variations of sulphur (**A**), phosphorus (**B**) and potassium (**C**) contents in Norway spruce wood. The samples were taken from mature forest trees of the Swiss Plateau (Frieswil, 740 m a. s. l., and Düdingen, 580 m a.s.l.), and analysed using LA-ICP-MS (LASER ablation inductively coupled plasma mass spectrometry). The results are presented as relative concentrations. The absolute concentrations, determined by foregoing ICP-OES measurements, provided sulphur contents ranging between 30-60 mg/kg,

phosphorus contents between 20-30 mg/kg, and potassium contents of about 320 mg/kg, thus confirming literature values for Norway spruce (Harju et al., 1997).

The data were pooled from three trees, 5 years per tree (D3 and D5: 1980-84, F1: 1931-35), leading to average profiles of 15 tree rings. One profile per element (S, P, K) was established, as well as the P/S quotient. All profiles are displayed in figure 5. The number of back-to-back laser shots (4-8) was dependent on the varying tree ring width. In order to obtain a temporal signal, each tree ring was divided in four distinct zones, usually used in dendrology: early earlywood (EEW), late earlywood (LEW), early latewood (ELW) and late latewood (LLW). The first LASER shot corresponds to EEW, the last to LLW. LEW and LLW are equally attributed to the laser shots in between. Due to the fact that data stems from three individuals at two different sites, the errors in figure 5 are in an acceptable range of 11.9% for S, 22.5% for P, 22.0% for the P/S quotient and 20.8% for K.

Decreased contents of sulphur in LEW, ELW compared to the other seasons EEW, LLW indicate that wood sulphur is used for the seasonal growth of the trees. Since growth and development of the new needle generation is supported by sulphur allocated from previous year's needles, but allocation of sulphur from source needles to the stem is low (Schneider et al., 1994, Schupp et al., 1992), wood sulphur may be used preferentially for growth and development of the stem in spring and may be diminished as a consequence of growth during summer. In autumn, when accrescence stops, sulphur reserves are built up again in preparation for the next growth year. A closer look at the sulphur content profile of 15 pooled tree rings reveals this behaviour indeed (Fig. 5., S-profile LLW).

Seasonal changes in the phosphorus content of the wood show similar fluctuations (Fig. 5.**,** P-profile), although the increase of P at the end of the season (LLW) is less pronounced than for S. These fluctuations may be interpreted as changing retrieval of phosphorus from the mycorrhizal roots: up to now, it was supposed that trees largely live from phosphorus stored in the mycorrhizal fungi (inorganic polyphosphates) and that phosphorus levels in the tree remain relatively constant (Bücking and Heyser, 1997). However, the present data show that in tree rings, there is also a slight build-up of phosphorus reserves in the wood towards the end of the growing season, which seems to be used during needle formation in the following spring. Subsequent rebuild of the foliage mobilises nutrient reserves, thus enhancing phosphate demand. The fluctuation of the seasonal variations (Fig. 2, 3, 4 **B**) could be explained by a changing requirement for ATP and other phosphorus containing compounds during wood formation and foliage renewal.

Interestingly, there is a strong linear relationship between phosphorus and sulphur (Fig. 5, P/S profile) with a statistical correlation R^2 of 0.994. This underlines the strong biochemical coupling between both elements. The P/S quotient becomes steadily smaller towards the end of the year, thus hinting at the fact that more P compounds are generally required for the tree functions, and that the rebuilding of the P reserve is less complete than for S.

Since potassium (Fig. 5., K-profile) is of special importance in leaves and at growing points, most of it needed during the growing season. Accordingly, the measured profile shows a decrease from EEW to LEW.

Discussion

The sensitivity and huge spatial resolution of LA-ICP-MS allows dendrochemical analysis on a seasonal timescale. The profiles from Sulphur, phosphorus and potassium obtained thereby show similar trends for all selected trees. But although all trees are from the Swiss Plateau,

they stem from two different forests. Having grown under natural conditions, there can be differences between the individuals. Such differences appear clearly when having a look at tree ring width. The trees D3 and D5 for instance are situated in the same forest, but at 100 m distance from each other. Therefore, each tree has its own physiological history, due to a slightly different competitive situation.

Nevertheless, pooling of data provides a clearer picture, providing deeper insight into macronutrient metabolism of conifers. Regardless of their biochemical coupling, sulphur and phosphorus display a change of their concentration in the wood of Norway spruce during the year that differs in extent and pattern. The fact that sulphur allocation to new needles is decoupled from wood sulphur supply could explain the more regular shape of the seasonal concentration shift, as well as its smaller intensity compared to phosphorus (see Fig. 5.). Varying concentrations of the latter element shows that, similarly to sulphur, a reserve is built in wood tissue, and that phosphorus supply of foliage in spring not solely depends on mycorrhizal storage and symbiotic release of phosphorus from polyphosphate. Despite the reserve in wood, phosphorus supply by mycorrhizal symbiosis remains crucial, since the phosphorus pool is not filled up again as efficiently as for sulphur.
The potassium content of plants being much higher than necessary for healthy growth, the reserves are built up quicker and to a higher level than for phosphorus and sulphur. The higher potassium content in the latewood therefore shows a surplus after the end of the growing season.
We conclude that LA-ICP-MS offers new possibilities for investigation of plant physiological mechanisms. As a flexible, multielemental method, it can be used for measuring several other elements of interest in plant biology, such as Ca, Mg and Mn, to mention a few.

Acknowledgements

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Figure captions

Fig. 1 Relationship between ^{13}C signal and wood density. LASER shots on latewood (right side of the diagram, higher density) lead to a weaker ^{13}C signal than shots on earlywood (left side, low density).

Fig. 2 Relative sulphur content (**A**), relative phosphorus content (**B**), relative potassium content (**C**) and wood density (**D**) in tree rings corresponding to the years 1931-1935. Tree from Frieswil, F1.

Fig. 3 Relative sulphur content (**A**), relative phosphorus content (**B**), relative potassium content (**C**) and wood density (**D**) in tree rings corresponding to the years 1980-1984. Tree from Düdingen, D3.

Fig. 4 Relative sulphur content (**A**), relative phosphorus content (**B**), relative potassium content (**C**) and wood density (**D**) in tree rings corresponding to the years 1980-1984. Tree from Düdingen, D5.

Fig. 5 Relative sulphur content (**S**), relative phosphorus content (**P**), P/S quotient (**P/S**, statistical correlation R^2 = 0.994) and relative potassium content (**K**) The time scale corresponds to early earlywood (EEW), late earlywood (LEW), early latewood (ELW) and late latewood (LLW). Data is pooled from 15 tree rings, 3 trees (D3 and D5: 1980-84, F1: 1931-1935) at 2 locations (Düdingen and Frieswil).
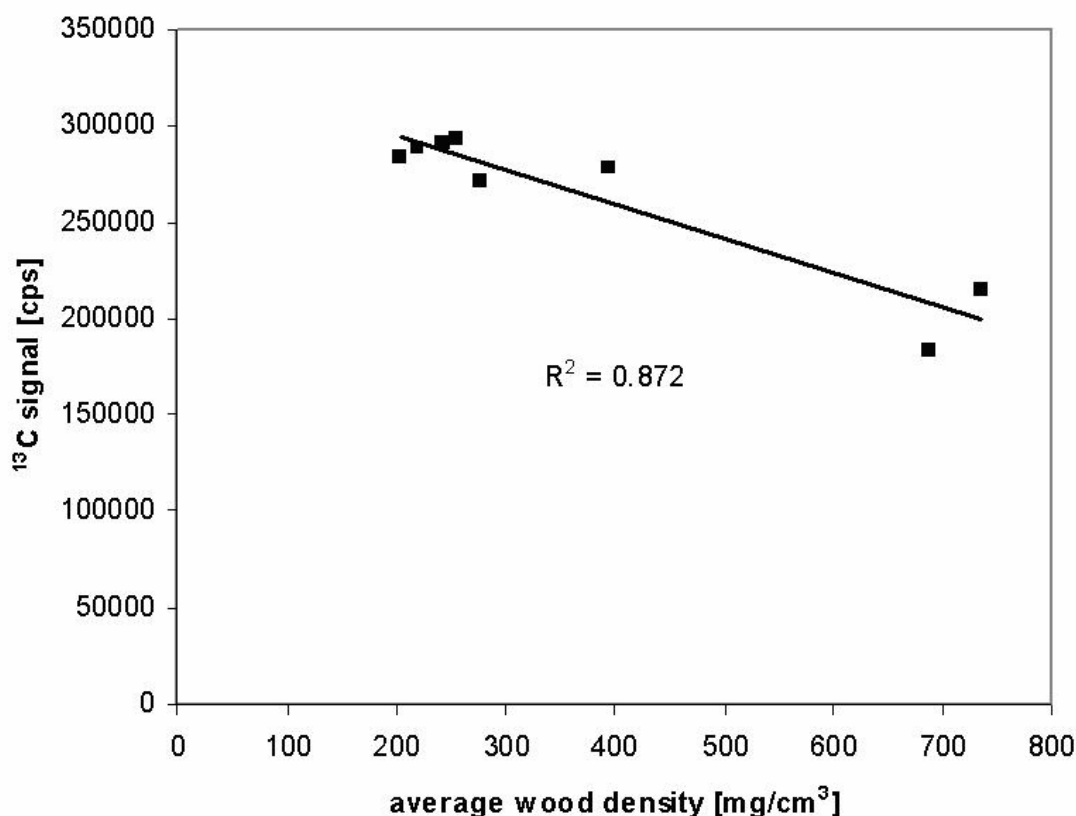
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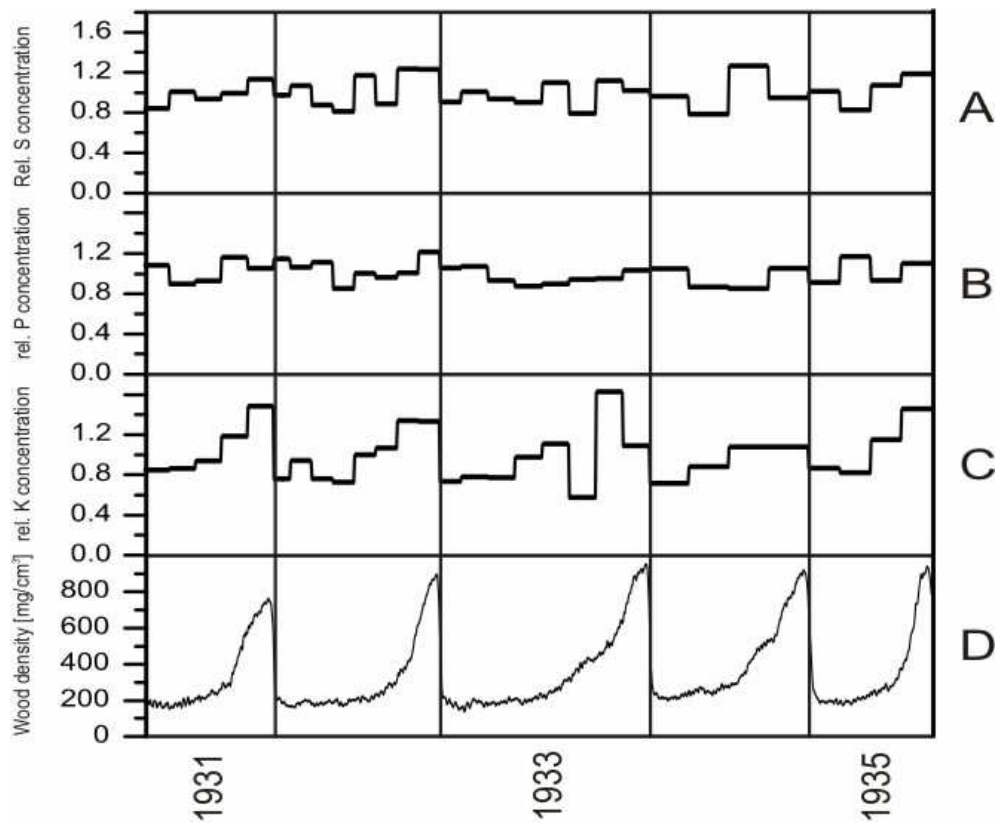
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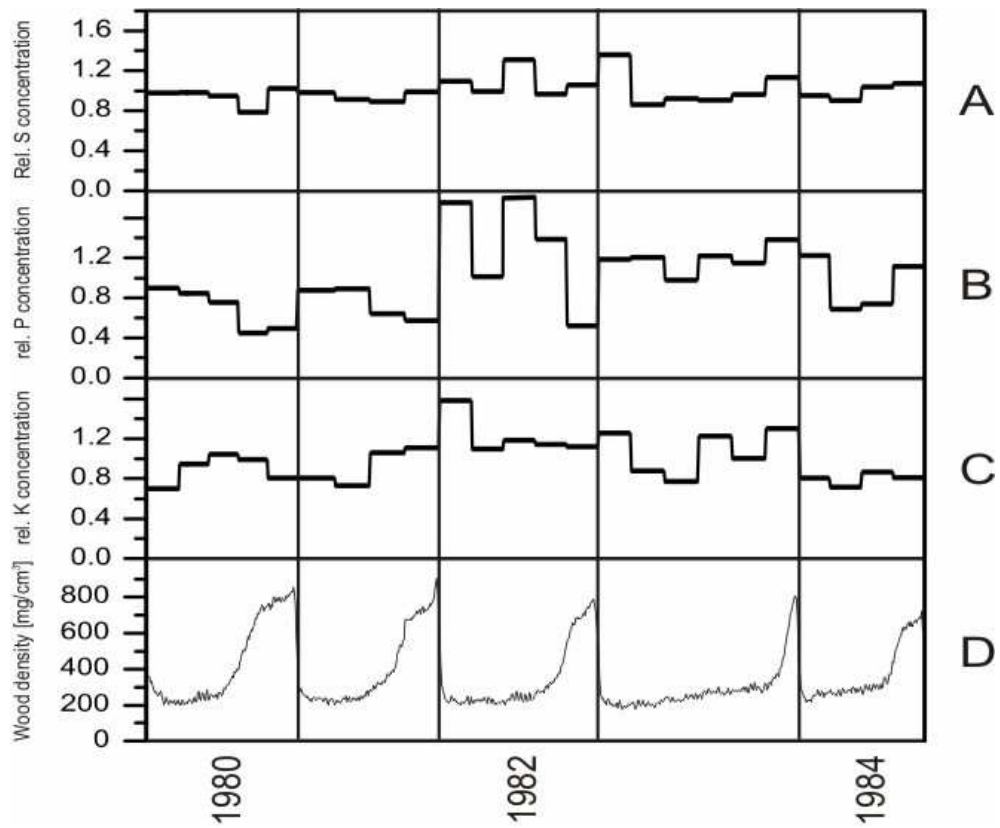
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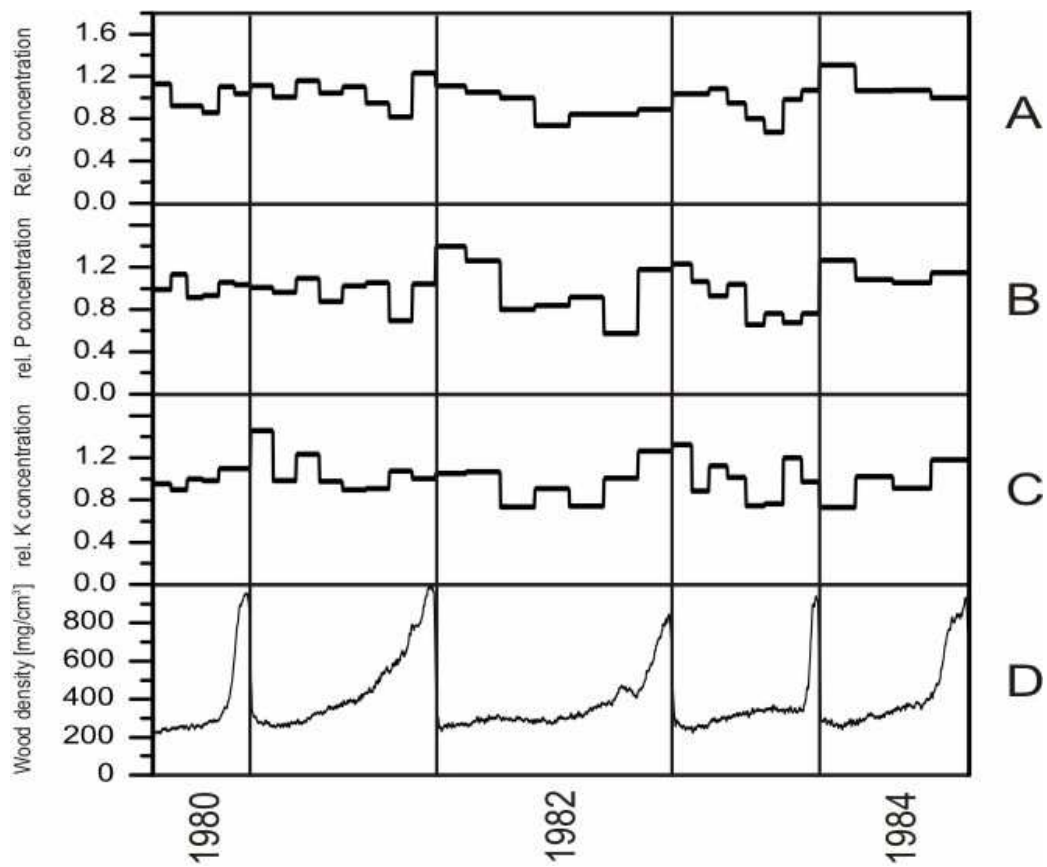
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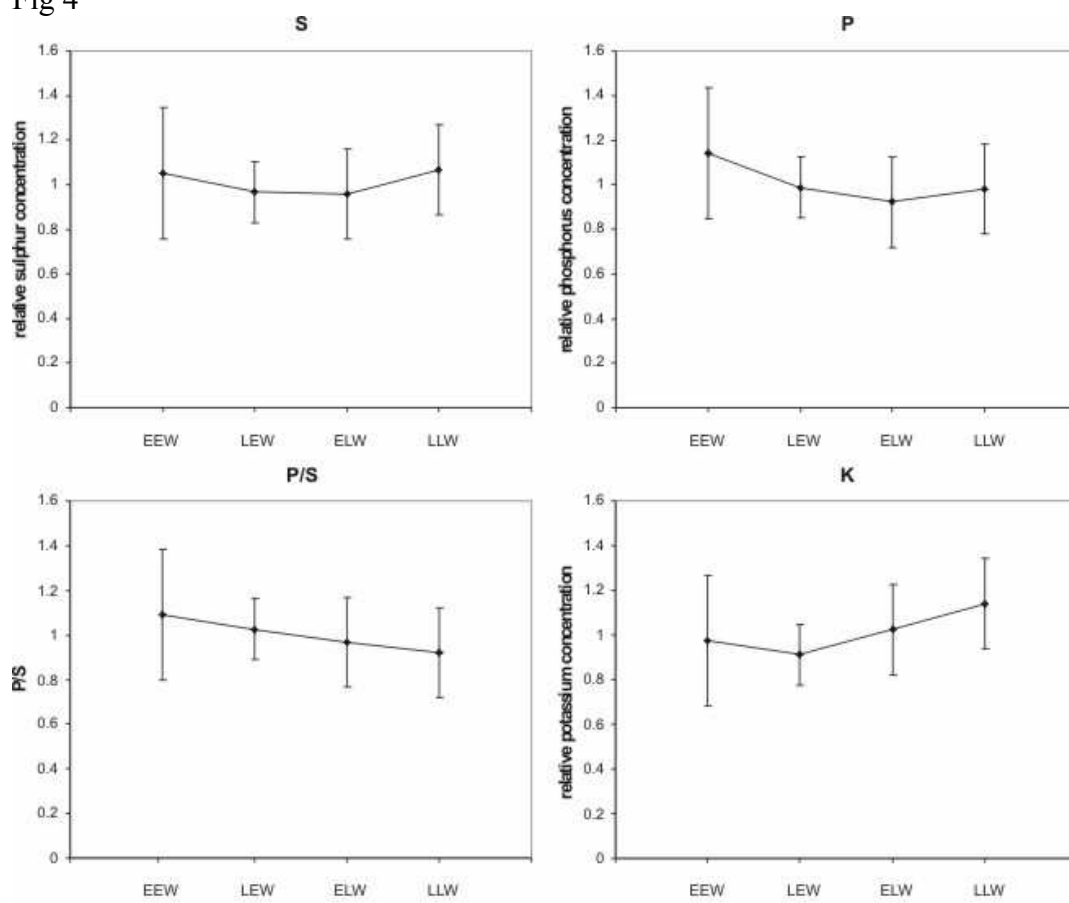
Fig 5

3.2 Norway Spruce as an Environmental Archive for Sulphur Dioxide & Supplementary Notes

Journal	Environmental Science and Technology
Manuscript ID	es0522175
Manuscript type	article
Date submitted by the author	4.11.2005
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Keywords	Sulphur Dioxide, Norway Spruce, Pollution, LA-HR-ICP-MS
status	submitted

Norway Spruce as an Environmental Archive for Sulphur Dioxide

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Norway spruce (Picea abies L. Karst.) is the dominant conifer species in Northern and Central Europe and can be found over a wide range of altitudes. Therefore, it is predestined as environmental archive. In this study, new aspects in dendrochemical analysis could be obtained using a hyphenated analysis method, especially developed for this task. LA-HR-ICP-MS allows spatially resolved and highly sensitive multi-element analysis in wood. Not only metals but also non-metals such as sulphur can be determined. This method was applied to tree ring investigations in Norway spruce with the goal to enlighten the applicability of trees as environmental archives for sulphur dioxide. Regional, environmental and atmospheric influences as well as plant physiological aspects are discussed to assess the possibilities and the limits of conifers as environmental archives.

Introduction

Environmental archives are valuable tools for retracing changes on a global scale. Aside from ice cores, lake sediments, peat bogs, coral reefs and speleothems, trees belong to the most popular archives. Their main advantage is their availability: Trees are widespread, can be reached in several latitudes and altitudes and even close to urban emissions. Besides, sampling is relatively simple compared to other archives, because sites can easily be reached. Additionally, wood being a robust material, sample loss and deterioration are averted. The formation of year rings in trees makes dating very accurate.

The selection of a suitable tree type has to be made carefully and with special attention to distribution and representativeness. Conifers are particularly adequate since they can be found all over the world and individual tree rings are especially easy to distinguish. The genus Picea is very common in the northern continents with the only exception of Africa (*1*, *2*). Related species can be found at other continents, e.g. Asia. Careful evaluation led to the choice of Picea abies (Norway spruce) here.

For archives analysis, methods are preferable which allow determinations of high spatial resolution combined with excellent detection power. Sample digestions with subsequent chemical analyses are suitable for determinations of metals and non-metals, but the spatial resolution is limited due to minimum required sample amounts for decomposition. Authors of previous studies digested 5 to 10 year segments followed by chemical analysis using $BaSO_4$ turbidity measurements (*3*), particle induced x-ray emission (*4*), atomic spectrometry methods (*5*), anodic stripping voltammetry (*6*) or neutron activation analysis (*7*). Thus, LASER ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is an optimal method for trace elements analysis because it allows highly sensitive and spatially resolved determination directly at solid samples. LA-ICP-MS was applied to investigations of metals in tree rings before (*8-13*). Some authors used Nd:YAG LASERS at 1064 nm wavelength and achieved relatively poor crater sizes of 2.5 mm to 500 μm (*8-10*). This allowed only analysis

of relatively wide tree rings e.g. of young trees. Investigations of smaller tree rings such as occurring often in alpine trees were not possible. Some authors worked with 266 nm and archived better spot sizes of about 100 to 150 μm (*11-13*).

All mentioned authors worked with LASERs coupled to quadrupole ICP-MS. Since this method only allows a mass resolution of about 0.7 to 1 amu (atomic mass unit) some elements (e.g. sulphur) can not be determined because of polyatomic interferences. This study presents a novel method especially developed for spatially resolved investigations of tree rings using the coupling of a 266 nm Nd:YAG LASER with a high resolution ICP-MS (LA-HR-ICP-MS). This method not only allows direct analysis with high spatial resolution but also sensitive determination of elements interfered otherwise. Even seasonal element profiles can be achieved; which shall be published soon.

The investigation of sulphur in tree rings is of special interest. Sulphur can be a pollutant (e.g. combustion processes or traffic) but is also occurring naturally (e.g. in soil). It is one of the essential macronutrients for plants, and plays an important role for protein synthesis (e.g. Ferredoxin, an iron-sulphur protein which has a key position in photosynthesis) and for the production of antioxidants like glutathione.

Three principal uptake mechanisms for SO_2 from the atmosphere are known: 1. via needles, 2. from the soil via roots after deposition, and 3. run-off water through lesion in the bark. The uptake as sulphate via the root system is the main incorporation mechanism into the stem. In heavily polluted areas, SO_2 can become an additional sulphur source beside natural sources. The compound enters the needles of conifers like Norway spruce via the stomata without regulatory control. It is then rapidly fixed into the amino acid cysteine by the sulphate assimilation pathway (*14*). Allocation of sulphur from source needles to the stem is low (*15*). Some of the accumulated sulphur is rather allocated to the new, developing needle generation (*16*). Nevertheless, falling needles, which remain enriched in sulphur, can contribute to heighten the soil sulphur concentration. Wet deposition of sulphite and sulphate is one of the most important sulphur sources for the soil. Due to the huge canopy surface of a Norway spruce tree, important amounts of aerosols and fog are deposited, and gasses washed out. This effect is known as crown interception and is three times higher in Norway spruce than in beech (Fagus sylvatica) (*17*). Subsequent accumulation of sulphur in the soil under Norway spruce is four times as important. This accentuation is due to the perennial foliage, which leads to uninterrupted crown interception in winter, where airborne sulphur from heating is at its highest. As a consequence, Norway spruce accumulates twice the amount of sulphur in its phytomass than beech.

The accumulation of anthropogenic sulphur has been studied before by Gaoming (*3*). He proposed that the high sulphur content measured in Chinese pine (Pinus tabulaeformis) from Chengde City, northern China, reflects the huge SO_2 emissions of this area. The results were based on analysis of 10-years wood segments, which reflects a relatively coarse time resolution compared to the superior spatial resolution of LA-HR-ICP-MS.

Even if the SO2 emission is on a regressing trend in central Europe, the global emission scenario shows a rising tendency for the globe. Developing countries depend on coal plants for covering the increasing energy demand. The high sulphur content of coal will lead to an enormous release of SO_2. Scenarios published in the latest report of the International Panel on Climate Change (IPCC 2001) predict a worldwide increase in SO_2 emissions, expected to exceed 100 megatonnes per year some time around 2020. Thanks measures like desulphurisation of crude oil and reduced use of coal, industrialised countries such as Switzerland managed to cut their emissions by a factor of 3 since they peaked in the late seventies and beginning of the eighties of the last century. The present study investigates whether such rapid changes are visible in Norway spruce. For this purpose, sulphur profiles were established by LA-HR-ICP-MS. The suitability of Norway spruce as environmental

archive will be discussed as well as the limits, with special regard to local immission situations in different regions and altitudes and plant physiological aspects.

Materials and methods

Selection of the tree species
Selection of an adequate tree type led to the choice of Picea abies *L. Karst* (Norway spruce), a dominant species in the northern, central and eastern parts of Europe. In the south, it extends to northern Greece and in the West to the Massif Central, France (*1*). Norway spruce is even naturalised in places such as in Britain or the Pyrenees Mountains (France). Moreover, it has established itself in the north-central United States, and in Canada (*2*). In Switzerland, Norway spruce represents 48% of all trees, according to the Swiss Federal Statistical Office 2004. A major advantage of this tree species is also its occurrence in high altitudes.

Sampling site description
Two sites with different SO_2 emission levels and altitudes were selected: one in the Swiss Plateau area of Düdingen (580 m a.s.l.), and the other at the alpine site of St Moritz (Engadine, 1900 m a.s.l.). Both forests are located near peat bogs, which provide data as independent environmental archives for comparison. In St Moritz, the drill cores were collected in a forest 100 m above and in 500 m distance of the peat bog. In Düdingen, the peat bog is 1 km distant from the forest, which is located in a distance of 500 m to the motorway A12 (Bern-Freiburg).

Soil sampling and sample preparation
At each site, a soil sample was taken between two trees using a garden corer. After having removed the loose foliage of the surface, 20 cm long cores were taken and put into plastic bags. The samples were then freeze dried until weight constancy and ground to a size of about 50 μm using a wolfram carbide vibrating cup mill (Fritsch, pulverisette 9).

Tree Sampling
In both forests, healthy, mature forest trees were selected. Before sampling, the circumference of each tree was determined. Trees of different size and cambial age were sampled in order to assess eventual ageing effects. Drill cores were taken at breast height using a 5 mm increment borer (Suunto). Gloves were used to avoid contamination. PhEur grade paraffin (Hänseler) was used for cleaning of corer and metal tongue. Sulphur freeness had been tested before by ICP-OES (inductively coupled plasma optical emission spectrometry) measurements.
The extent of the sapwood (humid zone) of each drill core was determined, immediately after sampling, because no visual differentiation between sapwood and heartwood is possible after drying. With respect to interpretation of the sulphur profile in a tree, the determination of the sapwood-heartwood boundary is mandatory.
The drill cores were verified to be free of pathogens (e.g. heartwood rot) and compression wood (i.e. darker reaction wood of higher density). The samples were then pressed into weighted plastic rails, previously cleaned with MQ water (bidest quality), and wrapped in a polyvinylidene chloride foil (Saran). Afterwards, they were stored in the rails and dried in an oven at 40°C until weight constancy. Water content of the wood was determined as weight loss after drying.

Radiodensitometry
For dating and radiodensitometry, additional drill cores from the same trees were taken 5 cm under the drill cores destined for LA. The wood density and tree ring width of the supplementary drill cores was measured at the installations of the Swiss Federal Institute for

Forest, Snow and Landscape Research, Birmensdorf (WSL). 1.2 mm thick splinters were sawn out of the drill core, at right angle to the direction of fibre. After having been conditioned at 20°C and 50% relative air humidity, the wood samples were x-rayed. The optical density of the resulting negative films was measured with a Dendro 2003 microdensitometer (Walesch Electronic). It corresponds to the volumetric-gravimetric wood density.

ICP-OES measurements of total sulphur
Microwave digestion of 5-years wood segments from in a 1:1 nitric acid and hydrogen peroxide mixture (HNO_3 65%, H_2O_2 30 %, both suprapure grade) was followed by ICP-OES analysis (Varian Liberty 150 AX Turbo and Varian VistaPro). A publication about these results is in preparation.

Element analysis of forest soil
The total elemental content of the forest soil samples was determined by wavelength-dispersive x-ray fluorescence spectrometry (WD-XRF) using a Philips PW 2400 XRF spectrometer with 3000 W maximum power, 60 kV maximum voltage and 125 mA maximum current. The analyses were performed using the "standard free" analytical program, UniQuant of Omega Data Systems, which gives quantitative results in the range 0.001 – 100 %. A total of 90 element lines were analysed with 4 – 8 s measurement time per line. The samples were directly determined as powders (diameter of analysed area: 24 mm). For powder samples, deviations in the order of 10-20% have to be expected. A total of 30 elements were analysed as non-ignited loose powders in helium under atmospheric pressure. Since the sample material is oxidic, the mass contents of the major elements were calculated as oxides. Undetected light elements such as hydrogen, carbon and oxygen must be included in the calculation procedure. Therefore, the loss on ignition (LOI) was additionally determined by heating ~25 g of each sample during 2 hours at 500° C to evaporate remaining water and volatile organic compounds. In order to assess the amount of organic sulphur in the soils, weight loss after burning was determined. The part that is best bioavailable corresponds to the volatile sulphur fraction.

Sample preparation for LA-HR-ICP-MS
Drill core pieces of 3 cm length were inserted into clean plastic rail pieces of corresponding size. After counting of the tree rings, a mark was applied onto the rail every 5 year rings. Two sample holding rails fixed in a metal holder were placed onto an aluminium socket in a special sampling cell designed by EMPA (*18*).

Development of the LA-HR-ICP-MS coupling
A Nd:YAG LASER Perkin Elmer/Sciex sampler 320, modified and completely redesigned at EMPA (*19, 20*), was used for LA. It was operated at a wavelength of 266 nm using a Q-switch delay of 300 μs. Pulse energy and repetition rate were optimised for the ablation of wood and resulted in final settings of 30 mJ for pulse output energy and 10 Hz for pulse repetition rate. The shutter was opened for 20 s, leading to 200 shots onto the samples. For the wood analysis, the best compromise between optimal signal intensity, accuracy and high spatial resolution was achieved by craters with an average diameter of 100 μm. The achieved spot sizes in this study are smaller than craters obtained by others who operated with Nd:YAG LASERs at 1064 nm (*8-10*). The typical spot sizes in these studies ranged between 2.5 μm and minimum 500 μm, which allowed only investigation of relatively wide tree rings e.g. of young trees. Narrow rings such as of trees in alpine regions or seasonal effects could not be determined. Other studies achieved smaller LASER spots of 100 to 150 μm diameter and operated the LASER at 266 nm (*11-13*).

Shorter wavelengths are advantageous. Investigations of the influence of wavelength on particle size distribution in LA have shown that higher accuracy of the ablation can be obtained by LASERs with shorter wavelength (*21-23*). This is due to a more homogeneous particle size distribution in the aerosol formed and a minimisation of non-stoichiometric effects occurring during sampling and aerosol transport.

In the present study, the LASER was coupled to a high resolution magnetic sector field ICP-MS (HR-ICP-MS) ELEMENT 2, ThermoFinnigan. It can be operated in the three resolution mode (low 300, medium 4000 and high 10000). For comparison, quadrupole-ICP-MS is limited to a resolution of 300 (about 0.7 to 1 amu). Thus, the sulphur isotope $^{32}S^+$ is overlaid by polyatomic interferences: mainly by $^{16}O^{16}O^+$. However, a coupling to a high resolution instrument enables to resolve this problem.

The relative abundances of other natural sulphur isotopes ^{33}S (0.75%) and ^{34}S (4.21%) are low compared to ^{32}S (95.02%). Therefore ^{32}S was used for establishing the annual sulphur profiles in wood. Before method development for transient signal acquisition, the sulphur signals were optimised to maximum intensities on the stable sulphur isotope ^{32}S. A validation of the performance was carried out daily by using doped wood and cellulose standards prepared at our laboratory. ^{13}C has been used for internal standardisation to obtain density indipendent signals. The natural ^{13}C variation in wood can be neglected, since it is in the magnitude of the per-mille range (*24*).

The instruments were interconnected by a self-designed adapter which allowed the use of helium as a transportation gas with allowance of argon for stable plasma conditions. Some studies demonstrated the advantages of helium regarding particle formation, particle distribution and transport efficiency (*25*). The ICP was operated under hot plasma conditions using a RF power of 1250 W, 15 l/min cooling gas and 0.8 l/min auxiliary gas.

Sulphur background level was efficiently reduced by careful elimination of potential contamination sources in the ablation cell, transportation tubing and torch as well as by regular cleaning of the sample cell before use.

The LASER was carefully adjusted using a microscope coupled to a camera system to ensure that the sampled zone was the same for each tree ring (early earlywood). Nevertheless, due to the slight seasonal variations in element content, it is not possible to perform multiple determinations in tree rings (*26*). The statistical error of the instrument was determined to 0.3% for ^{13}C (internal standard) and 1.2 % for ^{32}S.

Results and discussion

During the second half of the last century, the emissions of SO_2 in Switzerland changed significantly. Increasing mobility and comfort culminated in relatively high emission rates, particularly pronounced towards the end of the seventies and at the beginning of the eighties. Measures like desulphurisation of crude oil and a reduced use of coal resulted in a sustained decrease of SO_2 emissions.

The annual relative total sulphur concentrations for the two Düdingen drill cores are plotted in figure 1. The sapwood-heartwood transition zone is marked by a dashed vertical line for each drill core. Figure 2 shows the profiles for the two St. Moritz trees. Since measurement of the St. Moritz trees was performed within the sapwood zone, the transition zone is not visible on the diagrams. All sulphur profiles were obtained from ^{32}S signals normalised using the ^{13}C isotope as internal standard and are displayed as annual relative total sulphur concentrations from 1956-1988.

From 1954 to 1975, the Düdingen sulphur profiles D1 and D3 vary only slightly. Following the year 1975, a significant sulphur increase was observed in both trees, which lasts until 1980. Compared to this, the profiles of the St. Moritz trees S4 and S5 remain flat throughout

the measured period. Düdingen is a natural reserve in proximity of a motorway, and the trees are thus directly exposed to an emission source. In contrast, the trees from St Moritz grew in an alpine environment.

In all likelihood, surplus sulphur input is the reason for the peaks observed in the trees D1 and D3 (figure 1). It is noteworthy that both are located in a distance of 500 m from the motorway A12 (Bern-Freiburg). Thus, in the rural area of Düdingen, the dominant SO_2 source is probably mainly traffic. This hypothesis is supported by the fact that different sections of the A12 motorway have been inaugurated between 1971 and 1973, which correlates with the appearance of the sulphur peaks in the trees D1 and D3. Interestingly, the peaks appear when the SO_2 emission rate of traffic reached a maximum of 6266 tons per year in Switzerland, as evaluated by the Swiss Agency for the Environment, Forests and Landscape (SAEFL, figure 3 A).

As mentioned above, the profiles of the alpine region trees of St Moritz are much smoother, which was expected since SO_2 immission rates are expected as lower for alpine regions. This was proved in measurements performed by EMPA for the National Air Pollution Monitoring Network (NABEL). Results from locations comparable to Düdingen and St Moritz allowed the estimation of the local situations for both sampling sites. NABEL runs 16 measuring sites. The rural Härkingen (431 m a.s.l.) is comparable to Düdingen, because it is equally located in the Swiss Plateau (about 70 km away), at a similar altitude and also in direct neighbourhood of a motorway. The immission situation there is lower than in cities or agglomerations but higher than in predominantly rural locations without direct emission source. The influence of the direct source in Härkingen can be estimated by the observed increasing SO_2 immissions depending on the wind direction. Compared to northern winds, western winds (predominant wind direction on the Swiss Plateau) carry twice the quantity of SO_2 to the measuring station. The sampled trees in Düdingen are also situated on the south-eastern side of the motorway.
The annual average SO_2 concentration in the Alps is up to 10 times lower than in locations exposed to traffic. Thus, the alpine region Davos (1638 m a.s.l.), which is comparable to St. Moritz (about 10 km away) is much less polluted. The annual immission rates clearly show that the atmospheric SO_2 air concentrations depend on the measuring site. Thus, it is comprehensible that the St Moritz profiles are relatively flat compared to Düdingen .
The atmospheric SO_2 concentrations evaluated within the scope of the NABEL studies prove that each site has its individual immission profile. Thus, the emission profile for Switzerland does not reflect the local environmental situation, which is decisive for the potential exposure of the tree or another archive such as a peat bog.
The peat bogs of Düdingen (figure 3 B) and St. Moritz (figure 3 C) offer the possibility of independent comparison, due to their proximity to the sampled trees, just 1 km away. The sulphur profile found in a Düdingen peat drill core is very similar to the emissions evaluated by SAEFL (figure 3 A) (27), and corroborates the observations made in the Düdingen trees. Since there are no direct NABEL data for Düdingen, the peat bog results help retracing the immissions specific for this site.
In the St. Moritz peat bog (28) no significant peak was found at the period corresponding to the maximal SO_2 emissions in Switzerland, which corresponds to the tree profiles. These results also underline the fact that due to the Alps, the Engadine is secluded from emissions of the Plateau.
However, there are also differences between the two archives. Concentrations of sulphur are lower in trees than in peat bogs. An important difference is also that sphagnum mosses do not have a protective cuticle and thus take up SO_2 readily. For an ombrotrophic, raised peat bog, rain is the only sulphate source, while soil sulphate supply plays an important role for trees. This leads to the assumption that a higher soil background might affects the extent of the

sulphur content in wood. Measurements of the average total sulphur content in soil and drill cores support this idea (figure 4).

The Düdingen forest soil is greyish and clayey, while the darker St. Moritz forest ground contains more organic mater. Figure 4 shows clearly that the sulphur concentration in St. Moritz soil samples is almost two times higher (factor 1.9) than in the Düdingen soil. However, the difference between the respective wood sulphur concentrations is less pronounced (factor 1.2) which leads to the assumption that bioavailability of sulphur in the soils has an influence. This bioavailable part corresponds to the organic sulphur fraction (*29*) and its amount equates to the weight loss after burning of the soil samples. In St. Moritz the sulphur content was 0.23% before and 0.17% after burning, meaning a LOI of 0.06%. In Düdingen the values are 0.10%, 0.07% and 0.03%, respectively. Although total sulphur in St. Moritz soil is twice the amount of sulphur in Düdingen, the proportions of organic sulphur (calculated as LOI) are almost equal: 28.9% for Düdingen, 24.6% for St Moritz. This could explain why the differences in wood total sulphur content are not as big as the differences in soil total sulphur concentration.

A further difference between trees and peat bogs is that peat bogs seem to be more sensitive for atmospheric SO_2 variations at lower levels. This could be explained by the presumption that a high natural sulphur background in soil might mask the tree's responds to low levels of SO_2. Plant physiological mechanisms might have an additional influence in regulating processes for sulphur uptake. Thus, in the Düdingen trees only the maximum emission peak is distinct visible whereas in the peat bog profile also an increase from 1960 to 1965 and an almost constant level between 1965 and 1975 was observed. The exact threshold value could not be determined, since no NABEL data were available from Düdingen and St Moritz.

Since cambial age of both trees from each site is different, the ageing effect, which highly influences tree ring width, has no consequences on the sulphur profiles. In Düdingen, the samples D1 and D3 originate from trees with a cambial age of 101 and 96 years, respectively. In St Moritz, the sampled trees (S4 and S5) have a cambial age of 135 and 177 years, respectively. Heartwood formation as potential reason of the sulphur peaks can be excluded, since the peaks are both found in the sapwood zone, the extent of which is different for both trees.

The results from the Düdingen trees indicate that the amount of sulphur that remains in the wood tissue increases in case of elevated sulphur input. The idea is supported by the observation that wood is used for sulphur storage. Investigations on such seasonal concentration shifts in Norway spruce will be published soon (*26*).

After having been used for the growth of the trees in spring, the sulphur reserves are built up again in autumn. Thus, sulphur recuperation is not completed during the process of heartwood formation presented above. However, close to the cambium, where new cells are built during stem growth, a maximum in sulphur content was observed in all measured trees. It is followed by a rapid decrease (by a factor 1.5 to 2) in direction of the pith, which corresponds to protein decay and partial recycling of sulphate. Very high sulphur content is also found on the other side of the cambium: in the phloem. Bark sulphur content of all trees was always higher than xylem sulphur content by a factor up to 4. It is known that mineral compounds from water running along the stem can enter the phloem and thus lead to a generally higher nutrient content (*13*). The extent of the uptake by the cambium when salts pass through the bark is unknown. All of the above shows that compared to physiologically inactive archive like ice cores or speleothems, metabolism and physiology of the tree have to be taken into account in order to distinguish between natural and anthropogenic signals.

In view of the similarity of the peaks observed 1970-1980 in the Düdingen drill cores with those found in the peat bog at the same site, it can be concluded that Norway spruce is a

suitable archive for SO_2. The absence of a peak in the St. Moritz archives, peat bog as well as trees also supports these findings. However, there are some limitations. Due to the high natural sulphur background in wood, lower SO_2 immission rate can not be detected. Trees are less sensitive environmental archives than peat bogs. Thus, Norway spruce is not suitable for reconstructing low emission levels.

Furthermore, the sulphur content profile can be influenced by physiological processes. For instance, measurements not published so far showed that fungal decay or compression wood has a significant influence on the sulphur level. Therefore, only healthy trees should be used as archives. A further physiological aspect is that incorporation of SO_2 by the needles can reduces the sulphate uptake via roots substantially, even if it is not stopped completely (*30*). This might mitigate the tree's response to low SO_2 concentrations.

Some further aspects are also evident for data evaluations: 1) Extent of the sapwood must be known, as well as the transition zone to heartwood. 2) Wood density and tree ring width profiles must be obtained in order to assess abrupt changes in growth (logging leads to the so called clearing effect, which is an abrupt growth release in a tree freed from competition), since they may affect total sulphur content. 3) The high sulphur concentrations near the cambium are natural. It would be interesting to study the sulphur content near the cambium of trees lumbered before the maximum emissions in Switzerland, at the height of the peak, and shortly afterwards. This would allow assessing the extent of additional sulphur in wood tissues near the cambium. The particularly high sulphur contents (up to four times those of Swiss Norway spruce samples) found in the sapwood of Chinese pine growing in Chengde might indeed reflect high local pollution (*3*). However, part of this increase is natural and due to the proximity of the cambium.

Notwithstanding the limitations mentioned above, it can be concluded that Norway spruce and conifers in general are valuable archives for SO_2. Although interpretation is complex, sampling is straightforward and analysis is relatively fast.

Acknowledgements

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Figure captions

FIGURE 1: Annual relative total sulphur concentrations for the Düdingen drill cores D1 (A) and D3 (B). Dashed vertical line: sapwood-heartwood transition zone.

FIGURE 2: Annual relative total sulphur concentrations in the St. Moritz drill cores S5 (A) and S4 (B).

FIGURE 3: SO_2 emissions of the traffic in Switzerland in tons per year (SAEFL 1995, A), total sulphur content of the Düdingen peat bog PBD (B), and total sulphur content of the St Moritz peat bog PBS (C).

FIGURE 4: Sulphur contents of St. Moritz and Düdingen soil samples (white columns), compared to total S concentrations in the trees (black squares).
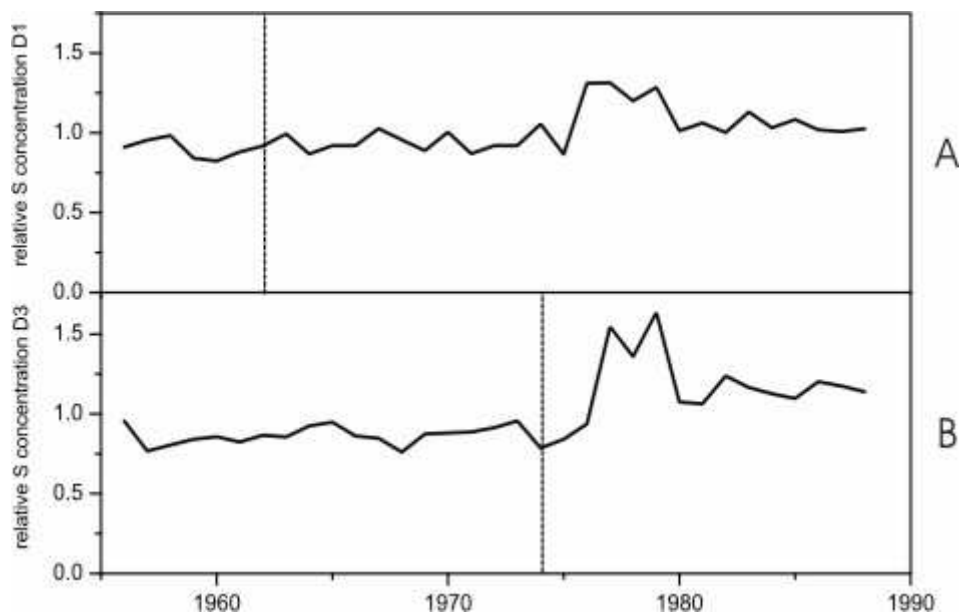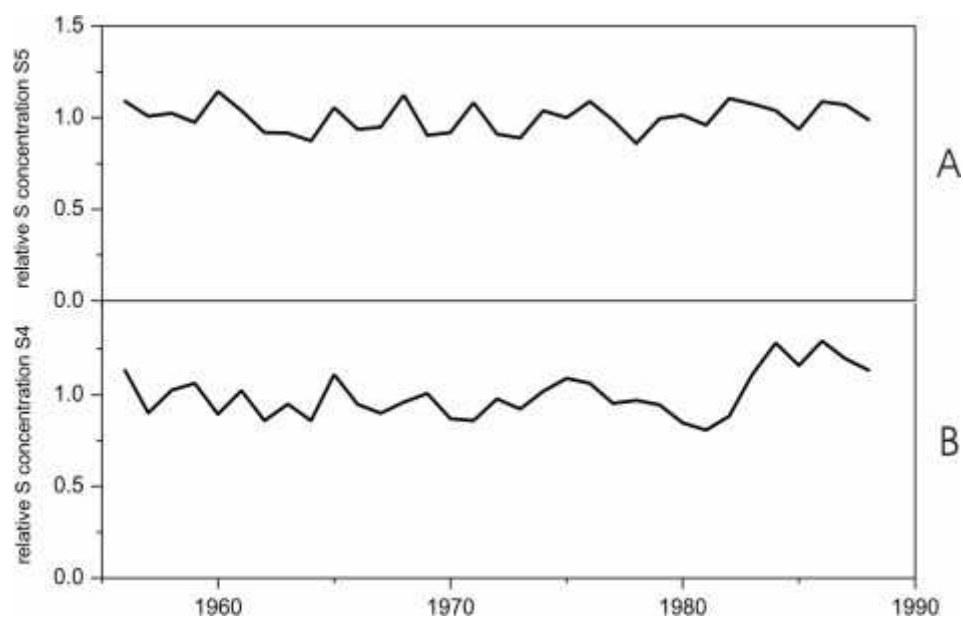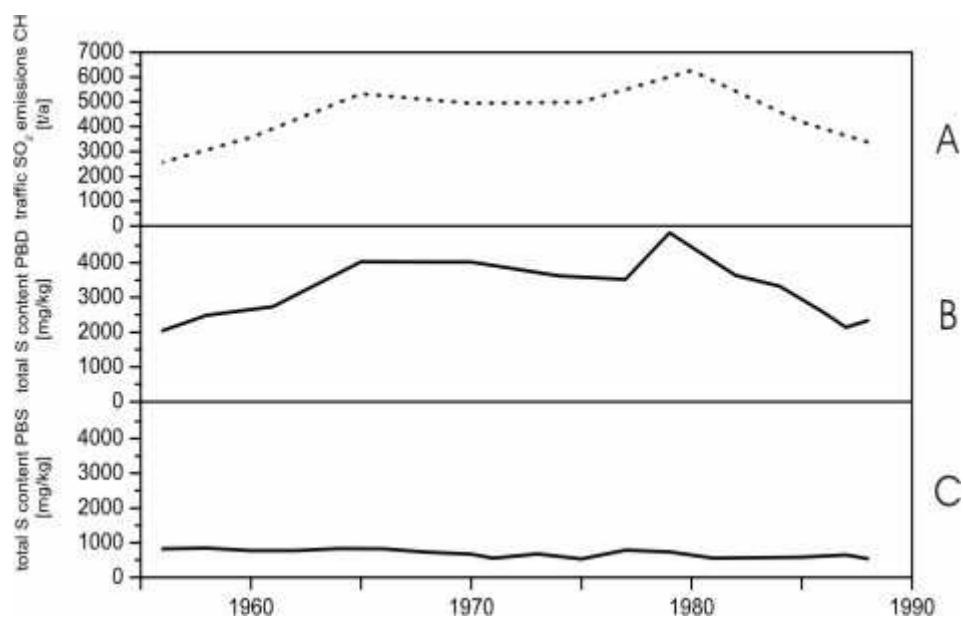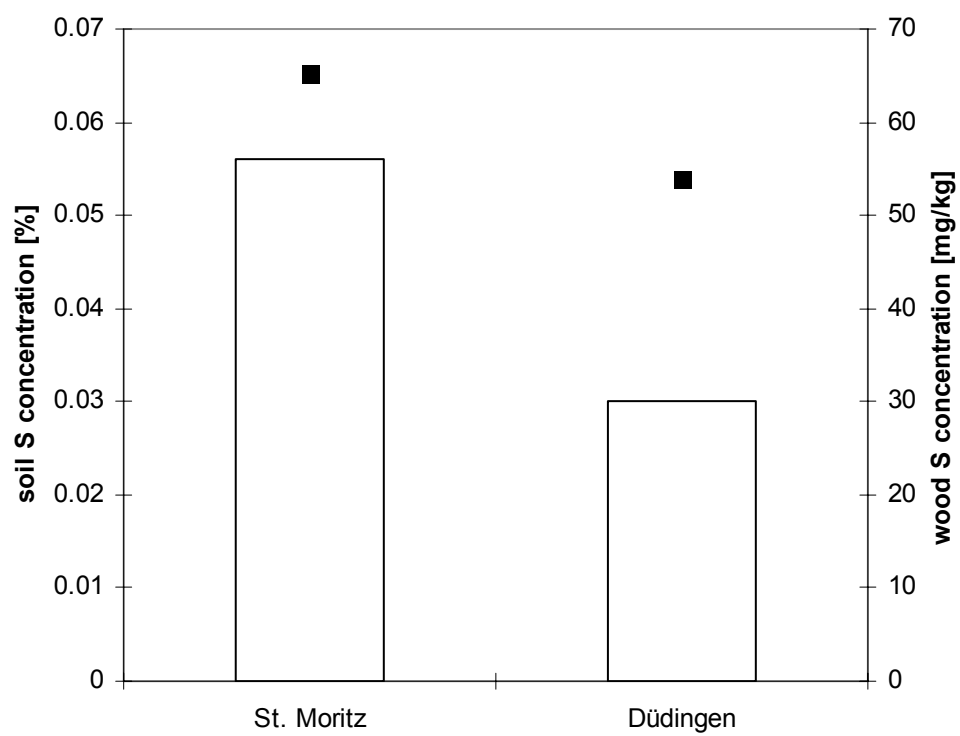

FIGURE 1

FIGURE 2

FIGURE 3

45

FIGURE 4

Supporting notes for: Norway Spruce as an Environmental Archive for Sulphur Dioxide

Supporting note 1: Interferences in ICP-MS

In the present study the Nd:YAG LASER was coupled to a modern high resolution magnetic sector field ICP-MS (HR-ICP-MS) ELEMENT 2, ThermoFinnigan. The Element 2 can be operated in the three resolution modes low, medium and high, which represent a resolution of 300, 4000, and 10000, respectively. The coupling to a HR-ICP-MS opens new advantages over conventional quadrupole couplings because this setting not only allows the determination of most of the metallic elements, but also the measurement of elements such as sulphur or phosphorus, which would not be possible otherwise due to mass interferences with their isotopes (e.g. ^{32}S, ^{31}P). For instance, a quadrupole-ICP-MS is limited to a resolution of about 300, which means about 0.7 to 1 amu. Some elements such as sulphur, phosphorus, chromium or the main isotope of iron can not be determined because of interfering elements. All sulphur isotopes are overlaid by polyatomic interferences: ^{32}S$^+$ mainly by ^{16}O^{16}O$^+$, ^{33}S$^+$ mainly by ^{16}O^{17}O$^+$, and ^{34}S$^+$ mainly by ^{16}O^{18}O$^+$, ^{17}O^{17}O$^+$. NO$^+$ and NOH$^+$ polyatomic or matrix depending double charged interferences also occur, but are a minor problem in case of sulphur determination. ^{36}S$^+$ (0.017 %) is interfered by isobaric ^{36}Ar$^+$ (Plasma gas), an interference which can not be resolved in a HR-ICP-MS either. However, a coupling to a high resolution instrument enables to distinguish between the signals of sulphur and the polyatomic interference in the medium resolution mode. Furthermore, it resolves the interference from ^{12}CH on ^{13}C, the carbon isotope used for normalisation. Table 1 gives an overview about typical interferences on the main isotope of sulphur as well as the necessary mass resolutions for the separation of the ^{32}S signal from polyatomic or double charged interferences.

TABLE 1: Characteristics of sulphur isotope ^{32}S (highlighted in grey) and the most probable polyatomic interferences

Isotope	exact mass m	relative abundance	Delta mass dm	m/dm
^{32}S$^+$	31.97207	95.018 range of natural variations (*1*): 94.537-95.261		
^{64}Zn^{++}	31.96457	48.89	0.02526	1266
^{16}O^{16}O$^+$	31.98983	99.52	0.017758	1801
^{15}N^{17}O$^+$	32.00223	0.2	0.012400	2581
^{14}N^{17}O^{1}H$^+$	32.00285	0.37	0.03078	1040
^{15}N^{16}O^{1}H$^+$	32.01003	0.04	0.03796	843

Because the relative abundances of the natural sulphur isotopes ^{33}S (0.75%) and ^{34}S (4.21%) are low compared to ^{32}S (95.02%), and thus lead to weak signals, only the latter was used for establishing annual sulphur profile in wood.

Reference:

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Supporting note 2: Tree characteristics

On the sites of Düdingen (D) and St. Moritz (S), mature, healthy trees of different sizes were selected. They were sampled in forests near peat bogs, in order to have access to data from independent environmental archives for sulphur dioxide. The following characteristics were determined: stem circumference, sapwood extent, water content of the wood and cambial age of the trees. They are presented for each individual in the table below, including the average year ring width.

TABLE 1: Characteristics of all sampled trees

Identification Code	Measured circumference [cm]	Measured sapwood width [cm]	Determined wood water content [%]	Sapwood zone [y]	Determined cambial age [y]	Average year ring width [mm]
D1	128	4.3	39.8	41	101	1.15
D3	185	4.7	45.6	29	96	2.49
S4	145	7	69.3	59	135	1.25
S5	153	3.8	70.1	66	177	1.02

With respect to interpretation of the sulphur profile in a tree, the determination of the sapwood-heartwood boundary is mandatory. The sapwood zone is the humid area in a tree where nutrient transport and storage are still active, whereas the heartwood is much dryer and does not contain any living parenchyma cells (i.e. storage cells) and active vessels anymore. Therefore, the concentrations of nitrogen, phosphorus and potassium in sapwood are usually higher compared to heartwood (*1*). Heartwood formation is progressive and due to apoptosis of xylem cells. Their decay begins shortly after the cambium and is accompanied by the release of proteins and the subsequent formation of a ring-like zone with higher amino acid concentration in the tree (*2*). Protein content of the cambium ranges between 0.42 - 0.56 %, and falls shortly afterwards to 0.2 – 0.3 % (*3*).
There is also a partial, selective migration of minerals and sulphate back to the sapwood through the rays (*4*). Additionally, bidirectional exchange between phloem and xylem takes place during long-distance transport along the stem. This is described for sulphate, and organic sulphur-containing compounds such as the amino acid cysteine and the tripeptide glutathione (*5*).
The water content and the sapwood zone extent seen in table 1 illustrate the water-conducting functions of sapwood. On basis of the densitometry analysis, the sapwood zones of the two alpine trees of St. Moritz were determined to contain 59 and 66 tree rings, whereas the Düdingen trees showed much smaller sap wood zones of 29 and 41 tree rings. This is also reflected in the higher wood water content determined for the St Moritz trees (wood water content: 69.3 and 70.1%, respectively) in comparison to the Düdingen trees (wood water content: 45.6 and 39%, respectively). This shows that mountain trees tend to conserve their parenchyma cells during a longer time. Their environment is tantamount to permanent stress, which results in slower growth at these altitudes. Therefore, tree rings of alpine trees are usually narrower, which was also confirmed in this study by radiodensitometry results. Accordingly, Spruces sampled in St. Moritz have the smallest circumference with respect to their age. A notable exception for Düdingen is tree D1, which has narrow tree rings and a small circumference. It was probably impaired in its growth by competing trees.

References:

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Supporting note 3: Local immisson rates

In Switzerland, SO_2 immission rates are much lower for alpine regions. This was shown in measurements performed by the EMPA for the National Air Pollution Monitoring Network (NABEL). Results from locations comparable to Düdingen on one hand, and St Moritz on the other hand allowed estimating the local situation for both sampled forests. Results of 10-minute determinations were averaged to monthly and then to annual immission rates. Figure 1 shows the annual average of SO_2 immissions determined at 8 national sampling sites from the NABEL air pollution monitoring program, and table 1 summarises the main characteristics of the different locations.

TABLE 1: Abbreviations (in order of appearance) and characteristics of the sites presented on figure 1, characteristics of the Düdingen and St Moritz sample sites. Polluted sites are highlighted in grey.

Identification code	Location	Altitude [m a.s.l.]	Category
JUN	Jungfraujoch	3578	High alpine
DAV	Davos	1638	Countryside, above 1000 m a.s.l.
RIG	Rigi	1031	Countryside, above 1000 m a.s.l.
PAY	Payerne	489	Countryside, under 1000 m a.s.l.
HAE	Härkingen	431	Countryside, motorway
ZUE	Zürich	410	City center, parking
BER	Bern	546	City center, main road
DUE	Dübendorf	433	Agglomeration
S4, S5, PBS	St Moritz	1900	Alpine
D1, D3, PBD	Düdingen	580	Countryside, motorway

It appears clearly that, depending on the measuring site, there are important differences in the atmospheric SO_2 air concentrations. Sites like Jungfraujoch (JUN), Rigi (RIG), Davos (DAV) are less polluted than the urban and suburban sites Zürich (ZUE), Bern (BER), Dübendorf (DUE) and Härkingen (HAE). The countryside site Payerne (PAY) has an intermediate position. This is likely due to its aerodrome. As it can be seen on figure 1, the annual average SO_2 concentrations can be 7 to 15 times lower in the Alps than in urban areas or locations exposed to traffic. The NABEL sites RIG, DAV and JUN are located in alpine regions, DAV being in the Engadine just like the St Moritz sampling site. The sampling site JUN is the highest NABEL site and represents a kind of background level in the alpine region. Thus, it is not surprising that the profiles of the trees S4 and S5 from St Moritz are flat compared to the trees D1 and D3 from the polluted site Düdingen.

Härkingen (HAE) is comparable to Düdingen because it is also located in the Swiss Plateau 70 km away. Furthermore, it is at a similar altitude and also in the direct neighbourhood of a motorway. The immission situation is lower than in cities or agglomerations but higher than in predominantly rural locations without direct emission source such as Payerne, where the main road is in some 100 m distance in the south-east of the NABEL measuring site. The obvious influence of a direct source such as the motorway in Düdingen or HAE is shown by data obtained at the HAE site. Its evaluation with respect to wind direction on the site is shown in figure 2.

On figure 2, it appears that western winds carry the main quantity of SO_2 to the measuring station. The apparatus is located a few meters away from the motorway, on its north-eastern side. Since west is the predominant wind direction on the Swiss Plateau, this results shows that traffic is the main SO_2 source in Härkingen. Similarly, the sampled trees and the peat bog in Düdingen are situated on the south-eastern side of the motorway, thus also receiving much of the SO_2 from western winds.

The atmospheric SO_2 concentrations evaluated at urban, suburban, countryside and alpine emplacements within the scope of the NABEL studies clearly show important differences between the sites (figure 1). It also proves that each site has its individual immission profile. Thus, the emission profile for Switzerland does not reflect the local environmental situation, which is decisive for the potential exposure of the tree or another archive

Figure captions

FIGURE 1: Annual average SO_2 immissions at 8 NABEL sites in Switzerland

FIGURE 2: average SO_2 mixing ratio per wind direction degree (0: north, 90: east, 180: south, 270: west) based on 10 minute data for the years 1994-95 (NABEL).

FIGURE 1

Haerkingen, 1994 to 1995
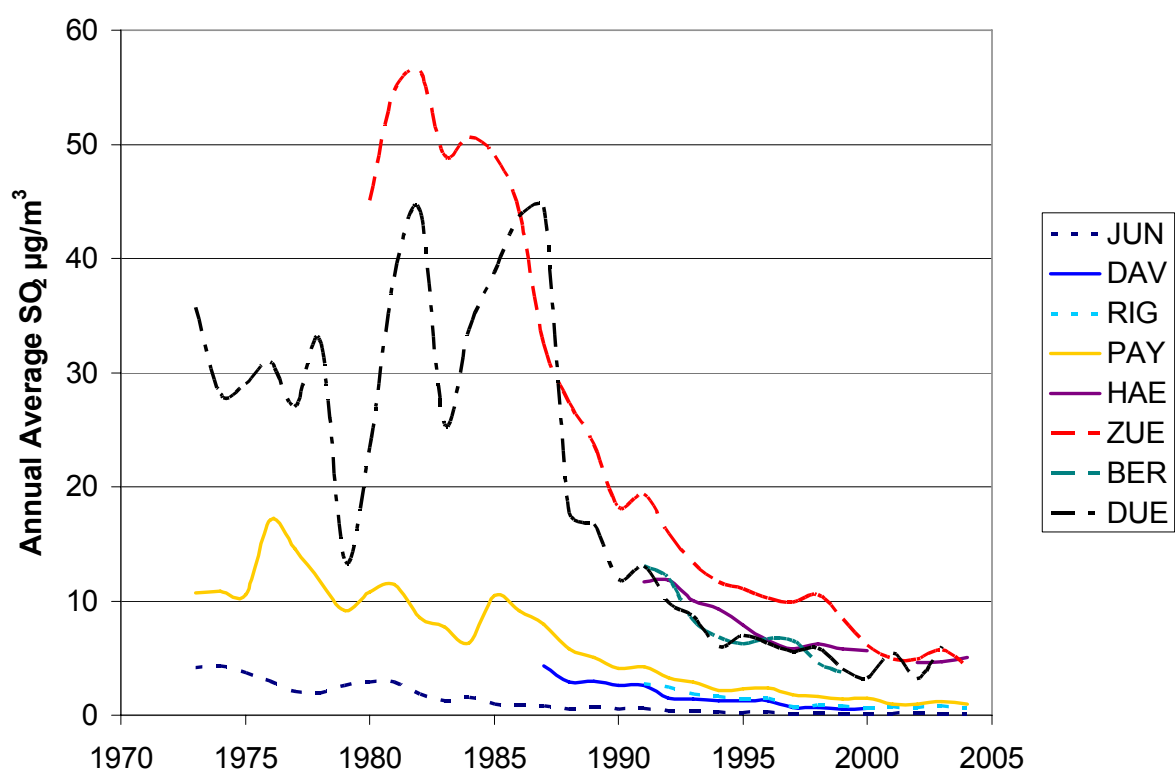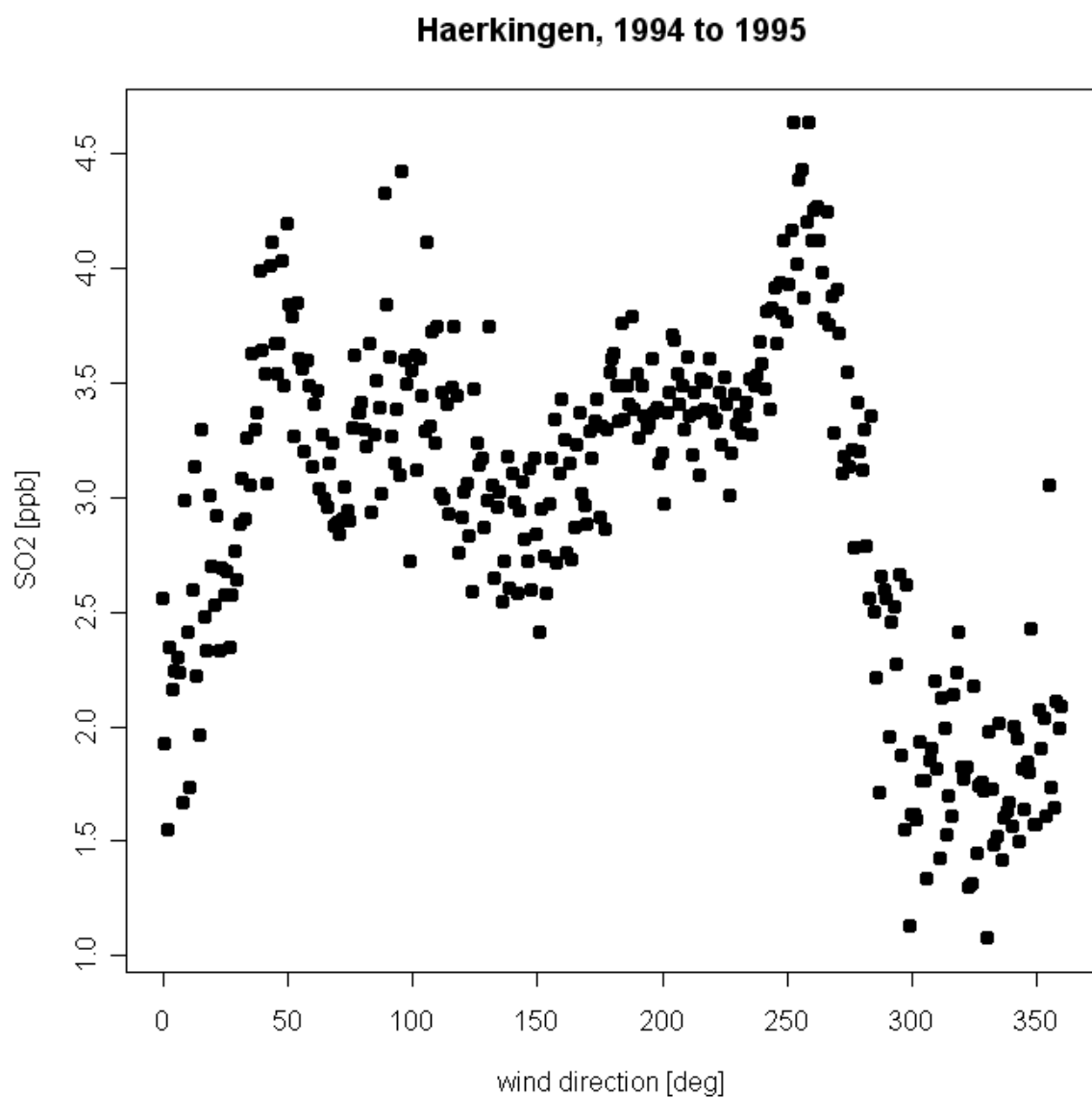
FIGURE 2

3.3 Dendrochemical Analysis of Norway Spruce Drill Cores by Optical Emission Spectrometry

Journal	Analytical and Bioanalytical Chemistry
Manuscript ID	ABC-01099-2005
Manuscript type	article
Date submitted by the author	11.12.2005
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Keywords	Sulphur Dioxide, Norway Spruce, Oxygen Bomb Digestion, Microwave Acid Digestion, ICP-OES
status	submitted

3.4 Dendrochemical Analysis of Norway Spruce Drill Cores by ICP-OES (Analytical and Bioanalytical Chemistry, submitted)

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Abstract Norway spruce (*Picea abies* L. Karst.) is the dominant conifer species in Northern and Central Europe; it is found over a wide range of latitudes and altitudes. It is therefore predestined for use as environmental archive. In the present study, methods were developed for profound dendrochemical investigation of this tree species as an environmental archive. Two independent digestion procedures were investigated regarding suitability not only for metal determination, e.g. for potassium, calcium or manganese, but also applicable for analysis of low sulphur content in wood. The procedure finally developed was applied to Norway spruce drill cores. Trees from two rural sites of the Swiss Plateau, both exposed to SO_2 immissions, were compared with an alpine site with low anthropogenic impact. The measurements confirmed the suitability of Norway spruce as an archive for sulphur dioxide. In contrast to their alpine counterparts, trees from the Swiss Plateau revealed a clear sulphur peak towards the end of the 1970's. The maxima reflect the SO_2 immission situation of this period. However, the sensitivity of conifers as environmental archive for sulphur is influenced by plant physiological aspects. Low SO_2 changes can not be retraced in the profiles. Furthermore, plant specific physiological changes such as formation of compression wood or fungal attack can have a significant influence on the sulphur content of the trunk. In contrast to the wood sulphur profiles, changes in the contents of K, Ca and Mn in forest trees are likely of natural origin. While potassium content increases significantly near the cambium, calcium and manganese concentrations decrease. These results indicate different storage strategies of these metals.

resolution inductively coupled plasma mass spectrometry · *WD-XRF*: wavelength-dispersive x-ray fluorescence spectrometry

Introduction

Trees are one of the most popular environmental archive types because they are widespread and can be found in remote as well as at highly populated places including cities, and even close to anthropogenic sources. Furthermore, the relatively easy sampling using increment borers and the low risk of sample losses or deterioration make trees very attractive for this task. Additionally, tree rings make dating very precise. In conifers particularly, individual tree rings can be distinguished easily. Conifers can be found in most parts of the globe. In the northern hemisphere, the genus *Picea* is very common, which was one of the main reasons, why *Picea abies* (Norway spruce) was selected for this study.

Sample digestion followed by chemical analyses is suitable for the determination of metals and non-metals in wood. In previous studies, digestions of 5 to 10 year drill core segments were performed, mainly for the determination of metals. Microwave acid digestion in Teflon$^®$ pressure bombs is commonly used in dendrochemical studies about heavy metals, using different acid mixtures.. A mixture of HNO_3, HCl and HF was proposed for bark [1], but the addition of HCl and HF is not compulsory for the digestion of wood. Digestion of wood in 50% H_2O_2 is also described [2]. Using quartz cups, one study preconised a mixture of HNO_3 and $HClO_4$ [3]. However, a digestion with perchloric acid is not necessary for the digestion of wood tissue. Moreover, the use is not preferable for sulphur analysis because of the relatively high sulphur concentration in $HClO_4$. Even in ultra pure purity grade acid the contamination is relatively high, e.g. max. 5 ppm SO_4 in $HClO_4$ 70 % Merck Suprapur$^®$ in contrast to max. 200 ppb SO_4 in HNO_3 65% Merck Suprapur$^®$. Digestion of wood powder by heating in HNO_3 on a heating block [4] is inadequate in the case of sulphur due to losses of volatile compounds. Ashing of wood samples and subsequent digestion with HNO_3 as described by Gaoming [5] has the major disadvantage of relatively high required sample quantities, which results in a relatively low time resolution of 10 years. Moreover, the procedure is time consuming and losses of volatile sulphur compontents during ashing procedure are possible.

Sulphur was only measured in the last-mentioned study; all other digestion methods described above were optimised for measuring metals. However, none of the above mentioned procedure was appropriate to fulfil the purpose of this work which was the development of a suitable digestion method not only for multielemental determination of metals but also for sulphur analysis at low concentrations. The optimisation is challenging due to the low sulphur content in wood of approximately 50 mg/kg. Therefore, two independent decomposition procedures were optimised and applied for sulphur analysis of wood samples, and compared using standard reference materials: microwave acid digestion and oxygen bomb combustion.

Investigation of sulphur in tree rings is especially interesting, since it is both a pollutant (mainly in form of anthropogenic SO_2) and a naturally occurring element (e.g. as SO_4^{2-} in soil). Furthermore, sulphur is one of the essential macronutrients for plants, which plays an important role for physiological processes such as photosynthesis, dissimilation and also for defence via the production of antioxidants like glutathione [6, 7].

Three principal uptake mechanisms for atmospheric SO_2 have to be taken into account: 1. via needles (leaves), 2. via roots after deposition, 3. via run-off water through the bark. Uptake as sulphate in the rhizosphere is the main mechanism of sulphur acquisition by plants. However, in heavily polluted areas, SO_2 can become an additional sulphur source. The trace gas enters the needles of conifers via the stomata, is removed from the substomatal cavity by solubilisation in the aqueous phase of the apoplastic space, and is taken up into the cytoplasm of surrounding cells [8]. Once inside the cell, either storage in the vacuole or assimilation into the amino acid cysteine takes place by the sulphate assimilation pathway [9, 10]. Allocation

of sulphur from source needles to the stem is low [11]. Instead, most of the accumulated sulphur is allocated to the following needle generation [12]. Falling needles, enriched in sulphur, can contribute to raising the soil sulphur concentration. Wet deposition of sulphur remains the most important sulphur sources of forests. Due to the huge canopy surface of a Norway spruce tree, important amounts of aerosols and fog are deposited. So-called crown interception is three times higher in Norway spruce than for a deciduous tree like beech (*Fagus sylvatica*) [13]. As a consequence, the sulphur accumulation in the soil under Norway spruce is four times higher than under beech. This is accentuated by the fact that perennial foliage leads to uninterrupted crown interception during winter, where airborne sulphur from heating reaches a maximum. Accordingly, Norway spruce accumulates two times more sulphur in its phytomass than beech.

Accumulation of anthropogenic sulphur in conifers has been studied e.g. by Gaoming [3]. The high sulphur content measured in Chinese pine (*Pinus tabulaeformis*) from Chengde, northern China, are believed to reflect the important industrial SO_2 emissions in this zone.

Sulphur is still a problem. Even if in industrialised countries such as Switzerland, desulphurisation of crude oil and reduced use of coal permitted cut emissions by a factor of 3 after they peaked around the eighties of the last century, the global emission rate still increases. Developing countries such as China depend on coal-fired power plants for covering their increasing energy needs. High sulphur content of coal then leads to an enormous SO_2 release. In the latest report of the International Panel on Climate Change (IPCC 2001), scenarios of a worldwide increase in SO_2 emissions were predicted.

In the present article, mineral content profiles of Norway spruce trees will be discussed, with a special focus on sulphur. Beside sulphur, especially the macronutrients potassium and calcium and profile of the micronutrient manganese were obtained. The results will be discussed with special regard to anthropogenic and plant physiological influences.

Materials and methods

Samples

Tree sampling and site description

Due to the reasons mentioned above, *Picea abies* L. Karst (Norway spruce), a dominant species in the northern, central and eastern parts of Europe, was chosen in the present study. In Switzerland, it represents 48% of all trees and also occurs in high altitudes.

Three forests were selected: two in the Swiss Plateau (Düdingen, 580 m a.s.l., Frieswil, 740 m a.s.l.), and one at the alpine site of St Moritz (Engadine, 1900 m a.s.l.). The SO_2 immission situation is different for each sampling site and is at its lowest in St Moritz. 100 underneath the sampled forest, there is a peat bog useful for comparison as an independent environmental archive. Düdingen has the highest pollution levels due to the proximity of the forest to the motorway A12 (Bern-Fribourg), 500 m away of the forest. As in St Moritz, there is a peat bog near the forest, 1 km away. In Frieswil, a rural site without direct SO_2 source, atmospheric concentrations of the pollutant are at an intermediate level.

In all sampling sites, mature forest trees were selected. The circumference of each tree was determined before sampling. Trees of different size were sampled in order to assess the consequences of possible ageing effects. Drill cores were taken at breast height using a 5 mm increment borer (Suunto). The contamination levels of possible cleansing agents for the corer were determined before the sampling campaign to ensure that the corer metal tongue was devoid of sulphur. Measurements using ICP-OES revealed that WD40, which contains 21% mineral oil, was not suitable because it is rich in sulphur. PhEur grade paraffin (Hänseler) was

finally chosen for cleaning and lubricating the sampling tools because of its low background contamination. It contains only traces of sulphur. Gloves were used to avoid contamination. In order to compare different analysis methods, six drill cores per tree were taken in four directions. One of them was used for LA-ICP-MS [14, 15], two for radiodensitometry [15], one for x-ray absorption spectroscopy [16], and the remaining two for the digestion and combustion techniques described in this study.

The extent of the sapwood zone of each drill core was determined immediately after sampling, since no visual differentiation between sapwood and heartwood is possible after drying. For interpretation of element profiles in a tree, determination of the sapwood-heartwood boundary is important.

The samples were then pressed into weighted and previously cleaned plastic rails, and wrapped in a polyvinylidene chloride foil (Saran). Afterwards, they were kept in the rails and dried at 40°C until weight constancy. Water content of the wood was determined as weight loss after drying.

Soil sampling, sample preparation and analysis

At each site, two soil samples were taken in proximity of the trees using a garden corer. After removal of foliage on the surface, 20 cm cores were collected and put into plastic bags. The samples were then freeze dried until weight constancy, followed by pulverisation to a grain size of about 50 µm using a wolfram mill (Fritsch, pulverisette 9). Finally, the samples were analysed using WD-XRF (wave-length dispersive x-ray fluorescence spectrometry) with a Philips PW 2400 XRF spectrometer. The "standard free" analytical program UniQuant (Omega Data Systems) was used for analysis, giving quantitative results in the range of 0.001-100 %. 90 element lines were analysed with 4-8 s measurement time per line. The powdered samples were determined directly (diameter of analysed area: 24 mm) A total of 30 elements were analysed as non-ignited loose powders in helium (atmospheric pressure). Because of the oxidic nature of the samples, the mass contents of the major elements were calculated as oxides (K_2O, CaO, MnO). Undetected light elements (H, C and O) must be included in the calculation procedure. This is why loss on ignition (LOI) was determined by heating ~25 g of each sample during 2 hours at 500° C to evaporate remaining water and volatile organic compounds. More Details can be found in a previous study [14].

Sample preparation

Two sample preparation procedures have been investigated for optimum sample decomposition. They had to allow not only a precise determination of metals but also an accurate quantification of sulphur: On one hand acid digestion using microwave, on the other hand a modified oxygen bomb combustion procedure which was originally developed for sulphur determination in oil or coal.

Development of an oxygen bomb combustion method

Oxygen bomb combustion was chosen for independent comparison with an acid microwave digestion. This procedure is routinely used for determining the halogen and sulphur content in coal and petroleum products. [17] It is well validated and comprises all relevant sulphur components including the volatiles. However, it was never applied to wood before. This type of sample is particularly challenging because of its low sulphur content of only about 50 mg per kg, which means about 60 times lower than in coal.

Combustion of the wood samples with oxygen was performed in a sealed decomposition vessel with catalytic coating. All sulphur compounds contained in the wood were converted to

sulphate during the rapid combustion. The oxygen pressure bomb combustion was performed using an Ika AOD 1 system and following the DIN 51577 procedure. Using a carefully cleaned scalpel, the drill cores were cut into 10 tree rings segments. A minimum of 70 mg wood sample were put into a quartz crucible, which was then placed into a holder. A cotton thread leading to the crucible was fixed to the ignition wire, and both sample and thread were moistened with about 1 ml ethanol p.a. for combustion support. 10 ml 1 M NaOH (p.a. grade) were dosed into decomposition vessel for absorption of the combustion gasses. Previous optimisation studies showed that no H_2O_2 is required for additional oxidation. The cover/crucible holder was then screwed onto the decomposition vessel. After filling the apparatus with oxygen (5.0 grade) up to 30 bar inner pressure (6 L), the combustion was started by ignition. Subsequently, the oxygen bomb was placed into a water bath for cooling and nodded in order to allow contact of the whole inner part with the absorption liquid. 10 min later, the apparatus was vented. Previous controls demonstrated that venting does not require the connection to a gas washing flask with supplementary absorption liquid. Before each new measurement, decomposition vessel and crucible holder were rinsed with MQ water (bidest purity grade) and dried. Combustions were all exempt of residue, except of a small amount of sooth in the glass crucible, which was wiped away with a towel. The presence of soot is tantamount to an incomplete combustion of the carbon fraction in wood, but does not signify incomplete sulphur oxidation. The use of one-way cellulose acetobutyrate crucibles was found to be unsuitable because they increased the blank value 2.6 times compared to quartz glass crucibles.

The combustion procedure was optimised and validated using sulphate solutions (1 mg/l), and standard reference materials (BCR 101, powdered spruce needles, 1700 ± 40 µg/g S). The recovery rates for synthetic sulphur samples ranged at about 97 %. For BCR 101, recovery rates up to 90 % of the certified value were obtained, but in average the recovery rates were only about 80 %. The optimisation of the reagent types and volumes showed that a careful optimisation of the NaOH concentration in the absorption liquid is important in terms of the recovery rates. The concentration was finally optimised to 1 M NaOH. Lower concentration resulted in much lower recovery rates. The findings point to a systematic error in the present digestion procedure.

The oxygen bomb combustion method was also applied to real wood samples. One tree per sampling site was selected. For tree samples, a compromise had to be made between minimal weight of the sample and best obtainable time resolution. Drill cores with a diameter of 5 mm had to be cut in 10 year segments in order to reach the required minimum sample weight of 70 mg. Due to the low sulphur content in wood, a sufficient sulphur level was only measured for one out of 3 drill cores. The only drill core providing enough sulphur content originated from a St Moritz tree. The sulphur concentrations in the drill cores from the Swiss Plateau trees displayed only values below detection limit. The use of a 12 mm drill core might solve the problem. Another possibility might be a minimisation of the combustion devices including vessel volume.

However, it appears that oxygen bomb combustion was not an optimal choice for the determination of sulphur in wood. The present procedure does not provide reliable results for the sulphur content of wood samples. Moreover, the detection limits were relatively poor and the required sample amounts were relatively high. An adequate minimisation of the required sample amount is challenging: the detection limits achievable so far for decomposition procedure of small sample amounts of 70 and 200 mg were not adequate for low sulphur contents in wood tissue.

The reagents might be a significant contamination sources since neither NaOH nor ethanol were available in high purity quality. Decomposition vessels could be an additional contamination source. However, further optimisations were discarded since minimisation of

the required sample amount is limited in the present composition system and the procedure is complex and time consuming. Better results were obtained by the microwave acid digestion described below.

Development of a microwave acid digestion

The main goal of the development of this digestion procedure was to achieve a good temporal resolution. Thus, the decomposition procedure was carefully optimised with respect to accurate quantification of very small amounts of sulphur in Norway spruce wood. Optimisation was particularly challenging because wood contains only an average of 5 μg S per 100 mg drill core piece. Therefore, digestion was optimised with special attention to minimum sample weight, optimum acid mixture, minimised sample losses and contamination risk.

Using a clean scalpel, the drill cores were cut into 5-10 tree rings segments, depending on the weight. A minimum sample weight of about 40 mg was required to ensure accuracy. The optimisation and validation of the digestion procedure was performed using standard reference material (BCR-CRM 101). The recovery rate amounted to 97%.

A 1:1 mixture (2 x 2.5 ml) of nitric acid HNO_3 65% and hydrogen peroxide H_2O_2, 30 % (both Merck suprapur®) was finally selected for the microwave digestion. All digestions were performed in a MLS ETHOS 1600 microwave oven controlled by software version easyWAVE3. In the space of 17 minutes, temperature was brought to 150°C and maintained during 10 minutes. Maximal pressure was set at 25 bar.

Hafez et al. (1991) [18], and Jeker et al. (2001) [19] recommended an addition of MgO to the nitric acid for microwave digestion of peat bogs. Aim was to avoid sulphur losses by the formation of $MgSO_4$. In order to verify is this addition is required for wood sample as well, several digestions in pure acid and with addition of increasing amounts of MgO (0, 22.5, 45, 90 and 135 mg/l) were performed using the BCR-CRM 101, as well as homogenised wood (S content: 50 mg/kg, characterised at EMPA) doped with sulphur compounds (one batch witch 20 mg/kg sulphate, the other with 20 mg/kg cysteine). However, a comparison with the certified values showed that an addition of MgO does not bring significant changes and is therefore not necessary for the digestion of wood samples. The amount of magnesium in Norway spruce wood is about six times superior to that of sulphur and therefore the mineral content of wood is probably high enough to bind volatile sulphur compounds. Optimisation of the procedure also showed that sample losses occurred when inner quartz vials were used. Even at relatively long ramp times, a temporary overpressure during wood digestion could not be avoided. The short-term overpressure caused the caps of the quartz glass vessels to lift, which led to loss of SO_2/SO_3 to the liquid surrounding the vials: the sample amount released could be detected in the outer MQ water/H_2O_2 mixture. With BCR-CRM 101, the chemical yield amounted to 93%, while for homogenised and characterised wood of lower sulphur content, it was 56 % only. Thus, best results were achieved when decompositions were directly performed in the PTFE vessels. For this reason, all later digestions were directly performed in the Teflon® bombs.

ICP-OES analysis

All analyses were performed using inductively coupled plasma optical emission spectrometry using a sequential Varian Liberty 150 AX Turbo with axial torch or a simultaneous Varian VistaPro with a radial torch under standard hot plasma conditions. For sulphur determination on the Varian Liberty 150 AX Turbo, the optical chamber had to be rinsed with nitrogen

because oxygen absorbs the sulphur emission line. On the Varian VistaPro, the optical chamber was rinsed with Ar.

The instruments were operated under standard hot plasma conditions. The microwave acid digestions were analysed using a standard quartz sample introduction system consisting from a Meinhard nebuliser and a Twister cyclonic spray chamber. For analysis of the alkaline solutions from the oxygen bomb combustion, a Varian HF kit had to be mounted because of the strongly alkaline solutions (about 0.4 M NaOH), which can cause quartz corrosion. The kit consists from a plastic V-groove nebuliser, a plastic spray chamber and a demountable torch with corundum injector tube.

Radiodensitometry

For dating and assessment of abrupt growth changes or reaction wood, supplementary drill cores from the trees analysed were taken in pairs and measured by radiodensitometry. Wood density and tree ring width of the additional drill cores were measured at the installations of the Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf (WSL). 1.2 mm thick splinters were sawn out of the fixed core, at right angle to the direction of fibre. After conditioning (20°C, 50% relative air humidity), the wood samples were x-rayed. Optical density of the negative films obtained was measured with a Dendro 2003 microdensitometer (Walesch Electronic). The optical density corresponds to the volumetric-gravimetric wood density.

Results and discussion

Sulphur

The principal focus of this study was to assess to which extent SO_2 immissions can be reconstructed using tree drill cores sampled at different sites. Due to the three uptake mechanisms mentioned above (uptake via roots, needles, and bark), which are amplified by the huge foliar surface, Norway spruce was predestined for this purpose. A further advantage is that the species is widespread in Switzerland and found at sites with completely different immission profiles, such as the Alps and the Swiss Plateau.

The emissions rates of SO_2 in Switzerland were particularly pronounced towards the end of the seventies and at the beginning of the eighties. Measures taken to reduce these emissions led to a sustained decrease of SO_2 pollution. The wood sulphur content in trees sampled in 3 distinct locations in Switzerland: Düdingen (countryside, near to a motorway), Frieswil (countryside) and St Moritz (Alpine), are displayed in figure 1. Average standard deviation of all pooled data was 7.7%, 14.5% and 10%, respectively. Such deviations have to be taken into account, since the sampled trees all are individuals, grown under natural conditions. Although the Düdingen trees have an average cambial age of 96 ± 4 years in average and the trees from Frieswil an average cambial age of 142 ± 6 years, the profiles look very similar.

There is only a slight variation in the Swiss Plateau sulphur profiles for the first three quarters of the 20th century (Fig. 1. A and B). In the tree rings corresponding to 1974-1984, a sulphur peak is visible. This corresponds to the period when SO_2 emissions reached their maximum in Switzerland, as evaluated by the Swiss Agency for the Environment, Forests and Landscape (SAEFL). Düdingen and Frieswil are both rural sites, separated by 20 km distance only, and reflect the immission situation of their respective region, which is why there is a slight shift between both peaks. The motorway A12 (Bern-Fribourg), which is next to the sampled Düdingen forest (Fig. 1. A), acts as a local sulphur dioxide source, as was shown by

measurements of further trees and the peat bog nearby [14, 19]. Although there is no direct pollution source in Frieswil (Fig. 1. B), deposition of anthropogenic sulphur occurs as well and is likely to be the reason for the peak.

Trees from the alpine forest show that the sampling site of St Moritz (Engadine) is secluded from the bulk of the SO_2 emissions in Switzerland. As corroborated by LA-ICP-MS measurements [14], St Moritz profiles do not display the characteristic maximum found in trees from the Swiss Plateau and corresponding to the immission maximum. Furthermore the profiles resemble sulphur content obtained from a St Moritz peat bog [20, 14]. As seen in fig. 1 B, the total sulphur content shows a generally higher S content. The reason is not anthropogenic origin, but a high natural background due to the elevated soil sulphur content in St Moritz compared to Düdingen [14]. This corresponds to the local immission situation, which is also reflected in peat bog drill cores taken nearby: measurements performed by EMPA for the National Air Pollution Monitoring Network (NABEL) showed that annual average SO_2 concentrations in the Alps are up to 10 times lower than on the Swiss Plateau [14].

In most recent years, i.e. close to the cambium where cell division occurs, maximum sulphur content was observed in all measured trees, including the alpine trees (fig. 1 C). This effect is natural and not due to anthropogenic SO_2 emissions, which continue to decrease steadily in Switzerland. The reasons of this rapid decrease by a factor of 1.5 in direction of the pith are protein decay during heartwood formation and recycling of sulphate. Apparently, sulphur recycling is not complete during this process, and wood is used for sulphur storage [15]. Thus, in case of higher sulphur input, as on the Swiss Plateau in the years 1970-80, the amount of sulphur remaining in the wood tissue increases (fig. 1 A and B). The trend line displays a clear upward trend of the wood sulphur content towards the second half of the century. Part of it is due to the natural higher sulphur content near the cambium (5 most recent years), but the principal reason is likely the SO_2 immissions of the Swiss Plateau. The upward trend in alpine trees (fig. 1 C) is much less pronounced, and principally due to the proximity of the cambium.

While in trees from the Swiss Plateau, the sapwood-heartwood transition zone was found around 1970 ± 6 years in average, in St Moritz trees, the average boundary ranged in the tree rings at 1941± 16 years. Thus, the period with maximum SO_2 emissions (1970-1980) is situated in the sapwood in both cases. The pollution peak which is visible in two types of archives from the Swiss plateau (Düdingen trees and peat bog, Frieswil trees) is likely not affected by water transportation and element translocation, since the conducting system is most efficient in the outermost tree rings of Norway spruce [21].

Two trees from the Frieswil forest showed deviating sulphur profiles. These tree profiles, presented as F1 (A) and F4 (B) in Fig. 2., were excluded from the calculations of the graph in Fig. 1. Reasons for the different profiles were investigated further. It is known that fungal infection can alter the cation content of heartwood [22]. Apparently, the sulphur content can be massively altered, too: In tree F1, the two huge S peaks found in wood segments corresponding to 1934-38, and 1949-53 corresponds with decayed tree rings. Microscopy of small slices of these two infected spots prior to acid digestion revealed the presence of a fungal pathogen, presumably *Ophiostoma piceae* or *Ceratocystis piceae* [23]. The exact fungal species is not determined yet. Further investigations are needed to estimate whether the huge increase is due to an accumulation of sulphur by the pathogen, or to a defence mechanism established by the tree.

In contrast, tree F4 shows very low S concentrations which related to a compression wood zone ranging from about 1940-1980. This type of reaction wood is found exclusively in conifers and built on the stressed side of the tree in case of mechanical pressure e.g. cause by wind, snow, slope or proximity of another tree. It distinguishes itself by its wider, denser and darker growth rings, compared to the wood on the opposite side of the tree. Compression wood contains up to 25 % less cellulose than normal wood [24] and the augmented lignin content provides more hydroxyl binding sites, which leads to higher cation content compared to the wood on the opposite side of the tree [25]. Thus, the variations of the total S content shown in the figure 2 are of physiological origin and presumably independent from the sapwood-heartwood transition zone as well. Manganese content was altered as well. Consequently, the trees F1 and F4 were not included in the calculations presented in the fig. 1 and 3.

Bark is exposed to precipitation and run-off water, and thus constantly enriched with minerals retrieved from the run-off water [26]. In the bark, nutrients can transiently be stored in parenchyma cells or/and can be subjected to long-distance transport. Substances poisonous for the tree can be exuded, e.g. as resin. Therefore, the element content of the bark is usually much higher than in wood tissue. The presence of sulphur in water running along the stem may lead to higher sulphate content of the bark [27]. Additionally, the phloem plays an important role for the cycling of sulphur in trees and contains sulphate and organic sulphur such as glutathione as well. Both sulphate and organic sulphur can be enhanced when sulphur availability is high [28]. For these reasons, the average bark sulphur content of seven trees from the three locations of the present study (423 mg/kg $\pm$ 42%) is about eight times higher than the average wood sulphur content (54 mg/kg $\pm$ 14%).

Potassium

High element concentrations in bark of about 2.4 g/kg $\pm$ 23% in average compared to average wood contents of 0.5 g/kg $\pm$ 28% (see fig. 3) were found for potassium, which confirms literature data [29]. These results are based on four trees from two locations, i.e. two trees at each site, Düdingen (Fig. 3. A) and St Moritz (Fig. 3. B).
Potassium is present in all parts of the tree and known as very mobile. Accordingly, large amounts are found in the radially extending ray cells (i.e. storage and conducting cells) [30]. Chiefly being used in young tissues, it plays important roles for colloid formation, enzyme activation and cell osmosis. The profile in figure 3 shows an upward trend. The highest potassium content was found in the 5 tree rings next to the cambium.

Since potassium is of essential importance for tissue growing, it is presumed that the highest concentrations are present in the active cells, i.e. in the sapwood region. Compared to Düdingen trees, where the sapwood-heartwood boundary is found around 1970 ($\pm$ 6 y), the transition zone in St Moritz trees is found at 1941 ($\pm$ 16 y). Thus, the potassium concentration is far less influenced by sapwood-heartwood transition than by the proximity of the cambium. The heartwood/sapwood concentration ratio (0.9) is roughly three times higher than literature values (0.33) [31].

Similarly to sulphur, crown interception enhances the potassium concentration in the soil underneath spruce trees. Due to dry deposition and wash-out phenomena, element concentration in the precipitation increases during its passage through the crown [13]. Interestingly, no correlation was observed between the potassium content in soil and its content in wood. This contrasts the significant correlation between wood sulphur content and soil sulphur concentration [14]. Average soil potassium content in Düdingen amounted to 6.4

g/kg (±3.4 %), thus being more than 6 times higher than in St Moritz (1 g/kg ± 15%). Surprisingly, wood from the Düdingen trees contains less potassium (0.26 g/kg ± 20%) than wood from St Moritz trees (0.42 g/kg, ± 29.5%). The fact that spruce trees from an alpine site with low potassium concentration in soil have the highest wood potassium content underlines the efficiency of potassium uptake by this species if needed. The fact that mountain environment is tantamount to permanent stress could explain the increased need for K of the St Moritz trees. Their sapwood zone comprises more tree rings (42 ± 16) than the sapwood of Swiss Plateau trees (33 ± 6) and contains more water (60.5 ± 7.7% compared to 43.6 ± 6.2%). Mountain trees tend to conserve their parenchyma cells during a longer time. Their growth is slower and their tree rings are therefore narrower.

Calcium

Similarly to sulphur and potassium, the highest calcium contents were found in the bark (10.4 g/kg, ± 26%) compared to the wood (0.72 g/kg ± 11.2%, see fig. 3 B), which was also observed by other authors [28]. Data of four trees from two locations (Düdingen and St Moritz, two trees at each site) were pooled for these results.

Calcium ions are used in cell wall synthesis and also during cell division. They influence the permeability of plant membranes, are used as second messengers for plant responses to signals (both environmental and hormonal) and regulate key enzymes in the cytosol [32]. In a recent study, it has been found that high calcium content in spruce wood and bark lowers the content of heavy metals [33]. This shows that calcium content in forest soils, reflected in wood and bark of trees, can interact with other metals and change their availability.

The uptake of calcium is even more efficient than that of potassium. In the phloem, it is less mobile than potassium and is strongly bond in the xylem [30], due to the numerous hydroxyl groups of lignin [25]. The higher the lignification grade, the higher the calcium content in wood.

Contrasting with the upward trend of potassium content, the wood calcium profiles in fig. 4 display a downward trend, particularly pronounced in the 10 most recent tree rings. As described above, the sapwood-heartwood boundary is found around 1970 for the Düdingen and Frieswil trees, and around 1941 for St Moritz trees. Thus, the declining trend is rather due to the proximity of the cambium than to sapwood-heartwood transition. The declining trend in calcium content of the wood from pith to cambium is likely due to lignification, which is incomplete in the sapwood. Düdingen trees have an average cambial age of 96 years (± 4 y), while for the trees from St Moritz, the average is 156 years (± 19 y). Despite this difference, the profiles from individual, healthy trees without growth irregularities are very similar. The heartwood/sapwood concentration ratio (1.2) corresponds to literature values (1.17) [31].

Unlike potassium, a correlation between calcium content in soil and wood was observed. Average soil calcium content in Düdingen (0.35 g/kg ± 0.7%) is almost 3 times lower than in St Moritz (1 g/kg ± 16.6%). Wood from the Düdingen trees contains slightly less calcium (0.71 g/kg, ± 12.7%) than wood from St Moritz trees (0.79 g/kg ± 4.2%).

Manganese

For manganese higher element content (1.2 g/kg ± 39.5%) were found in bark as well, compared to the total concentration in wood of about 0.18 g/kg ± 24.6% in average (see fig. 3

C). These results were obtained by analysis of four trees from two locations (Düdingen and Frieswil, two trees at each site).

Manganese ions activate a number of enzymes such as decarboxylases and dehydrogenases [34] in plant cells. They are considered to be moderately mobile in wood [25]. The profiles of manganese in Norway spruce wood (fig. 5) are very similar to the calcium profiles (fig. 4), but with a steeper downward trend towards cambium. Minimum values are located in the outermost 12 tree rings, where sap transport is particularly efficient.

In analogy to sulphur and calcium, the manganese content in soil correlates with the content in wood. Average soil manganese content in Düdingen (0.5 g/kg ± 35.4%) is slightly lower than in Frieswil (0.6 g/kg ± 95.8%). Düdingen trees contain slightly less manganese (0.17 g/kg ± 21.4%) compared to Frieswil trees (0.20 g/kg ± 29.7%). The two Frieswil soil samples where taken in 40 m distance from each other. The fact that distribution of metals is not homogeneous in forest soils could explain the high difference in manganese content [35].

Conclusion

Trees are challenging archives and the interpretation is complex, since anthropogenic and natural physiological signals can be intertwined. Pollutants, changes in soil chemistry and climate not only influence tree growth but also have an impact on xylem chemistry. Thus, for a correct interpretation of element content profiles, knowledge about tree physiology is mandatory. Physiological factors of the tree such as heartwood formation, pathogens and reaction wood can be identified from the chemical content of tree rings and must therefore be taken into consideration.

Norway spruce wood is a suitable archive for sulphur dioxide. As shown in the present article as well as in a further publication [14], the requirements postulated by Walkenhorst and Hagemeyer for retrospective bioindication in trees [36, 37] are met in the case of sulphur:

1) a constant relationship between element supply and accumulation
2) storage in tree rings constricted to a short time period
3) storage in a limited, known number of tree rings
4) no retroactive remobilisation of element
5) identical radial distribution in different spots of the stem
6) identical radial distribution in the stems of different trees of the same sampling site.

Additionally, we show that further influencing factors have to be taken into account:

1) The signal provided by the outermost 10-12 tree rings can not be interpreted for the archive, since sap transport is very active in this zone.
2) The transition zone from sapwood to heartwood must be determined directly after sampling.
3) Wood density and tree ring width profiles must be obtained for recognition of growth changes (sudden growth of a tree liberated from competition may alter its chemical profile drastically).
4) In tree rings near the cambium, high sulphur and potassium contents, and low calcium and manganese contents are natural.

In trees exempt of pathogens and reaction wood, S, K, Ca and Mn show quite uniform patterns that allow making a general physiological interpretation of the profiles for Norway spruce, and, in the case of S, to monitor past pollution events.

Acknowledgements

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Figure captions

Fig. 1.: Pooled total S content profiles (1934-2003, with trend line) from 3 locations. Vertical dashed line: sapwood/heartwood transition zone. 2 trees from Düdingen, average standard deviation 7.7% (A); 3 trees from Frieswil, average standard deviation 14.5% (B); 2 trees from St Moritz, average standard deviation 10% (C).

Fig. 2.: Total S content profiles (1929-2003) from Frieswil. Vertical dashed line: sapwood/heartwood transition zone. Tree F1, affected by fungal disease (A); Tree F4 with a distinct compression wood zone (B).

Fig. 3.: Pooled total K content profiles (1934-2003, with trend line) from 2 locations. Vertical dashed line: sapwood/heartwood transition zone. 2 trees from Düdingen, average standard deviation 20% (A); 2 trees from St Moritz, average standard deviation 29.5% (B).

Fig. 4.: Pooled total Ca content profiles (1934-2003, with trend line) from 2 locations. Vertical dashed line: sapwood/heartwood transition zone. 2 trees from Düdingen, average standard deviation 12.7% (A); 2 trees from St Moritz, average standard deviation 4.2% (B).

Fig. 5.: Pooled total Mn content profiles (1934-2003, with trend line) from 2 locations. Vertical dashed line: sapwood/heartwood transition zone. 2 trees from Düdingen, average standard deviation 21.4% (A); 2 trees from Frieswil, average standard deviation 29.7% (B).
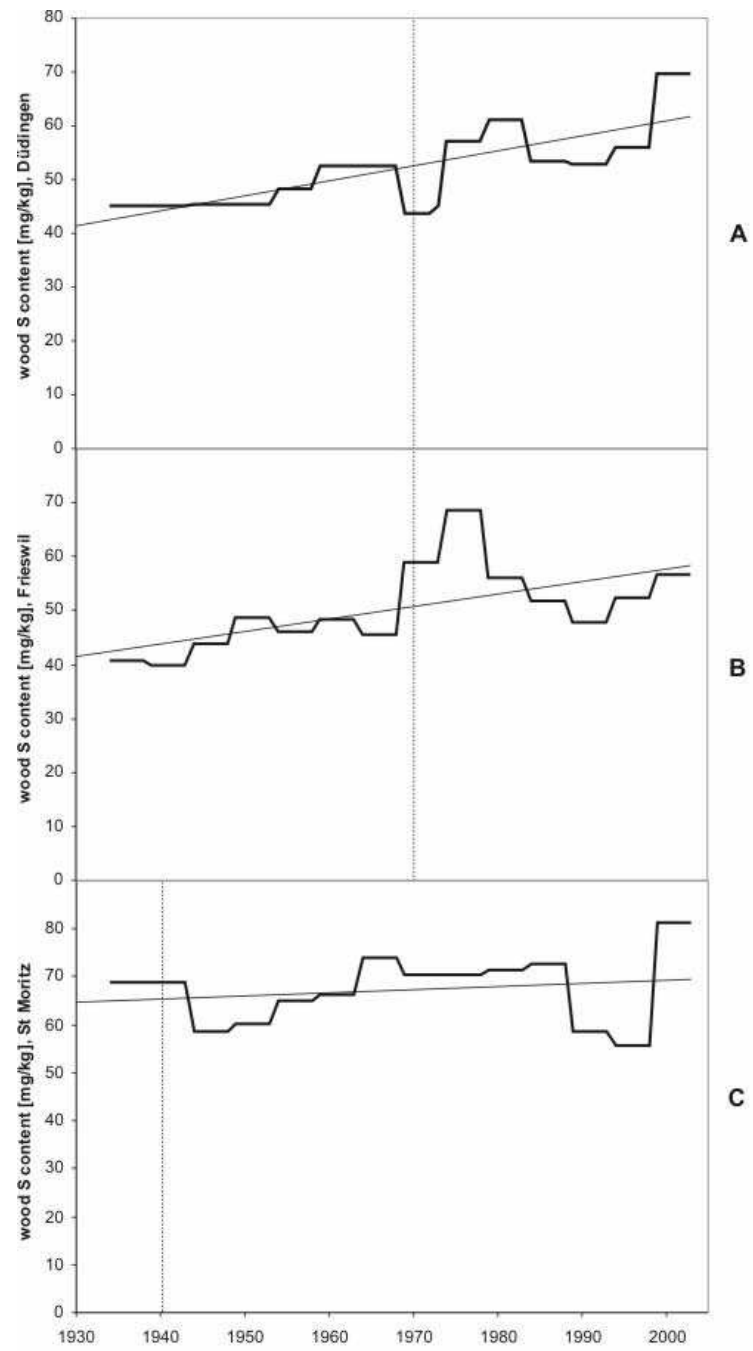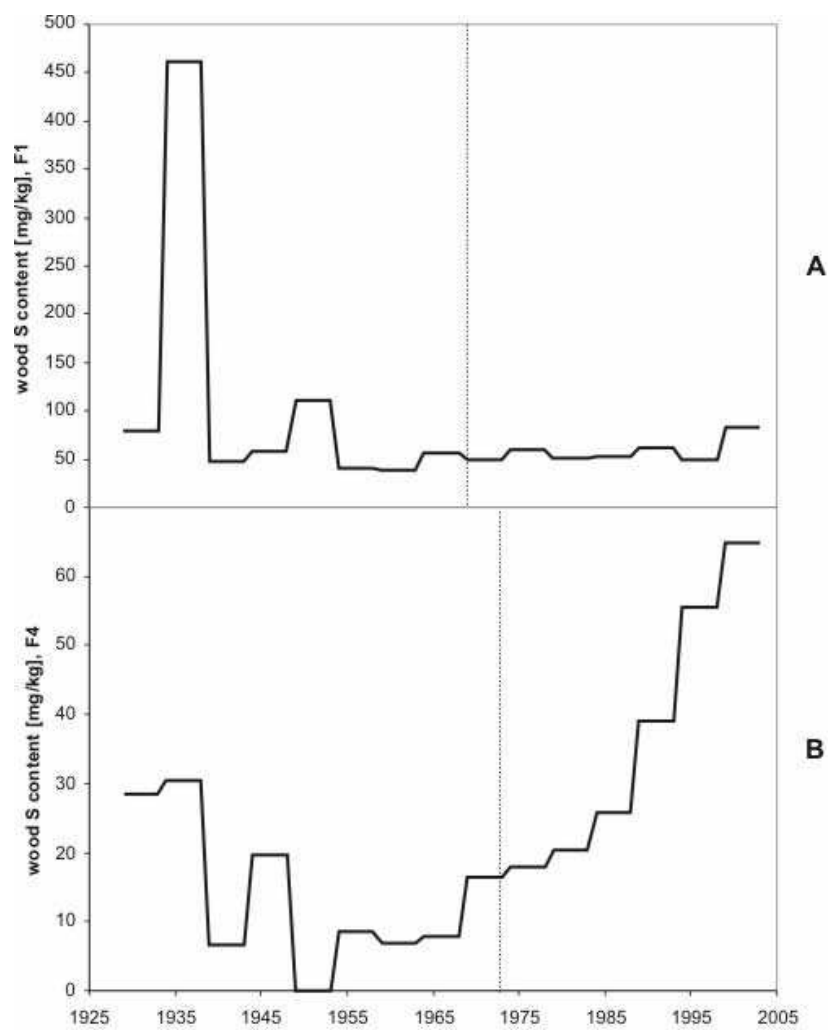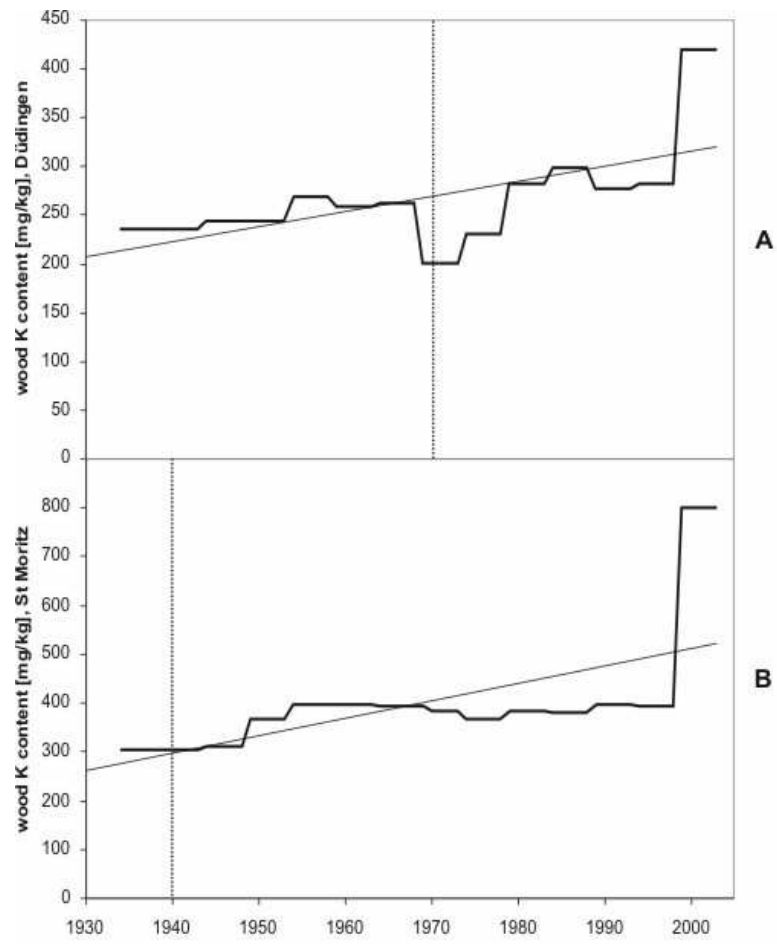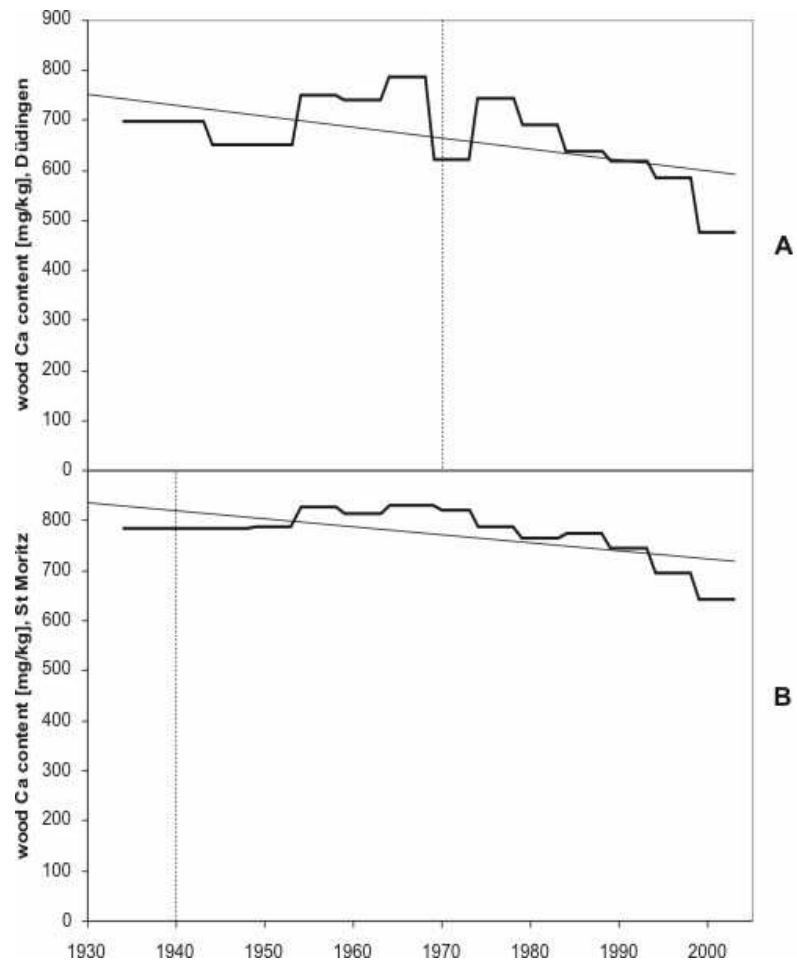
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.
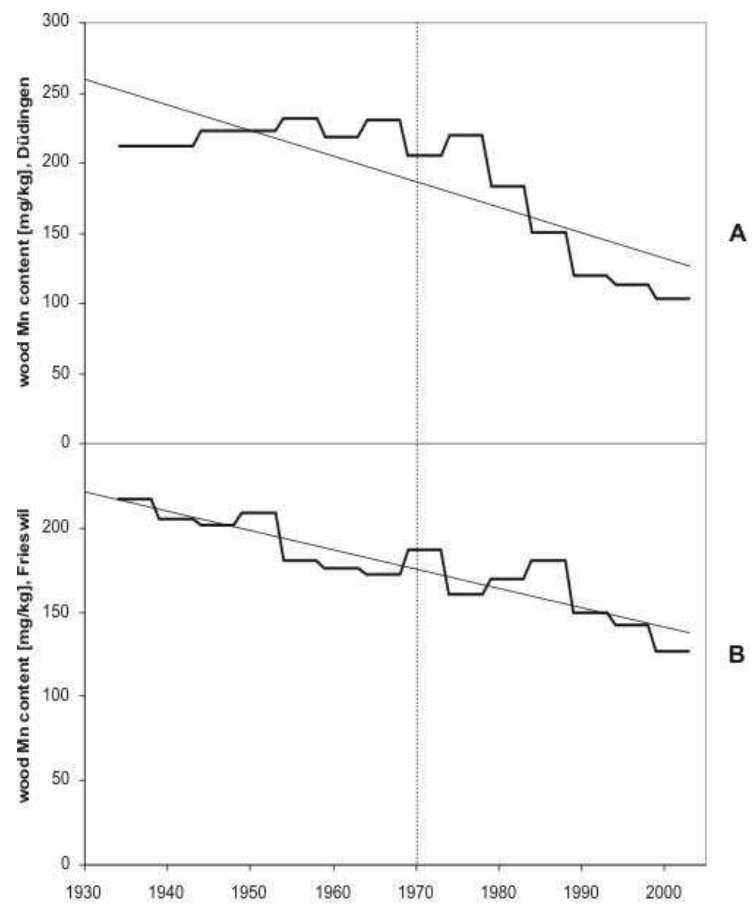
Fig. 5.

3.4 X-rays reveal sulphur functional group profiles in spruce wood (to be published in the near future)

Manuscript type	article
Date submitted by the author	To be submitted
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Keywords	Sulphur Nutrition, Sulphur Metabolism Norway Spruce, XAS
status	To be submitted

X-rays reveal sulphur functional group profiles in spruce wood

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Profiles of the major sulphur functional groups in mature Norway spruce wood tissue have been established for the first time. The big challenge was the development of a method suitable for sulphur speciation in samples with very low sulphur content such as Norway spruce wood (<100 ppm). This became possible by L-edge X-ray absorption spectroscopy in total electron yield (TEY) detection mode with thin gold-coated wood slices from drill core pieces. Functional groups were identified using sulphur compound spectra as fingerprints. Latewood of single year rings revealed reduced sulphur groups, particularly, inorganic sulphide, organic disulphide, and methylthiol, among highly oxidized sulphur forms. Sulphur group-specific intensity profiles of Norway spruce wood from two forests of the Swiss Plateau revealed very similar annual variations and distinct common maxima in the 1986 year ring. These maxima can be interpreted as a consequence of atmospheric sulphur pollution that apparently is put into spruce wood as an archive.

Sulphur is an essential nutrient for growth and development in all living organisms. For plants, it is predominantly available to the roots in the form as sulphate[1]. The primary function of sulphur is, however, connected with protein structure based on the constituent amino acids, cysteine and methionine. Therefore, sulphate has to be reduced and has to be incorporated into the organic precursors of these amino acids. Sulphate reduction and assimilation proceeds through several enzymatic catalyzed reduction steps[2], yielding sulphur in oxidation stage (-II) (sulphide). It is largely connected to photosynthesis as an energy source and, for this purpose, is mainly localized in green tissues. Therefore, sulphate taken up by the roots is transported to the leaf parenchyma cells and further into the chloroplasts. Sulphide produced in the chloroplasts is finally combined with O-acetylserine to cysteine, the primary amino acid in plants. Cysteine functions as precursor for the synthesis of all reduced sulphur compounds, including methionine from which plant protein and anti-oxidants (glutathione)[3] are produced. Sulphur exists in plants in a considerable variety of functional groups that have been intensively studied in leaves (needles), xylem and phloem sap, but are largely unknown in wood. For trees, the functional groups are of particular significance, because of its storage and mobilisation capacity and for heartwood formation.

To analyze functional groups of sulphur in wood, we chose Norway spruce, the dominant tree species in northern and central Europe that represents 48% of all trees in Switzerland. Mature trees were sampled in forests of the Swiss Plateau, one near Düdingen (580 m a.s.l.) and the other near Frieswil (740 m a.s.l.), 20 km apart. Sulphur L-edge TEY spectra with ~50 nm

gold-coated wood slices from the Düdingen tree revealed four major sulphur forms (see Methods) in latewood regions: (1), highly oxidized sulphur, either depicting inorganic sulphate (SO_4^{2-}), organic sulphonate ($R\text{-}O\text{-}SO_2^-$), or sulphate ester ($R\text{-}O\text{-}SO_3^-$), (2), organic disulphide (RSSR) represented best by DL-cystine as model compound, (3), methylthiol ($R\text{-}S\text{-}CH_3$) by DL-methionine or S-methyl-L-cysteine, and, (4), inorganic sulphide (S^{2-}) [R=organic group]. Sulphur form- representing compounds were selected from a broad (27-fold) sulphur compound database. A typical background-corrected sulphur spectrum for latewood from Düdingen tree year ring 1989 is given in Figure 1.

Intensity profiles for the sulphur forms probed with the Düdingen tree for time period 1971-1989 are shown in Figure 2a, those with the Frieswil tree between 1968-2001 in Figure 2b. The profiles pertain to two wood slices per tree site. Each slice was measured in a similar manner during single runs. Düdingen slices covered periods 1971-1976 and 1983-1989; Frieswil slices, 1968-1977 and 1986-2002, respectively.

The various sulphur forms probed in the wood tissues (Figs. 2a and 2b) comply with sulphur compounds reported for other tree parts, albeit in different relative abundances. Xylem sap of Norway spruce exhibits inorganic sulphate amounts outweighing that of reduced glutathione plus cysteine by a factor of c. 50[4,5], whereas needles reveal much lower sulphate-to-total-sulphur contents (~16%)[6]. Our analysis of latewood tissue, on the other hand, showed comparably large amounts of highly oxidized and reduced sulphur forms, together with inorganic sulphide-like species normally expected in active needles. The presence of the latter species in conifer wood tissue has not been reported so far. In both figures (2a, 2b) it also appears that the amount of each sulphur form varies very similarly with time. Thus, sulphur storage in the stem/trunk seems not to be restricted to one particular sulphur form, as concluded from xylem sap analyses[4,5], but sulphate is the dominating species when compared to the needles[6].

Focussing on the results shown in Figure 2b, elevated sulphur levels in wood are natural close to the cambium (~5 most recent years) where cell division occurs and sap transport is very active, but are expected to decrease gradually in the direction of the heartwood due to protein decay and partial recycling of sulphate. On the other hand, both figures (2a, 2b) show elevated amounts with each sulphur form in physiologically inactive heartwood and a common maximum in living sapwood with year ring 1986, irrespective of tree origin and cambial age (Düdingen tree ~96 years, Frieswil tree ~142 years). The first observation may have a wider significance as most developmental cell deaths in plants recruit a pathway of cell dismantling similar to the one found in Norway spruce wood[7] and in its embryogenesis[8]. The explanation for the common maxima in the 1986 latewood tissue is complex as natural physiological and anthropogenic signals can be intertwined. Their unravelling is important as scenarios presented in the latest report of the Intergovernmental Panel on Climate Change (IPCC 2001)[9] predict a worldwide increase in SO_2 emissions. In this context we note that the observed common maxima for year ring 1986 show a remarkable coincidence with successful Swiss efforts to cut SO_2 emissions drastically around 1985 after they had peaked for about ten preceding years, as evaluated by the Swiss Agency for the Environment, Forests and Landscape[10]. As the Norway spruces studied in the present work were exempt of pathogens, reaction wood and clearing effects[11], the maxima with the 1986 year ring underline the possibility of monitoring past pollution events and to study the impact of SO_2 pollutants on the xylem chemistry.

METHODS

Tree sampling.

Mature trees from Swiss Plateau forests near Frieswil and Düdingen were sampled using a 5 mm increment borer (Suunto). Drill cores were taken at breast height. Sampling aimed at minimal contamination by using hand gloves and sulphur-free oil (PhEur grade paraffin; Hänseler) to preserve and clean corer and metal tongue of the borer. The extent of the sapwood zone of each drill core was determined immediately after sampling as no visual differentiation between sap- and heartwood is possible after drying.

Sample preparation and data acquisition.

Approxi-mately 0.1 mm-thick wood slices were cut from ~3 cm long drill core pieces using a microtome (American Optical Corporation). To minimise surface charge effects during X-ray irradiation[12], each slice was coated with ~50 nm Au using a metal sputter coater (Cressing-ton 208HR; I=40 mA; P=2 Pa of Ar). The Au-coated slice was taped at both ends to a metal holder using charge-conducting carbon stickers. Stickers were also used to measure sulphur model compounds. Sulphur L-edge TEY spectra were acquired on the 6 m TGM beamline at Synchrotron Radiation Center (SRC) located at the 1 GeV Aladdin storage ring, Stoughton, WI (USA) using a narrow X-ray beam spot (~2x0.15 mm^2). With Au-coated wood tissue, TEY currents were typically some pA's large with noise ~10%. With the Düdingen tree, we measured latewood regions from single year rings, with the Frieswil tree, late- and earlywood regions from two consecutive year rings.

Background correction of sulphur L-edge spectra.

Sulphur compound spectra generally exhibit more than one peak on top of a slowly developing edge step with increasing energy, often showing fine peak features (doublets, triplets) at low energy and broad oscillations in the after-peaks region. Fine peak features with reduced sulphur compounds are mainly found between 162-170 eV, low oxidized sulphur between 166-183 eV, and highly oxidized sulphur species seldom appear below 170 eV. With wood, the background signal was found to vary dynamically with photon energy, likely, due to charging artefacts[12]. Background, edge step and other broad spectral contributions in the after-peaks region were removed numerically with each sulphur compound and wood spectrum using a cubic polynomial expansion with energy. The expansion functionality was also used in the fitting of the already roughly corrected wood spectra to selected sulphur model compounds as a further refinement of the complicated background.

Selecting sulphur group-representative compounds.

The background-corrected spectra with Düdingen latewood showed two featureless peaks between 160-170 eV, and a third around 183 eV (Figure 1). Latter peak can be attributed to highly oxidized sulphur species, particularly, sulphate, sulphonate, or sulphate ester, whereas the peaks below 170 eV depict reduced sulphur forms. It also appeared that major peaks might overlap with the wood spectra. Principal component analysis (PCA)[13] with wood spectra between 160-170 eV indicated three uncorrelated variables accounting for about 90% of variance. This estimate was corroborated by sulphur K-edge fluorescence scans with a fresh Düdingen wood slice for year ring 1986, showing three peaks consistent with sulphide, sulphite and sulphate. The width of the sulphate peak and the asymmetry to lower energy also suggest the presence of sulphonate. (Courtesy: Adam Webb and Prof. Ronald Cavell from the

Canadian Beam Line at SRC). Fitting the wood scan to sulphur K-edge compound spectra showed that the asymmetric peak is also reproduced very well by that of sulphate ester only. Thus, for the quantitative fitting of the sulphur L-edge wood spectra, we selected four sulphur group-representing compound spectra on the basis of energy positions and relative peak intensities within a large (27-fold) database, comprising inorganic, alkyl and aryl sulphides, heterocyclic, thiophenic and mercaptanic sulphur, sulphonates, and inorganic sulphate. Many spectra were digitized from coal and asphaltene-oriented literature[14-16], but background-corrected here.

Quantification of sulphur functional groups in wood spectra. Each wood spectrum was fitted simultaneously to four selected sulphur compounds after normalising the largest peak in the latter spectra to unity. Each compound spectra were allowed to shift within ±1 eV to compensate for possible energy calibration differences within the literature and our data. Fitting Düdingen latewood spectra proved unproblematic. Frieswil spectra were complicated by additional contributions from earlywood sulphur species, typically, appearing between 166-170 eV. These contributions were often small and reproduced best by DL-methioninesulphoxide (sulphoxide; R_1SOR_2) and occasionally also reduced gluthatione (thiol; RSH). Their spectra were included in the fitting of the Frieswil data to raise the accuracy in the four sulphur compounds found suitable for fitting the Düdingen latewood spectra. The earlywood related sulphur species are not commented in this article. Assuming the cross section for sulphur comparable with wood and model compound spectra, the error in the fitted sulphur form-specific amounts were estimated ~20%, at best, considering signal-to-noise ratio and spectral overlap between the reduced sulphur forms.

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Acknowledgements

Figure captions

Fig. 1. High resolution (ΔE=0.2 eV) S L-edge TEY spectrum with latewood for Düdingen tree year ring 1989 after subtracting edge jump and other broad contributions, together with calculated and constituting sulphur group-specific spectra in their fitted amounts.

Fig. 2. Sulphur group-specific content profiles (in TEY current units) derived from background-corrected latewood spectra as a function of time for Norway spruce from the Düdingen (a) and Frieswil site (b).
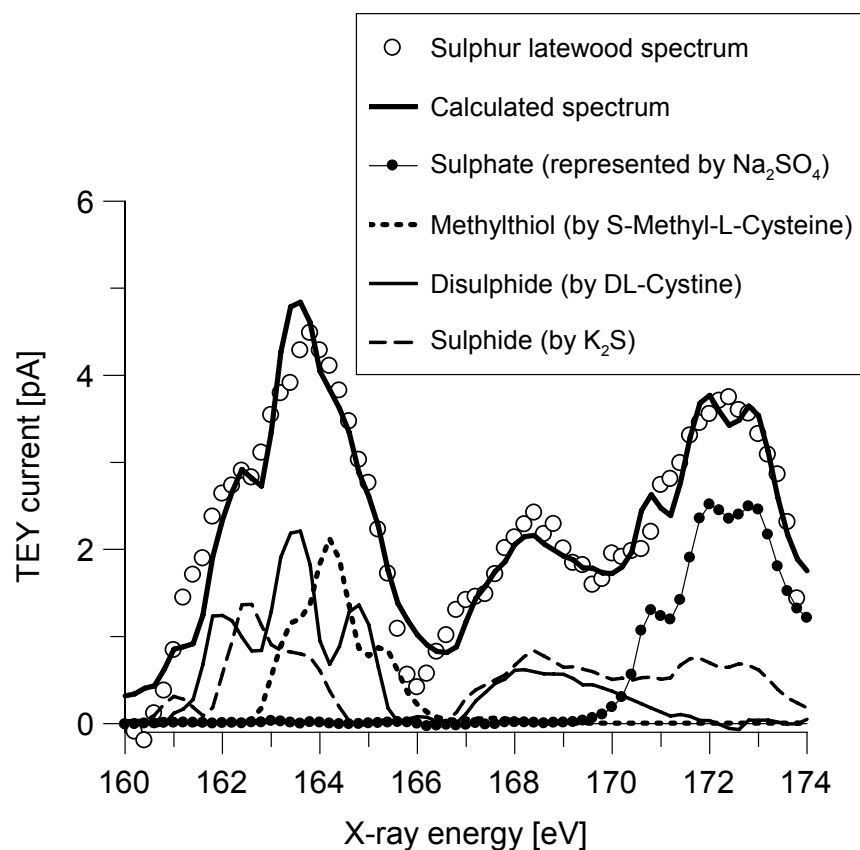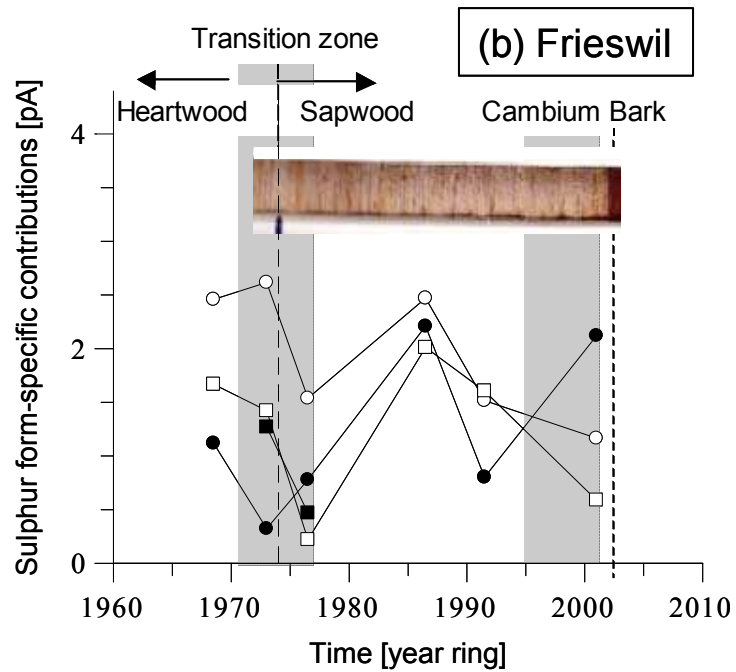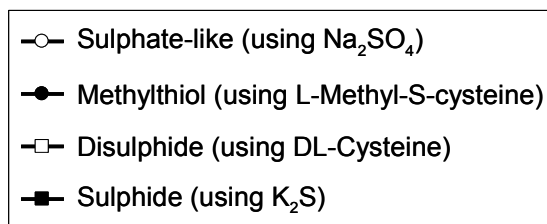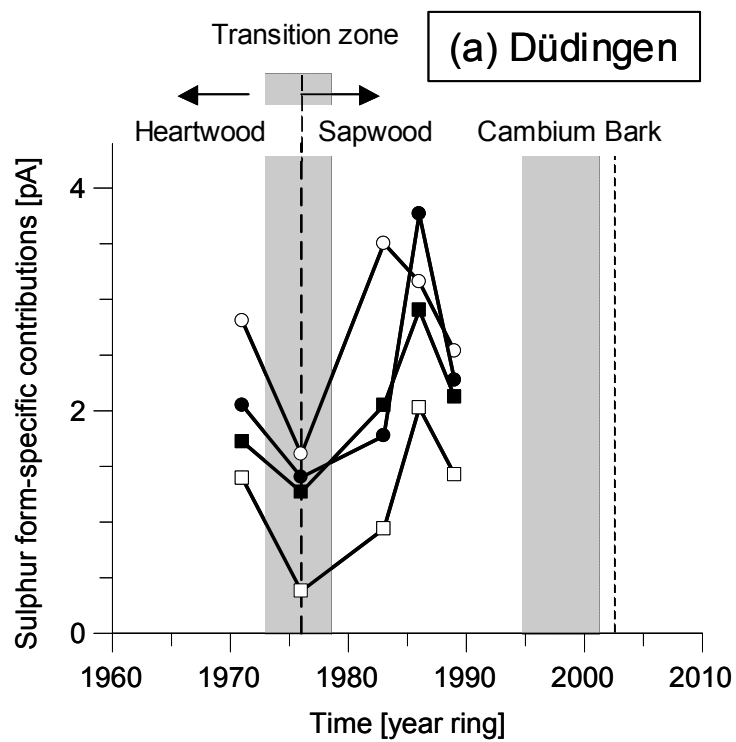

Fig. 1.

Fig. 2.

3.5. Unpublished results: Isotopic Signatures of $\delta^{34}S$ in a Düdingen Peat Bog Profile

As mentioned in chapter 1.5, the sampled Düdingen forest is situated near a peat bog. Both environmental archives retrace the local SO_2 immissions, mainly due to the motorway A12 in proximity. Additionally to the total S profile, analysis of stable sulphur isotopes can be used to assess anthropogenic influences on an ecosystem. Fractionation of the stable sulphur isotopes ^{34}S (4.21%) and ^{32}S (95.02%) can lead to a change of the $\delta^{34}S$ signature (ratio of heavier to lighter isotopes). This can reveal biological changes as well as anthropogenic influence in an ecosystem.

In this study, the inverse correlation between total sulphur content and $\delta^{34}S$ signal found in a Düdingen peat bog drill core confirms other measurements [*Jeker, 2001*]. In a peat bog, the inverse correlation of $\delta^{34}S$ signal and S content is due to dissimilative sulphate reduction. This process is stimulated in case of increased sulphur input and the formation of H_2S discriminates against the heavier isotope ^{34}S. Bacterial reduction of sulphate causes the dissolved H_2S to become enriched in ^{32}S, while the sulphate becomes enriched in ^{34}S. This fractionation occurs because the heavier isotope preferentially remains in sulphate, where sulphur is bonded more strongly. The reduced sulphur, which is depleted in ^{34}S, is then built in sphagnum mosses and other plants, which generates a low $\delta^{34}S$ signal. Fractionation does not solely occur during biological processes. Oxidation of SO_2 to SO_4^{2-} in the atmosphere discriminates against the lighter isotope ^{32}S. Thus, sulphate aerosols are enriched with ^{34}S. When SO_4^{2-} concentration is low, $\delta^{34}S$ values are influenced by several minor sources and therefore have a greater range of values. At higher concentrations however, $\delta^{34}S$ values approach that of the dominant sulphur source in the area [*Mast et al., 2001, Case and Krouse 1980*]. Sulphur isotope signatures can therefore be used to distinguish between natural and anthropogenic S sources, if their difference in isotopic composition is large enough. In the Czech Republic for instance, high $\delta^{34}S$ aerosol values were measured at relatively unpolluted sites, while low $\delta^{34}S$ values were recorded in the heavily polluted north [*Novàk et al., 2000*]. In order to be certain of how well the stable sulphur isotope profile reflects $\delta^{34}S$ values of industrial emissions, the provenance of the fossil fuels and coal used during the 20th century should be known. This information could provide the mean $\delta^{34}S$ value of the fossil fuels used in Switzerland. In mineral oils, high sulphur content corresponds to a low $\delta^{34}S$ [*Maruyama et al., 2000*]. Thus, high SO_2 emissions due to diesel consumption before the introduction of fuel desulphurisation could have lead to a lowered $\delta^{34}S$ signal.

The measurement of $\delta^{34}S$ in peat bog samples requires the oxidation of all sulphur compounds and subsequent precipitation of the sulphate as $BaSO_4$. 500 mg of freeze-dried and ground peat sample were digested in Teflon[®] bombs with a MLS ETHOS 1600 microwave oven (software: easyWAVE3). The peat sample was digested with 4 ml HNO_3 65%, 3 ml H_2O_2 30%, 1 ml $HClO_4$ 70% and additional 0.0025 g MgO. After centrifugation, the supernatant was decanted and 2 ml $BaCl_2$ 10% were added. The resulting white precipitate was centrifuged and put into a 2 ml plastic vial. There it was washed several times with MQ water before being dried in an oven at 120°C. 450 µg +/- 50 µg of the residue were then weighted in a tin cylinder together with 5 mg V_2O_5. The measurement of the isotope ratios were made at the centre for environmental research Leipzig-Halle (UFZ, operator: M. Gehre).
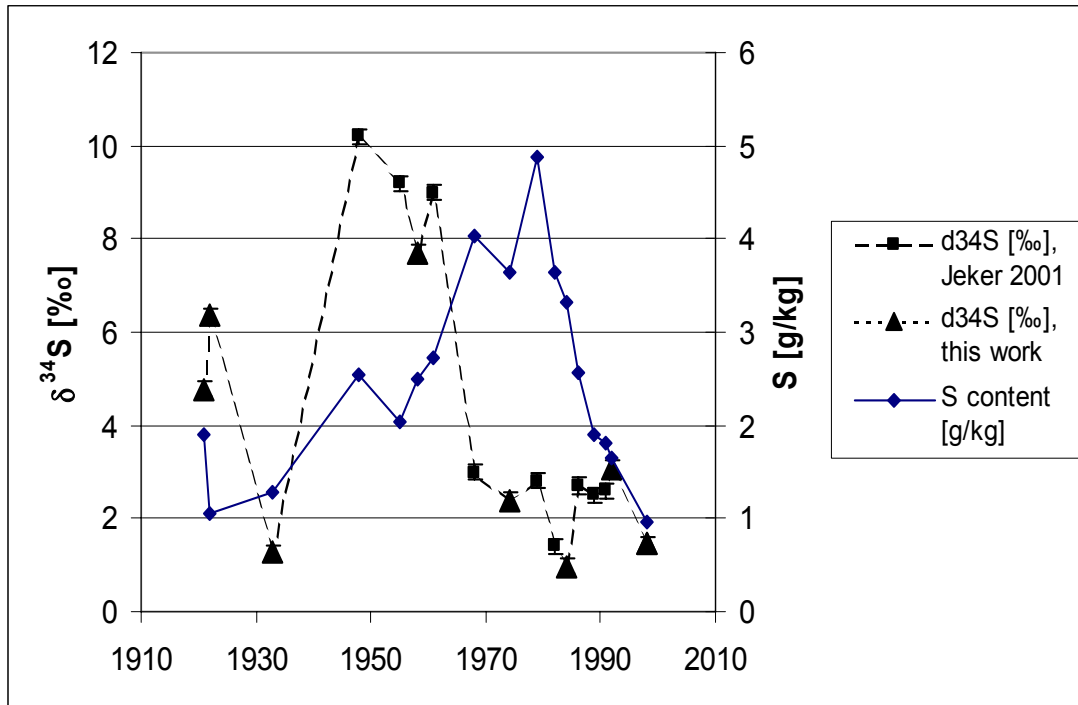
Fig. 2.: S content (Jeker 2001) and δ³⁴S ratio of a Düdingen peat core (the S content peak corresponds to the high SO₂ emissions 1950-1990 as evaluated by the SAEFL).

The obtained results seen on figure 1 (triangles) corroborate the previous measurements (quadrangles [*Jeker, 2001*]) and show a negative correlation between S content and $\delta^{34}S$ value. Yet there seems to be a deviance from this tendency at the points corresponding to 1933 and 1998.

The low $\delta^{34}S$ values from 1968 to 1992 are likely due to dissimilative sulphate reduction triggered and stimulated by the massive sulphur input, due to the proximity of the peat bog to the A-12 motorway (built in the 1970ies). The increase of anthropogenic sulphur probably contributed to lower the $\delta^{34}S$ signal, since mineral oil with high sulphur content has a low δ^{34}, and aerosols from polluted areas can have low $\delta^{34}S$ values [*Novàk et al., 2000*].

It would have been interesting to determinate the stable isotope ratio of sulphur in the trees. However, the S content in wood is too low compared to peat bog (see chapter 3.5.). Thus, precipitation of $BaSO_4$ is not feasible.

4.   Outlook

LA-HR-ICP-MS is a fast and reliable method. However, the determination of drill cores by LA-HR-ICP-MS is relatively time consuming because of the necessary sample preparation (cutting of the drill cores into 3 cm segments and the marking of the year rings), the manual adjustment of the samples and the complex data evaluation. Therefore, instrumental improvements at the LASER system, such as an automation of the sample adjustment, a special cell design for drill cores and an automated data evaluation would be advantages.

Due to the cell size, the length of drill core pieces is limited to 3 cm, which only allows the measurement of 20-45 successive years, depending on tree ring width. A maximum of two such 3 cm pieces can be put into the cell. A cell which allows a complete covering of a drill core, which has a typical size of about 30 x 1 cm, and therefore, measurements of a sample in its integrity, would be ideal and desirable for tree ring analysis. The all-too frequent opening of the sample cell leads to certain shifts of the background concentrations (e.g. of ^{32}S, ^{13}C). Thus, the most reliable results come from samples measured in one single run.

However, the designing of such a sample cell for drill cores would be very challenging because not only sample dimension aspects but also an optimised sample transport, low contamination risk, aerosol dilution for optimum signal shape etc. must be taken into account. Bleiner studies various influence factors on the cell design [*Bleiner, 2002, Bleiner and Altorfer, 2005*]. The mounted cell developed at EMPA for authentication analysis of antique objects is not suitable for sulphur determination because of the required plasticine sealing material with high sulphur content [*Devos et al, 1999*].

Further minimisation of the sulphur background level would be also desirable. Consequent banning of adhesive foil and plasticine from sample holder and cell interior already helped reducing sulphur background considerably. However, some sulphur might still exhaust from the glue used to fix glass and metal components of the current sample cell together. This problem became apparent after necessary reparation of the cell. The outgassing sulphur could be minimised after heating it in an oven at 70°C during 72 hours. Nevertheless, a cell body completely made of metal would likely further diminish sulphur background – an option which is not preferable for metal analysis in multi-element determination. However, there are other possible sulphur background sources beside the cell, such as sealing rings used in the torch of the ICP-MS. The quality of the carrier gas is of less importance because the tested helium qualities of 4.6 and 5.0 grade showed only little differences. A significant influence might be introduced by outgassing of oil from the interface and backup pumps in the ICP-MS. The use of a low-sulphur-oil might lower the sulphur background additionally.

Since the LASER redesigning at EMPA was in progress during the whole thesis work, several LASER system improvements were implemented during this time and more are in preparation. Thus, an automation of the sample adjustment is planned using a cell table operated by a step motor as well as an automated triggering of the ICP-MS instrument start and the development of a program for automated data evaluation. Automation (i.e. a stepping motor piloted by computer) would be a great improvement, avoiding manual adjustment using the microscope after each single shot. The automated adjustment of the shutter (after 20 s), which is now implemented in the software, was a true improvement, but a coordinated starting of the ICP-MS and the LASER by a synchronised triggering would also be important. While measurements with annual resolution are already laborious due to the lacking automation, the determination of seasonal resolution is very time consuming. Furthermore, it leads to an enormous amount of data because for each parameter, a transient signal of each single shot is obtained. 13 Elements were acquired (C-isotopes ^{12}C and ^{13}C, S-isotopes ^{32}S, ^{33}S, and ^{34}S, and isotopes of the elements ^{24}Mg, ^{27}Al, ^{31}P, ^{39}K, ^{43}Ca, $^{55}Mn55$, ^{66}Zn, ^{85}Rb, ^{88}Sr, ^{138}Ba, ^{208}Pb) plus $^{40}Ar_2^+$ and $^{16}O^{16}O$ for intensity monitoring: this is tantamount to a complete transient signal (duration: 106 s) of 18 isotopes for each single LASER spot measurement.

Evaluation of this data had to be made manually using the Microsoft Excel™ program, subtracting the corresponding background median from the median of the ablation signal (duration: 20 s, see chapter 2.6). Since the first data were acquired without the synchronised shutter, the maxima were not located distinctly. However, as already mentioned above, further improvements of the LASER system are planned.

Since no commercial standards are available for LA-ICP-MS measurements of S in wood, calibration pills had to be self-prepared. Due to a lack of time, calibration remained in the optimisation stadium. Therefore, all results presented in this study are relative concentrations. In order to allow quantification of the measured elements, this issue should be resolved. The wood pellet standards did not give the required solidness for an adequate LASER ablation. Additional adding of cellulose might overcome the problem.

An interesting topic of further investigations would be the determination of sulphur isotope ratios. As described in chapter 3.5 for peat bogs, the $\delta^{34}S$ pattern gives remarkable hints about the origin of the sulphur. A magnetic sector field HR-ICP-MS provides a possibility to measure isotope ratios but even if the accuracy is better than for quadrupole-ICP-MS, the sequential determination is not sufficient for determining very small differences. Thus, multi-collector inductively coupled mass spectrometry (MC-ICP-MS) or thermion mass spectrometry (TIMS) would be the methods of choice for precise isotope ratio analysis. Tests for peat bogs with solutions issued from microwave acid digestion were described in chapter 3.5, but isotope ratio determination for sulphur in wood could be not performed within this thesis. However, the determination would be challenging because the ^{34}S content in wood is very low, so the determination of small changes in isotopic composition (e.g. change of the $\delta^{34}S$ signature) is not trivial and development of the analytical method would be probably time consuming. The determination of isotope ratios in solutions could be possible, but LA-MC-ICP-MS coupling would be ideal for obtaining data with a high spatial resolution.

The investigation of organic sulphur components in wood would be a further interesting research aspect. The time of flight secondary ion mass spectrometry (TOF-SIMS) might offer the possibility to perform organic sulphur analysis on the wood surface. However, preliminary tests with the current TOF-SIMS at EMPA gave no satisfying data. But further method development or the use of a more recent instrument might lead to lower detection limit and better results.

Concerning radiodensitometry, complete wood density and tree ring width datasets were obtained from 18 trees stemming from three locations. Valuable climatic information about the last 150 years might therefore be included into a dendrochronology database of a measuring network. This data could perhaps be used, together with the remaining corresponding drill cores for potentially programming an optical sensor (mounted in the observation optic), which in combination with a stepping motor would considerably accelerate the measurements, thus rendering LA-ICP-MS suitable for routine dendrochemical measurements.

Oxygen bomb combustion could be further optimised concerning background and sample losses by use of ultrapure grade chemicals and carefully pre-cleaned vessels only. The use of quartz crucibles devoid of asperities would facilitate cleaning of the soot resulting from combustion and hence lower background sulphur level. Additionally, since this method requires a relatively high sample quantity, the use of a 12 mm increment borer (which causes greater damage to trees though) would provide more sample material without lessening time resolution.

Similarly, a 12 mm increment borer would provide more material for determination of the $\delta^{34}S$ value in wood (see previous chapter). S isotope composition of freeze-dried and homogenised samples could then be determined using an elemental analyser coupled to a stable isotope mass spectrometer.

In the near future, the inauguration of a X-ray absorption spectrometry (XAS) beam line at the Swiss Light Source (SLS, PSI Villigen), similar to one used at the SRC (see chapters 2.8, 3.4), would allow to optimise S speciation. More wood samples and longer time series could be measured in order to complete the physiological and environmental information obtained in this study.

5.   Conclusion

Trees can be used as archive for sulphur but the use is restricted to higher variations of the SO_2 immissions. Minor anthropogenic SO_2 impacts and variations are overlaid by plant physiological processes. Overall, it has to be remarked that trees are challenging archives and the interpretation is complex since anthropogenic and natural physiological signals can be intertwined. Not only climate and pollutants influence the sulphur uptake but the soil chemistry has a certain impact. Since trees are living beings, plant biology and xylem chemistry has to be taken into account. Moreover, physiological factors of the tree such as heartwood formation, pathogens and reaction wood can have a strong influence on the sulphur pattern in a tree. Thus, for a correct interpretation of element content profiles, knowledge about tree physiology is mandatory.

Therefore, additionally to the six biological conditions for retrospective bioindication in trees stated by Hagemeyer, Walkenhorst and Lohrie [*Walkenhorst and Hagemeyer, 1997, Hagemeyer and Lohrie, 1995*], further conditions have to be fulfilled for the retracing of sulphur in trees:

Conditions by Hagemeyer, Walkenhorst and Lohrie:

1. a constant relationship between element supply and accumulation
2. storage in tree rings constricted to a short time period
3. storage in a limited, known number of tree rings
4. no retroactive remobilisation of element
5. identical radial distribution in different spots of the stem
6. identical radial distribution in the stems of different trees of the same sampling site.

Additional conditions required for sulphur retracing in tree archives:

1. The outermost 10-12 tree rings can not be used for archive interpretation, since sap transport is very active in this zone.
2. In tree rings near the cambium, the accumulation or depletion of certain elements is natural, e.g. close to the cambium high sulphur contents were detected
3. The transition zone from sapwood to heartwood must be determined directly after sampling.
4. Wood density and tree ring width profiles must be obtained for recognition of growth changes
5. Several samples from different trees should be taken in order

Basically, two types of archives can be distinguished, the static archives which are more or less unchangeable and constant, such as dripstone (stalactite, stalagmite) or arctic ice cores, and archives which can be influenced by external factors such as trees.

Interpretation of trees as an environmental archive is complex. They may be considered as adaptive systems, but this model simplifies reality coarsely. Due to their high life expectancy, they also have to be seen as long term system. This means that one particular event affecting

their physiology (e.g. anthropogenic S input) can be influenced by events before (e.g. drought). Additionally, there is a big difference between investigating seedlings or young trees from greenhouses (as for numerous S nutrition studies) and mature trees grown in a forest (this study). Since trees are not isolated organism, anthropogenic signals investigated in old forest trees are always accompanied by natural signals. This is due to the fact that interactions occur at several levels: with other organisms (congeners, symbiotes, pathogens, animals), with climate, soil conditions. It is important to watch out for such influence factors, in order to identify the anthropogenic signal with certainty.

Individual differences between trees of the same site are possible. Norway spruce in particular is a species where such differences within the population are important. Clones grown in the same greenhouse develop individual characteristics [*von Ballmoos, 2003*]. In the natural environment, the species displays an astounding genetic diversity, which leads to a pronounced polymorphism (comb shaped, brush-like and plated spruce). There are further differences between individual concerning growth, vegetation rhythm, wood consistence, and resistance against frost, insects, pathogens, immissions and drought.

Thus, by analysing old trees grown in forests, the exactitude of a laboratory and greenhouse experiment will never be achieved. But more than enough, this is counterbalanced by the fact that relevance of the acquired data is much higher. Furthermore, by sampling several trees at each site, good replication can be achieved, as well as repeatability by the inclusion of different sampling sites.

According to Körner, the best approach in an environmental study is to ask small questions and to get the maximum of information available from a sampling site [*Körner, 2003*]. Thus, six individuals (replication) from three different sites were sampled (repeatability). In order to have an interdisciplinary approach, several methods were applied for each tree. A relatively complete survey of how trees react to their changing environment can only be obtained by following these rules and with knowledge coming from different branches of study.

While the measurements presented here showed that there is no clear generality for elements like Mg, Zn, Cu, Cr, Ba and Al, the behaviour of S, K, Ca and Mn is very similar in each tree. In trees from the Swiss Plateau, the anthropogenic signal due to the maximal emission in the 1970ies, 1980ies is clearly identifiable. Thus, it can be concluded that Norway spruce is suitable as an environmental archive for sulphur dioxide but as mentioned above, the use is limited to large impact and variations because the minor concentrations are interfered by plant physiological effects.

6.   Summary

The aim of this PhD thesis was to respond to three questions:

1. whether trees are suitable environmental archives for retracing anthropogenic sulphur dioxide impacts
2. if they can reflect different local immission situations
3. how sulphur profiles found in trees differ from those found for peat bogs at the same sampling sites

The study was founded on the previously investigated fact that plants show an increased sulphur uptake in regions with high environmental sulphur immissions, which suggests that a constant relationship between sulphur supply and accumulation exists and that storage in wood might occur. In principle, three uptake mechanisms into trees are known (also see chapter 3.3):

1. uptake via needles
2. uptake via roots
3. uptake through the bark from water running down the stem

However, the main impact mechanism into the stem occurs via the roots whereas the input via run-off water is small. Plant physiologists assume that no significant transport occurs from the needles back to the stem.

For the investigation of the subject described above, a tree species representative for the northern hemisphere was selected (see chapter 3.2). For studying the influence of regional immission situations at different locations, a second precondition was that the selected tree species is present as well in the Swiss plateau as in alpine regions. Thus, Norway spruce was chosen as the most appropriate for this task. Moreover, the metabolism of this species has been the subject of numerous studies about its physiology, which is advantageous since plant biology had to be taken into account for data interpretation.

Sampling sites were chosen with respect to data material available for peat bogs which had been studied by Jeker for the Düdingen peat bog (Swiss plateau, 580 m a.s.l.) and by Schreier for the St Moritz peat bog (Alps, 1900 m a.s.l.) [*Jeker 2001, Schreier 2005*]. The forest of Frieswil (Swiss plateau, 740 m a.s.l.) was chosen as third sampling site representing a rural immission situation without direct local emission source (see chapter 1.5). The local sulphur dioxide immissions were comparable to NABEL (Swiss National Air Pollution Monitoring Network) monitoring sites (see chapters 1.2 and 3.2):

1. Payerne (countryside, 489 m a.s.l., comparable to Frieswil)
2. Härkingen (countryside, 431 m a.s.l., close to a motorway, comparable to Düdingen)
3. Davos (alpine region, 1638 m a.s.l., comparable to St Moritz, Engadine)

First of all, a suitable sampling procedure was applied using a 5 mm increment borer. Contamination of the samples was avoided by using sulphur-free lubricant and cleaner for the borer, and by wearing gloves for all manipulations (see chapter 2.1). Tree circumference as well as the extent of the heartwood-sapwood zone was determined in the field. Only healthy and mature forest trees were selected for the archive investigations. Careful selection of the sampled trees is important: results of this PhD thesis showed that trees with compression wood or with fungal pathogens have an altered S profile (see chapter 3.3). In order to assess the influence of the soil type on wood sulphur content, samples were taken from the soil close to the selected tree. The soil samples were later characterised using WD-XRF (wavelength dispersive x-ray fluorescence spectrometry) to assess the natural sulphur background as well as the general composition of the soils at the different sampling sites (see chapters 2.9, 3.2 and 3.3).

For all trees, measurement of cambial age, tree ring width and wood density was performed using radiodensitometry (see chapter 2.5). The knowledge of density was especially important for the LASER ablation high resolution inductively coupled plasma mass spectrometry (LA-HR-ICP-MS) determinations. By these means, the correlation between wood density and ^{13}C intensity could be controlled.

For the obtainment of total sulphur content profiles of the drill cores, two independent digestion procedures were developed: Oxygen bomb combustion (chapter 2.4) and an acid microwave digestion (chapter 2.3) were compared with respect to best suitability for sample preparation and best achievable time resolution for tree rings. The digestion procedure had to be appropriate not only for metal determination but also for the analysis of the low sulphur contents (20-100 mg/kg) in the wood. Beside complete digestion, important aspects for the optimisation were minimisation of the contamination risks and avoidance of losses due to volatile sulphur species. Microwave acid digestion gave the best results and the final

procedure was applied to the Norway spruce drill cores sampled at three sampling sites described above (see chapter 3.3). Analysis of the digested samples was performed using inductively coupled plasma optical emission spectrometry (ICP-OES) for sulphur and selected metals. The profiles found in wood were compared to the profiles obtained for the Düdingen and St. Moritz peat bogs, as well as to the local SO_2 immission rates of similar NABEL measurement sites.

However, the best spatial resolution achievable for the digestion procedure was 5-10 years, since a minimum sample amount of about 40 mg is required for reliable analysis. Therefore, a novel analysis method was developed using a Nd:YAG-LASER coupled to a high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS). This enabled determination of sulphur and selected metals in tree ring samples with excellent spatial resolution. After successful coupling and method development, sulphur profiles for drill cores from the three sampling sites were obtained (see chapter 3.2). Since LASER ablation high resolution inductively coupled plasma mass spectrometry (LA-HR-ICP-MS) provides multi-element analysis, selected elements such as magnesium, aluminium, phosphorus, potassium, calcium, manganese, zinc, rubidium, strontium, barium and lead were measured as well.

The carbon isotope ^{13}C was used for internal standardisation of the LA-ICP-MS measurements to correct density variations. A linear relationship between wood density and the carbon content in wood was proved by the strong correlation found between wood density and the ^{13}C signal of the LA-ICP-MS measurements (see chapter 3.1).

The obtained sulphur profiles were compared to the findings of the investigations using the acid digestion method, as well as to the profiles found in peat bogs. Regional, environmental, atmospheric and plant physiological influences were taken into account to assess the possibilities and the limits of conifers as environmental archives for sulphur dioxide.

For Düdingen, the sulphur profiles found in tree rings reflected the local emission situation and were similar to those found in the peat bogs. Both archive types showed the characteristic sulphur increase in the seventies with a maximum at the late 1970ies and the early 1980ies, followed by a decrease. This profile is analogous to NABEL immission measurements found at similar sites close to the sampling sites. However, the pattern found for the Düdingen peat bog was more structured than the profiles found for the Düdingen trees.

In contrast, neither the St. Moritz trees nor the St Moritz peat bog showed the characteristic sulphur maximum. The wood S and peat bog S profiles were in fact both relatively flat, thus corresponding to the knowledge that the Engadine is secluded from the bulk of SO_2 emissions of the Swiss Plateau (see chapter 3.2). The findings correspond also to the local immission situation at the alpine NABEL location where also the SO_2 immission level was much lower compared to those found at sampling sites of the Swiss plateau. However, the St. Moritz peat bog showed a more pronounced variation in its S profile than the compared trees. The peat bog S level decreases since the beginning of the last century and remains relatively flat, whereas the tree S profile is relatively flat over the complete profile. The higher S concentrations in the first half of the 20th century have been explained by consumption of coal augmenting locally with the beginning of the industrialisation [*Schreier, 2005*]. Nevertheless, this supposable augmentation of the SO_2 immission is not visible in the St Moritz trees.

A reason for the differences found between both archives is probably the influence of the natural background from the soil as well as the physiology of the trees (since sulphur is a macronutrient in plants). Additionally it has to be taken into account that the sulphur content of wood (20-100 mg/kg) is much lower than for peat bogs (1000-5000 mg/kg, [*Jeker, 2001*]).

From the results found in this PhD thesis, it can be concluded that small SO_2 immission rates (typical for alpine sites) and smaller variations are often overlaid by plant physiological effects. Sulphur from the soil can have an influence on the determinable threshold levels and therefore on the detectable changes. Thus, a retracing of low sulphur dioxide immission rates might be not possible at locations with high natural sulphur background in soil.

Furthermore, the results showed a naturally high S content in the tree rings near the cambium. This is due to the high transport flux in this region. Therefore, the 10-12 outermost tree rings of a drill core cannot be interpreted for archive relevant data evaluation. On the other hand, the boundary of the sapwood-heartwood transition zone seems to have less effect on total S content than expected.

Unlike S, Ca and Mn, the K content in wood did not correlate with concentrations found in soil samples (see chapter 3.3). K content was significantly higher in wood of mountain trees, although the K content in the St Moritz soil was low. These findings might reflect a tendency of trees living in a stressful environment (e.g. the Alps) to store as much nutrients as possible and to take them up with more efficiency.

The highest element contents for S and K were found in the 5 years next to the cambium, regardless the location of the tree. As mentioned above, sap transport occurs mainly in the 10-12 outermost year rings. In contrast, the Ca content was lowest in the 10 y next to the cambium. It was very similar for all sampled trees, independent from the sampling site. This element appears to be strongly linked to the lignin content, and hence to the progress of wood formation. Mn profiles showed a pattern which was similar to those of Ca, but with a steeper increase in direction of the pith just after the cambium. Generally, the contents of all elements were higher in bark, which might be caused by its strong exposure to salt rich water running along the stem.

In a next step seasonal profiles within single year rings were obtained, exploiting the high spatial resolution capability of the novel LA-HR-ICP-MS method. Depending on the year ring width, four to eight determinations spots could be achieved within one year ring which provided the possibility to assess variations in the early earlywood, the late earlywood, the early latewood and the late latewood zone. The measurements with seasonal resolution revealed that element contents in tree rings change with the seasons (see chapter 3.1). The profiles of sulphur, phosphorus and potassium showed distinct, varying patterns. Pooling of data from 3 Swiss Plateau trees reveal that sulphur might be used for growth and development of the stem which begins in spring. The reserves are diminished in summer and built up again in autumn, at the end of the growing season. The phosphorus content fluctuates in a similar way. However, the build-up of a reserve in autumn was less pronounced for phosphorus. Both elements displayed a linear relationship, due to their strong biochemical coupling (see chapter 1.3). Potassium, being of particular importance at all growing points in the tree, also shows a content decrease from spring to summer, followed by a reconstitution of the provision in fall.

The possible physiological impact of anthropogenic S oversupply was investigated as well. Speciation analyses of organic sulphur compounds by XAS were carried out for the Norway spruce wood samples from two Swiss Plateau trees (Düdingen and Frieswil, see chapters 2.8 and 3.4). Sulphur L-edge TEY (total electron yield) spectra obtained by XAS measurements revealed four major sulphur forms in latewood regions:

1. highly oxidised sulphur (inorganic sulphate, organic sulphonate, or sulphate ester)
2. organic disulphide (e.g. cystine)
3. methyl thiol (e.g. methionine or S-methyl-L-cysteine)
4. inorganic sulphide

These sulphur forms correspond to compounds found in other parts of Norway spruce (e.g. in needles). However the fact that sulphide is also present in wood was new. Frieswil spectra showed contributions from certain species (methionine sulphoxide) found in earlywood only, whereas other components (glutathione) were reduced.

The profiles of the different S compounds varied with time, but followed a similar pattern for both studied trees. The found elevated sulphur levels in wood close to the cambium are of natural as already described above. However, the explanation for the common maxima in the

tree rings corresponding to 1986 is complex. It might reflect a reaction of the trees to the drastic reductions of SO_2 emissions in Switzerland around 1985 after their sustained maximum in the late 1970ies, early 1980ies, but to investigate this theory, more data are needed.

For the use of trees for retracing anthropogenic sulphur dioxide impact, several conditions have to be met (see chapter 5). Furthermore, plant physiological effects and natural sulphur background from the soil have to be taken into account. However, it can be concluded that Norway spruce is suitable as an environmental archive for sulphur dioxide, although its use is limited to larger impacts and variations.

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8. Acknowledgements

9. Appendix

N.B.: Due to the huge datasets, most of the data is available in electronic form only. CD ROMs are procurable from Prof. Dr. U. Krähenbühl (University of Bern) and Dr. A. Ulrich (EMPA Dübendorf).

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9.1   Seasonal Profiles of S, P and K by LA-ICP-MS (chapter 3.1)

LA-ICP-MS results with seasonal resolution
N.B.: all values for S, P and K content are normalised with 13 C

Düdingen tree D3

LASER shot	av. wood dens. [mg/cm3]	13C [cps]	rel. S content	rel. P content	rel. K content
1984 a	616.8	65345	1.1	1.1	0.8
1984 b	315.6	70763	1.0	0.7	0.9
1984 c	277.1	78027	0.9	0.7	0.7
1984 d	258.9	74038	1.0	1.2	0.8
1983 a	498.8	60110	1.1	1.4	1.3
1983 b	290.8	68574	1.0	1.1	1.0
1983 c	270.3	72720	0.9	1.2	1.2
1983 c	237.6	77136	0.9	1.0	0.8
1983 d	207.5	75299	0.9	1.2	0.9
1983 e	219.4	75882	1.4	1.2	1.3
1982 a	686.7	52659	1.1	0.5	1.1
1982 b	339.3	79473	1.0	1.4	1.1
1982 c	239.3	79417	1.3	1.8	1.2
1982 d	222.8	82634	1.0	1.0	1.1
1982 e	246.1	83649	1.1	1.8	1.6
1981 a	734.5	52667	1.0	0.6	1.1
1981 b	382.0	80426	0.9	0.6	1.1
1981 c	236.7	91109	0.9	0.9	0.7
1981 d	244.0	90038	1.0	0.9	0.8
1980 a	782.9	51472	1.0	0.5	0.8
1980 b	618.5	75119	0.8	0.4	1.0
1980 c	298.4	87759	0.9	0.8	1.0
1980 d	232.0	93811	1.0	0.8	0.9
1980 d	249.8	87552	1.0	0.9	0.7

Düdingen tree D5

LASER shot	av. wood dens. [mg/cm3]	13C [cps]	rel. S content	rel. P content	rel. K content
1984 a	698.6	82509	1.0	1.1	1.2
1984 b	367.2	86726	1.1	1.1	0.9
1984 c	307.7	86055	1.1	1.1	1.0
1984 d	279.1	79275	1.3	1.3	0.7
1983 a	649.9	56119	1.1	0.8	1.0
1983 b	341.7	57669	1.0	0.7	1.2
1983 c	347.0	80065	0.7	0.8	0.8
1983 d	335.2	74491	0.8	0.7	0.7
1983 e	316.0	84918	0.9	1.0	1.0
1983 f	291.5	78829	1.1	0.9	1.1
1983 g	253.0	77630	1.0	1.1	0.9
1983 h	274.6	81146	1.0	1.2	1.3
1982 a	642.3	66691	0.9	1.2	1.3
1982 b	421.2	66978	0.8	0.6	1.0

1982 c	334.9	81525	0.8	0.9	0.7
1982 d	289.6	80730	0.7	0.8	0.9
1982 e	292.4	80096	1.0	0.8	0.7
1982 f	290.1	85677	1.0	1.3	1.1
1982 g	271.7	89195	1.1	1.4	1.1
1981 a	872.8	54721	1.2	1.0	1.0
1981 b	632.5	65298	0.8	0.7	1.1
1981 c	493.3	85073	0.9	1.1	0.9
1981 d	396.2	79463	1.1	1.0	0.9
1981 e	350.2	87705	1.0	0.9	1.0
1981 f	298.1	80508	1.2	1.1	1.2
1981 g	265.3	73726	1.0	1.0	1.0
1981 h	293.4	80383	1.1	1.0	1.5
1980 a	633.4	73539	1.0	1.0	1.1
1980 b	280.0	85806	0.9	0.9	1.0
1980 c	254.8	84283	0.9	0.9	1.0
1980 d	251.5	83936	0.9	1.1	0.9
1980 e	234.6	92532	1.1	1.0	1.0

Frieswil tree F1

LASER shot	av. wood dens. [mg/cm3]	^{13}C [cps]	rel. S content	rel. P content	rel. K content
1935 a	688.7	183252	1.2	1.1	1.5
1935 b	277.7	270261	1.1	0.9	1.1
1935 c	203.2	283098	0.8	1.2	0.8
1935 d	243.1	290445	1.0	0.9	0.9
1934 a	735.5	214807	0.9	1.1	1.1
1934 b	396.4	277991	1.3	0.9	1.1
1934 c	256.7	292859	0.8	0.9	0.9
1934 d	233.7	287993	1.0	1.0	0.7
1933 a	841.2	242886	1.0	1.0	1.1
1933 b	520.9	209069	1.1	1.0	1.6
1933 c	389.2	235718	0.8	0.9	0.6
1933 d	262.9	264858	1.1	0.9	1.1
1933 e	205.4	288874	0.9	0.9	1.0
1933 f	191.4	291515	0.9	0.9	0.8
1933 g	180.1	292440	1.0	1.1	0.8
1933 h	212.7	296858	0.9	1.1	0.7
1932 a	787.7	200707	1.2	1.2	1.3
1932 b	432.5	260307	1.2	1.0	1.3
1932 c	265.0	283681	0.9	1.0	1.1
1932 d	209.0	282441	1.2	1.0	1.0
1932 e	189.3	309195	0.8	0.9	0.7
1932 f	184.5	296461	0.9	1.1	0.8
1932 g	184.1	281806	1.1	1.1	0.9
1932 h	208.1	298771	1.0	1.1	0.8
1931 a	685.7	229640	1.1	1.1	1.5
1931 b	394.7	292887	1.0	1.2	1.2
1931 c	229.0	296199	0.9	0.9	0.9
1931 d	183.2	301232	1.0	0.9	0.9
1931 e	180.8	293465	0.8	1.1	0.8

Typical element profile of a single year
Data pooled from 15 tree rings, 3 trees (D3, D5, F1) at 2 locations (Düdingen and Frieswil).

tree ring region	av. wood dens. [mg/cm3]	rel. S content	rel. P content	rel. K content
EEW	243.3	1.1	1.1	1.0
LEW	244.4	1.0	1.0	0.9
ELW	368.8	1.0	0.9	1.0
LLW	703.7	1.1	1.0	1.1

9.2 Annual S profiles by ICP-MS (chapter 3.2)

LA-ICP-MS results with annual resolution
N.B.: all values for S content are normalised with ^{13}C

year	rel. S content D1	rel. S content D3	rel. S content S4	rel. S content S5
1988	1.03	1.14	1.13	0.99
1987	1.01	1.17	1.20	1.07
1986	1.02	1.20	1.29	1.09
1985	1.08	1.10	1.16	0.94
1984	1.03	1.13	1.28	1.04
1983	1.13	1.17	1.11	1.07
1982	1.00	1.24	0.88	1.10
1981	1.06	1.06	0.81	0.96
1980	1.01	1.07	0.85	1.01
1979	1.29	1.64	0.94	0.99
1978	1.20	1.36	0.97	0.86
1977	1.31	1.55	0.95	0.99
1976	1.31	0.94	1.06	1.09
1975	0.86	0.84	1.09	1.00
1974	1.06	0.79	1.02	1.04
1973	0.92	0.96	0.92	0.89
1972	0.92	0.91	0.98	0.91
1971	0.87	0.89	0.86	1.08
1970	1.00	0.88	0.87	0.92
1969	0.89	0.87	1.01	0.90
1968	0.96	0.76	0.96	1.12
1967	1.03	0.85	0.90	0.95
1966	0.92	0.86	0.95	0.94
1965	0.92	0.95	1.11	1.06
1964	0.87	0.92	0.86	0.87
1963	0.99	0.85	0.95	0.92
1962	0.92	0.87	0.86	0.92
1961	0.88	0.82	1.02	1.04
1960	0.82	0.86	0.89	1.14
1959	0.84	0.84	1.06	0.97
1958	0.98	0.80	1.03	1.02
1957	0.95	0.77	0.90	1.01
1956	0.91	0.95	1.13	1.09

9.3   Characterisation of the LA-ICP-MS reference pellets by ICP-OES

added S standard [mg/kg]	total S in cellulose pellets [mg/kg]
0	18.9
4	21.5
20	32.9
50	68.1
100	110.8
200	204.6

added S standard [mg/kg]	total S in wood pellets [mg/kg]
0.0	62.5
4.0	61.4
20.0	77.1
50.0	99.5
100.0	155.0
200.0	251.7

9.4   Soil element content for K, Ca, Mn and S determined by WD-XRF (chapters 3.2 and 3.3)

WD-XRF results of soil samples

For K, Ca and Mn, the values determined after heating are shown
For S, the LOI (loss on ignition)

2 soil samples were taken per forest. For each site, 3 is near the trees 1-3, 6 near the trees 4-6

Element	Düdingen 3	Düdingen 6	Frieswil 3	Frieswil 6	St Moritz 3	St Moritz 6
K [%]	0.63	0.66	0.47	0.67	0.09	0.11
Ca [%]	0.35	0.35	0.68	0.48	0.89	1.12
Mn [%]	0.03	0.06	0.10	0.02	0.03	0.08
S [%]	0.08	0.03	0.28	0.24	0.14	0.13

9.5 Microwave acid digestion: effect of MgO addition on S recovery

added MgO [mg/l]	total S (wood doped with 20 mg/kg S Standard) [mg/kg]
0	69.4
0	70.1
22.5	70.1
22.5	66.9
45	77.7
45	72.6
90	76.1
90	71.7

MgO [mg/l]	total S (wood doped with 20 mg/kg cysteine sollution) [mg/kg]
0.0	74.1
0.0	71.3
22.5	77.8
22.5	70.1
45.0	84.4
45.0	69.4
90.0	71.5
90.0	65.1
135.0	74.2
135.0	75.1

9.6 K, Ca, Mn and S contents determined by Optical Emission Spectrometry (chapter 3.3)

The values presented in this table are the average from two trees at each site
All results in mg/kg
Düd: Düdingen, StMo: St Moritz, Fri: Frieswil

year	K Düd	K StMo	Ca Düd	Ca StMo	Mn Düd	Mn Fri
1999-2003	420	801	475	644	104	127
1994-1998	282	393	587	694	113	142
1989-1993	278	399	617	743	120	150
1984-1988	300	381	640	775	150	181
1979-1983	282	383	690	765	184	170
1974-1978	231	366	745	789	220	160
1969-1973	201	383	621	820	206	187
1964-1968	263	392	788	832	230	172
1959-1963	259	398	740	814	219	176
1954-1958	269	397	749	829	232	181
1949-1953	243	366	652	788	223	209
1944-1948	243	310	652	786	223	201
1939-1943	236	303	698	783	212	206
1934-1938	236	303	698	783	212	217

The values presented in this table are the average from two trees at each site
Exeptions: Frieswil (3 trees), F1 (fungal decay), F4 (compression wood)
All results in mg/kg
Düd: Düdingen, StMo: St Moritz, Fri: Frieswil

year	S Düd	S Fri	S StMo	S F1	S F4
1999-2003	70	57	81	82	65
1994-1998	56	52	56	49	56
1989-1993	53	48	59	61	39
1984-1988	53	52	73	53	26
1979-1983	61	56	71	52	20
1974-1978	57	68	70	59	18
1969-1973	45	59	70	49	17
1964-1968	52	46	74	56	8
1959-1963	53	48	66	39	7
1954-1958	48	46	65	41	8
1949-1953	45	49	60	110	0
1944-1948	45	44	59	57	20
1939-1943	45	40	69	47	7
1934-1938	45	41	69	461	30

9.7   Mg, Zn, Cu, Cr, Ba and Al contents determined by Optical Emission Spectrometry

The values presented in this table stem from individual trees
All results in mg/kg
D: Düdingen, S: St Moritz, F: Frieswil

year	Mg D1	Mg D5	Mg F6	Mg S1	Mg S4
1999-2003	244.0	267.1	367.0	367.3	308.2
1994-1998	181.4	225.5	314.8	398.3	420.1
1989-1993	230.7	220.8	307.3	321.7	511.9
1984-1988	304.8	175.6	322.5	321.7	488.1
1979-1983	319.4	208.0	253.6	358.1	469.5
1974-1978	355.7	179.7	331.2	358.1	265.3
1969-1973	351.4	131.3	279.3	280.3	265.3
1964-1968	157.1	224.3	327.2	280.3	505.3
1959-1963	157.1	229.2	252.6	466.3	449.7
1954-1958	179.1	209.5	265.8	578.3	266.8

year	Zn D1	Zn D5	Zn F1	Zn F2	Zn F3	Zn F4	Zn F6	Zn S4
1999-2003	5.6	5.3	36.1	11.5	10.1	6.9	7.3	8.4
1994-1998	6.8	5.9	17.4	8.6	7.7	7.8	5.9	6.7
1989-1993	7.4	6.0	21.4	9.8	8.5	8.8	8.8	12.0
1984-1988	7.4	7.1	12.4	10.5	11.4	7.9	9.0	8.4
1979-1983	6.3	5.9	14.6	8.2	12.2	8.4	5.9	7.8
1974-1978	6.6	6.2	15.1	11.1	12.2	7.0	10.2	10.7
1969-1973	8.4	2.8	13.8	11.7	11.3	6.4	12.0	10.7
1964-1968	12.8	3.0	14.7	16.6	9.0	9.2	8.5	8.5
1959-1963	12.8	4.4	15.9	12.7	14.5	8.1	11.6	11.1
1954-1958	11.1	3.3	8.5	12.4	11.3	6.9	5.2	9.9

year	Cu D1	Cu D5	Cu F1	Cu F3	Cu F6	Cu S1	Cu S4
1999-2003	2.0	1.4	18.1	6.4	2.5	7.7	3.0
1994-1998	1.3	1.1	5.4	2.1	1.6	2.3	3.2
1989-1993	1.8	1.0	4.6	4.6	2.3	4.2	3.6
1984-1988	1.4	1.0	2.0	1.7	2.4	4.2	2.6
1979-1983	1.6	1.2	4.6	1.6	1.6	1.8	2.3
1974-1978	1.4	1.0	2.1	0.7	1.7	1.8	1.6
1969-1973	1.5	0.6	4.7	0.7	1.6	1.5	1.6
1964-1968	0.9	1.0	1.1	1.2	1.9	1.5	2.3
1959-1963	0.9	1.3	1.2	0.7	3.1	2.1	1.9
1954-1958	1.0	0.8	0.8	0.4	1.7	1.9	1.4

year	Cr F1	Cr F2	Cr F3
1999-2003	4.4	1.4	0.6
1994-1998	3.4	0.8	0.8
1989-1993	1.9	1.1	0.6
1984-1988	1.3	0.8	1.0
1979-1983	1.4	1.1	1.0
1974-1978	1.3	1.1	1.2
1969-1973	1.5	1.2	0.7
1964-1968	0.8	0.7	0.7
1959-1963	0.8	0.8	1.0
1954-1958	0.7	0.9	1.1

year	Ba F1	Ba F2	Ba F3	Ba F4	Ba S1	Ba S4
1999-2003	7.7	4.0	3.2	5.8	13.4	41.9
1994-1998	7.4	4.7	3.7	6.5	14.4	45.3
1989-1993	8.7	6.4	5.8	6.3	15.7	52.2
1984-1988	8.0	5.7	9.5	6.6	15.7	54.2
1979-1983	9.8	6.3	6.7	7.1	17.1	54.3
1974-1978	10.0	6.9	6.6	9.5	17.1	56.8
1969-1973	8.8	8.8	8.6	9.8	16.5	56.8
1964-1968	14.7	9.0	8.5	11.9	16.5	57.4
1959-1963	18.3	9.5	8.1	10.6	14.1	56.2
1954-1958	10.1	7.1	7.6	9.7	13.8	n.a.

year	Al D1	Al F1	Al F2	Al F3	Al F6	Al S4
1999-2003	1.5	76.0	8.2	4.9	3.3	4.6
1994-1998	1.1	4.5	15.5	15.4	3.7	8.9
1989-1993	1.0	10.8	6.5	2.1	5.4	6.5
1984-1988	0.7	3.8	7.5	1.8	2.4	3.6
1979-1983	n.a.	6.7	12.3	8.3	2.5	2.6
1974-1978	1.0	4.7	6.2	2.2	3.2	5.3
1969-1973	0.7	7.0	6.8	3.4	2.4	5.3
1964-1968	2.3	5.6	5.8	2.3	6.2	3.6
1959-1963	2.3	5.6	6.1	4.6	5.5	5.7
1954-1958	2.6	7.0	5.4	5.4	3.4	5.4

9.8   S group-specific contents derived from background-corrected latewood spectra

All results are in total electron yield (TEY) current units (pA)

Düdingen tree D5 (latewood from one tree ring)

year	sulphate	methylthiol	disulphide	sulphide
1989	2.54	2.28	1.43	2.13
1986	3.16	3.77	2.03	2.90
1983	3.50	1.77	0.94	2.05
1976	1.61	1.40	0.38	1.27
1971	2.81	2.05	1.39	1.72

Frieswil tree F6 (early- and latewood from two consecutive tree rings)

year	sulphate	methylthiol	disulphide	sulphide
2000-2002	1.17	2.12	0.59	n.a.
1991-1992	1.52	0.80	1.61	n.a.
1986-1987	2.47	2.21	2.01	n.a.
1976-1977	1.54	0.78	0.22	0.47
1972-1974	2.62	0.32	1.42	1.27
1968-1969	2.46	1.12	1.67	n.a.

9.9   Isotopic Signatures of δ^{34}S in a peat bog from Düdingen

peat sample	age	δ^{34}S [‰]
DME 2/3	1998	1.5
DME 5	1992	3.1
DME 12	1984	1.0
DME 18	1974	2.4
DME 23	1958	7.7
DME 33	1933	1.3
DME 39	1922	6.4
DME 40	1921	4.8

9.10 Tree characteristics

The tree characteristics presented in this table are the averages of six drill cores per tree

Sample ID	circumference [cm]	water content [%]	sapwood [cm]	sapwood [y]	cambial age [y]
D1	128	39.9	4.8	42	101
D2	200	49.7	6.5	36	94
D3	185	45.9	4.9	19	96
D4	148	55.5	6.5	32	90
D5	165	43.0	7.1	28	96
D6	179	42.1	5.5	27	98
F1	250	44.6	5.8	32	134
F2	245	50.1	6.8	24	141
F3	235	46.0	5.7	30	150
F4	185	33.6	3.3	24	147
F5	168	34.9	5.2	30	142
F6	166	37.6	4.3	35	137
S1	191	54.9	6.0	56	148
S2	149	55.4	6.1	54	150
S3	130	57.5	3.3	84	182
S4	145	70.0	7.3	38	135
S5	153	70.7	4.3	74	177
S6	133	54.6	5.1	64	142

10.   Contributions to conferences

Attended conferences

Symposium Nanotoxikologie 2006, Center for Xenobiotic and Environmental Risk Research (XERR), University of Zurich
Swiss NCCR Climate Summer School 2005, Grindelwald
TRACE – Tree Rings for Archaeology, Climatology and Ecology, Dendrosymposium 2005, University of Freiburg
EMPA Forschungsmarktplatz 2004, EMPA Dübendorf
TRACE – Tree Rings for Archaeology, Climatology and Ecology, Dendrosymposium 2004, WSL Birmensdorf
Swiss NCCR Climate Summer School 2003, Grindelwald

Talks

TRACE 2005
PSI Seminar 2005
TRACE 2004
EMPA Seminar 2004
PSI Seminar 2004
EMPA Seminar 2003
PSI Seminar 2001

Posters

Swiss NCCR Climate Summer School 2005, Grindelwald
Forschungsmarktplatz EMPA 2004, EMPA Dübendorf
Berner Umweltforschungstag 2004, University of Bern
Swiss NCCR Climate Summer School 2003, Grindelwald

11. List of publications

Barrelet T, Ulrich, A, Rennenberg, H, Krähenbühl, U (2005)
Dendrochemical Analysis of Norway Spruce Drill Cores by Optical Emission Spectrometry
Anal Bioanal Chem (submitted).

Barrelet T, Ulrich A, Rennenberg H, Zwicky CN, Krähenbühl U (2005)
Norway Spruce as an Environmental Archive for Sulphur Dioxide
Env Sci Tech (submitted).

Barrelet T, Ulrich A, Rennenberg H, Krähenbühl U (2005)
Seasonal Profiles of Sulphur, Phosphorus and Potassium in Norway Spruce Wood: New Light shed on Tree Physiology
Plant Biol (accepted).

Struis RPWJ, Ludwig C, Barrelet T, Krähenbühl U, Rennenberg H (2005)
X-rays reveal sulphur functional group profiles in spruce wood
Nature (submitted).

Barrelet T, Ulrich A, Krähenbühl U
Effects of pathogens and compression wood on sulphur content in Norway spruce
Annual report of the Laboratory for Radiochemistry and Environmental Chemistry, PSI, University of Bern, 2005.

Barrelet T, Ulrich A, Krähenbühl U
Quantification of Sulphur in Picea abies by LA-ICP-MS
TRACE - Tree Rings in Archaeology, Climatology and Ecology, Vol.3 - Proceedings of the DENDROSYMPOSIUM 2004 in Birmensdorf/CH.

Barrelet T, Ulrich A, Krähenbühl U
Spatial sulphur quantification in Picea abies using LA-ICPMS
Annual report of the Laboratory for Radiochemistry and Environmental Chemistry, PSI, University of Bern, 2004.

Barrelet T, Ulrich A, Krähenbühl U
Quantification of Sulphur in living Picea abies (Norway Spruce)
Annual report of the Laboratory for Radiochemistry and Environmental Chemistry, PSI, University of Bern, 2003.

Barrelet T, Krähenbühl U
Measurements of the isotopic signatures of $\delta^{34}S$ in a peat profile of Düdingen (Swiss midlands)
Annual report of the Laboratory for Radiochemistry and Environmental Chemistry, PSI, University of Bern, 2002.

Barrelet T, Krähenbühl U
Jörisee III (GR): Sediments of a High Alpine, Oligotrophic Lake as a Climatic Archive
Annual report of the Laboratory for Radiochemistry and Environmental Chemistry, PSI, University of Bern, 2001.

12. Curriculum Vitae

Timothée Barrelet
29.1.1976, Swiss nationality

Education

1996 – 2005	Studies in chemistry at the Department of Chemistry and Biochemistry, University of Bern
2001 – 2005	Ph.D.thesis: "Norway spruce as an environmental archive for sulphur dioxide". (Prof. Dr. U. Krähenbühl, Dr. A. Ulrich)
2000 – 2001	Diploma thesis „Jörisee III: Sedimente eines hochalpinen, oligotrophen Sees als Klimaarchiv" (Prof. Dr. U. Krähenbühl)
1991 – 1996	Grammar school of Bern-Neufeld; Maturity Type B (Latin)

Experience

2001 - 2005	Research Assistantship at the University of Bern, Laboratory for Radiochemistry and Environmental Chemistry. Techniques and collaborations: 1. LA-HR-ICP-MS at the EMPA Dübendorf 2. Microwave acid digestion and oxygen bomb combustion of drill core pieces followed by ICP-OES measurement 3. Radiodensitometry at the WSL in autumn 2003 4. L-edge X-ray absorption measuring campaign in February 2004 at the Synchrotron Radiation Centre in Wisconsin, USA (in collaboration with the PSI and the University of Wisconsin)
2002 - 2005	Teaching of environmental chemistry at the Swiss College of Agriculture, Zollikofen
2004	Officer at the laboratory of Spiez (first lieutenant)
2001	Swiss Air Force officer school, Dübendorf
2001	Teaching of food chemistry at the Grammar School Bern-Neufeld
2000	Research Assistantship at the University of Bern, Laboratory for Radiochemistry and Environmental Chemistry. Techniques: 1. Grain Size Separation of Alpine Lake Sediments by Sedimentation in Acetone. 2. PGAA Analysis of Particle Filters

1999   Translator for the electoral campaign of the list of the independents

Languages

French: mother language
German: second language
English: fluent
Italian: basic knowledge

Music

Co-founder of the band Magnetfisch (1998, label: Helvetic Production, distributor: VSM, official homepage : www.magnetfisch.org)