Seasonal variability in isotopic signature of leaf water and related water compartments

An ecophysiological perspective

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Annotation:

Stable isotope composition of bulk leaf water is a useful proxy for geochemical and biological processes and is, thus, appreciated in ecological research and global modelling. The mechanisms determining the abundance of different isotopes in leaf water are not fully understood. The research presented in this thesis aimed to describe variability in leaf water isotopes which occurs under natural conditions and to distinguish which factors generate such variability. For that purpose, field sampling, water extraction, stable isotope analysis and measurements of several additional environmental and physiological characteristics were carried out at one sampling site during three subsequent growing seasons. The results obtained point out that oxygen isotopes rather then hydrogen isotopes have greater potential to be used in higher scale applications. Apart from that, several interesting ecophysiological interpretations of isotopic data have been made.

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Poděkování/ Acknowledgements

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Contents

Foreword

Stable Isotopes and Global Networking

Stable isotope techniques represent a modern approach which enables to trace hidden biological processes and are therefore widely used in ecology, plant physiology and other realms of natural science. Stable isotopes could be implemented in research either directly as a tool during experiments (e.g. when labelling with artificially introduced source with known isotopic composition) or by monitoring natural abundances of isotopes in a system of interest. Besides this, stable isotope techniques are utile at nearly all scales - from molecular processes to global budgets. As molecules that contain different isotopes (sometimes called isotopomers) obey certain physical or biochemical rules, we can predict their behaviour and possible compartmentation within an environmental system and distinguish possible limitations and processes which govern the system.

Scientific community should take an advantage of both approaches – monitoring and manipulative experiments which aim to test critically hypotheses. Unfortunately monitoring is often held in contempt (Nisbet 2007). Some scientists and funding agencies respect only hypothesis-based experimental research and neglect the significant contributions and achievements of rigorous monitoring measurements. In an ideal case, these two approaches could supplement each other. Sometimes a hypothesis to be tested by an experiment is expressed based on the results of monitoring measurements. Furthermore, development and improvement of some analytical techniques, acquisition of the data for global modelling or revealing of some important long-term processes (e.g. climate change, increase in temperature and carbon dioxide concentration, El Niño effect) could be attributed to the achievements of monitoring science.

Monitoring is very useful presuming it produces reliable data. Therefore, high measurement accuracy and reproducibility must be demanded. This should include a well-defined and tested methodology and detailed sampling protocol. But what is probably even more important, the data integrated and interpreted together. The hidden pattern often emerges after the data of the same type but from different parts of the globe are interpreted together. Last but not least, it is always advantageous when the

data series are as long as possible because sometimes only long-term records enable to discover a real trend or pattern in the data.

Stable isotope composition of chemical compounds which play a dominant role in geobiochemical cycles represents a typical kind of data which are worth monitoring. We are able to predict isotopic composition of some specimens based on our knowledge of isotopic composition of source and fractionation factors characteristic for certain processes or reactions occurring when the specimen is formed from its source (e.g. isotopic composition of water evaporating from the lake could be computed knowing isotopic composition of water in the lake, water vapour present in the air, temperature and relative humidity). However, environmental systems are so complex that it is currently beyond our ability to describe entirely all of them with mathematical equations. By studying isotopic data harvested worldwide under different environmental conditions, we can puzzle out which processes are crucial to establish isotopic equilibrium in our specimen or we can gather a collection of empirical data which can be consequently used in a model. In the former case monitoring provides a tool for assessing hidden mechanisms while in the later case it builds a bridge across an unknown area which we will hopefully understand later. It has been proved many times that stable isotope data represent invaluable inputs into models of global carbon, nitrogen, sulphur and water circulations.

Several international networks which should coordinate acquisition of stable isotope data were conceived during the last decades. Moisture Isotopes in the Biosphere and Atmosphere (MIBA) network is one of them. The MIBA was conceived by the International Atomic Energy Agency (IAEA) in Vienna in May 2004. The primary goal of this network is to complement well-established and successful global networks (the Global Network of Isotopes in Precipitation, GNIP, and Global Network of isotopes in Rivers, GNIR) with the stable isotope data related more to the biosphere. MIBA's sampling protocols are designed for acquiring of isotopic signals of leaf and xylem water, as well as soil water and air water vapour. We are currently not able to predict these data reliably. Having a database of such data would help us to bypass this uncertainty and improve our understanding of many important aspects of environmental systems functioning (e.g. partitioning evapotraspiration fluxes, partitioning of annual carbon fluxes). Better understanding of leaf water isotopic signal should be the most important outcome of the MIBA project because abundance of ^{18}O isotope in leaf water imprints into CO_2 and O_2 molecules. Consequently, oxygen isotopic composition of

these molecules carries information on their origin which can be used to distinguish how terrestrial versus marine vegetation contributes to the global carbon budget.

The IAEA invited scientists form many countries to participate in the MIBA program. Laboratories which routinely carry out stable isotope measurements were encouraged to become subcoordinators, so that the people who wish to participate but do not have appropriate instrumentation get the possibility to have their samples measured. A worldwide network of sampling sites has been established. The Stable Isotope Laboratory running under the Departments of Plant Physiology and Ecosystem Biology, University of South Bohemia did not stand aside. From the initiative of Jiří Šantrůček, (Department of Plant Physiology, Faculty of Science, University of South Bohemia), the MIBA sampling has been conducted at the following sites: Brloh, Třeboň, Bílý Kříž (all in the Czech Republic), Czersky (Russia, North-East Siberia, mouth of the Kolyma river) and Orange Walk (North of Belize, Central America) at least for one year.

In this thesis, I analyze and interpret the data acquired during the Brloh field sampling campaign in three subsequent vegetation seasons. Our research was based on the monitoring of isotopic signals of different water fractions. In addition, we conducted measurements of some environmental and physiological characteristic which were expected to be explanatory for the measured isotopic signature. I present (i) the hypotheses which we developed based on the literature and our previous experimental work, (ii) methods we used during our research, (iii) results and conclusions obtained and (iv) the future prospective and suggestions for the experiments and measurements which could be made in support of our conclusions.

1/ Introduction

This introductory chapter describes in short what we investigated and why (Motivation). Further, fundamentals of stable isotope theory are outlined followed by more detailed description of behaviour of different isotopologues[1] in relevant environmental systems. Next section of this chapter focuses on differences in sun-exposed and shaded leaves with respect to isotopic fractionation. At the end of this chapter, the working hypotheses and our expectations are listed.

1.1 Motivation

Isotopic composition of bulk leaf water appeared to be a useful quantity for scientists investigating carbon, oxygen and water fluxes within the ecosystems or even within the whole globe (Farquhar et al. 1993, Yakir & Sternberg 2000). Since the isotopic signal of leaf water imprints into other molecules, the "history" of e.g. carbon dioxide or oxygen molecule can be traced by measuring their isotopic composition. Using this information, partitioning between photosynthesis and respiration or marine and terrestrial photosynthesis is possible. Therefore, substantial effort has been made to understand which factors determine the leaf water isotopic signal. Even though several models have been offered, the issue desires further investigations. We tried to improve the current state of knowledge by monitoring seasonal dynamics of stable isotopes in leaf water and related water compartments (i.e. stem and soil water) under natural conditions. Since the leaf water isotopic signal is influenced by isotopic composition of source water (i.e. stem water) and environmental conditions, one would expect to find changes in isotope abundances within the season. The values used in global modelling are inevitably averaged estimates, thus, knowing the real variability of the value would help to eliminate possible mistakes, or at least, be aware of them.

The research was conducted at a forest site near Brloh, the Czech Republic. The site is located in the centre of Blansky Les Nature Reserve which should ensure relatively pristine environment close to a natural state. A beech tree (*Fagus sylvatica* L.) was chosen as an experimental plant as it represents a dominant species at the site and would

[1] Isotopologues are molecules of the same chemical species which differ in isotopic composition of their atoms (e.g. H_2O, $H_2{}^{18}O$, ^2H_2O are the most natural abundant isotopologues of water). In contrast, the term isotope refers to a single atom. For the sake of simplicity, I sometime use a collocation 'water isotope' which is erroneous in this regards but still generally used in the literature.

naturally occur there even without human intervention. Samples of beech twigs and leaves together with soil samples were collected in approximately two weeks intervals during three subsequent growing seasons. Basically, we followed the MIBA sampling protocol (see Material and Methods for details). However, we extended the sampling to address the differences caused by contrasting light conditions (sun-exposed and shaded leaves and twigs, soil samples from the forest understory and open meadow). We expected to found differences in isotopes due to different evaporative demand, photosynthetic activity etc. of sun and shade leaves. In addition, several environmental and physiological characteristics (e.g. air and leaf temperature, relative humidity, irradiance, plant water potential) were measured while sampling to characterize the conditions at the site. Finally, carbon isotopic composition of leaf and twig dry matter was assessed because it carries information on stomatal conductance and photosynthetic processes.

In this thesis, I comment on seasonal variability in isotopic composition of soil, stem and leaf water. I try to reveal the factors which determine the isotopic signal and extract any additional information carried by stable isotopes.

1.2 Stable isotope composition and fractionation

Isotopes of the same element differ in the number of neutrons which are present in their nucleus. As a result, isotopes are characterized by a slightly different atomic mass, which is manifested by their different behaviour during many physical and chemical processes. As a consequence, isotopes (or rather isotopologues) are distributed unequally in the nature. This phenomenon is referred as isotopic fractionation and can be principally divided into three categories: kinetic, equilibrium and diffusive fractionation. In kinetic fractionation, the chemical reaction is usually faster for isotopically lighter substrates due to higher frequency of thermal vibrations compared to their heavier counterparts. Therefore, the product of the reaction contains more light isotopes than the substrate[2]. Equilibrium fractionation is caused by the fact that lighter molecules easily enter the higher energy state (e.g. from liquid phase to gas phase) than the heavy ones. The last type of fractionation could be attributed to the faster diffusion rates of the lighter molecules.

[2] unless the substrate is fully consumed. If this happens the product will retain the isotopic composition of the substrate.

Isotopic composition (δ) of a sample is usually expressed as an abundance ratio of heavy to light isotopes (R_{sample}) and compared with this ratio in an internationally accepted standard ($R_{standard}$):

$$\delta[‰] = \left(\frac{R_{sample}}{R_{\text{s tan } dard}} - 1 \right) \cdot 1000 \qquad \text{Eq.1.1}$$

where R=[^{18}O]/[^{16}O] for oxygen (and analogically for other isotopes). Negative δ values indicate that the sample is depleted in heavy isotopes in comparison with the standard, whereas positive δ values mean that the sample is isotopically enriched.

Sometimes it is advantageous to express the isotopic composition of a sample (product) with respect to the isotopic composition of its source (substrate). Such a notation (Δ, often called discrimination), points out how the reaction (the source to product transition) discriminates against the heavy isotope. Positive Δ values indicate that isotopically light molecules of substrate are preferred in the reaction. Consequently, the product is depleted in heavy isotopes in comparison with the substrate.

$$\Delta[‰] = \left(\frac{R_{source}}{R_{product}} - 1 \right) \cdot 1000 \qquad \text{Eq.1.2}$$

Relationship between Δ and δ can be derived:

$$\Delta[‰] = \left(\frac{\delta_{source} - \delta_{product}}{\delta_{product} + 1000} \right) \cdot 1000 \qquad \text{Eq.1.3}$$

which can be usually approximated by:

$$\Delta[‰] = \left(\delta_{source} - \delta_{product} \right) \qquad \text{Eq.1.4}$$

There are two possible ways of describing a reaction or a process in terms of the change in isotopic composition. First, it can be described by the so called isotope effect (sometime also referred as fractionation factor) α defined as:

$$\alpha = \frac{R_{source}}{R_{product}} \qquad \text{Eq.1.5}$$

where R is the ratio of heavy to light isotope as mentioned above. α is usually close to unit. Therefore, it is desirable to introduce another quality, the fractionation, ε, which expresses the deviation of α from unity. Hence,

$$\varepsilon = \alpha - 1 \qquad \text{Eq.1.6}$$

Combining with the Eq.5 and multiplying by 1000 we arrive to a definition similar to Eq.2. Therefore, the fractionation ε (‰) is conveniently used in equations defining the change in isotopic signal between source and product. See Eq.1.9 & 1.10 later in the text for instance. A comprehensive description of stable isotope fundamentals is contained in Ehleringer, Hall & Farquhar, eds, chapters 3-5(1993) .

1.3 Water in atmosphere and biosphere

Water present on the Earth circulates through different physical states and masses of water in the given state can move across great distances. About 90% of the global water flux is realized by evaporation from the ocean and subsequent rainout back into the ocean. Only 10% of water vapour originating in evaporation from the ocean moves inland and mixes with vapour evaporating from soil and transpiring from plants. When the vapour is cooled down, it condenses and rain or snow occurs. At this stage, the water cycles several times in terrestrial part of hydrological cycle. The terrestrial water cycle can be in short described as follows. First, rain water recharges soil. Part of the water is taken up by plant roots, flows through vascular tissues into the leaves, evaporates (probably in a substomatal cavity) and escapes from the plant in form of vapour via open stomata. This process is called transpiration. The rest of the water evaporates from the soil surface or percolates into the groundwater. Surface runoff can occur when the soil is saturated with water or when the precipitation is extremely intensive. The global cycle closes when water returns into the ocean as river or groundwater runoff.

Observing stable isotope composition of different water compartments could help us to trace the pathways of water circulation. Waters of the oceans are isotopically more or

less homogenous with δ-values close to 0‰[3]. In contrast, the meteoric waters (namely the atmospheric moisture, the precipitation, groundwater, rivers) have negative δ-values. Water evaporating from the sea surface is isotopically depleted. This limited water vapour reservoir is subjected to so-called Rayleigh distillation during which the heavy water molecules condense and rain out. Therefore, the reservoir (clouds) becomes progressively depleted in the heavy isotopes. In turn, the precipitation and water derived from them (e.g. rivers) are isotopically depleted in comparison with the waters of the ocean. Apart from that, isotopically enriched water bodies could be found. These are represented mainly by the water in lakes or similar water reservoirs and the leaf water. The enrichment arises as a result of intensive evaporation which leaves heavy isotopes behind in the liquid phase (Mook 2000).

Since we know the fractionation coefficients characteristic for the processes employed in the hydrological cycle, it is possible to predict isotopic composition of miscellaneous water bodies under different conditions and at different sites all over the world.

1.3.1 Isotopic composition of precipitation

Precipitation represents the primary input of water into the ecosystem as it recharges groundwater, soil water and surface water bodies. Craig (1961) showed that isotopic composition of the meteoric waters (e.g. rain, snow and river water) from diverse geographic locations follows a linear trend which can be approximated by the equation:

$$\delta^2 H = 8 \cdot \delta^{18} O + 10 \qquad \text{Eq.1.7}$$

This empirically derived line is known as the global meteoric water line (GMWL) and describes isotopic composition of water bodies which were not subjected to surface evaporation. As the GMWL represents a global average, δ^2H and δ^{18}O of samples collected at a specific locality lie on the line which usually has slightly different parameters (slope and intercept). The local meteoric water lines (LMWL) differ as a consequence of specific meteorological, topographical or orographical conditions characteristic for the given sampling site. However, the deviation from the GMWL is usually small. The slope of the MWL is determined by the ratio between equilibrium

[3] Oceanic water known as VSMOW (Vienna Standard Mean Oceanic Water) is now an internationally accepted reference standard for hydrogen and oxygen isotopic measurements

fractionation factors of hydrogen and oxygen isotopes (Table 1.1) for the rain condensation process which slightly varies with temperature. The intercept with δ^2H axis referred as deuterium excess (d-excess) is given by the humidity and temperature conditions.

As MWLs depict only the relation between δ^2H and $\delta^{18}O$ of precipitation, we can be interested in spatial and temporal variation of these values. By analyzing data from the GNIP database, it has been revealed that isotopic composition of precipitation changes with latitude, altitude, continentality and season. More specifically, the latitude effect is responsible for change of about -0.6‰ in $\delta^{18}O$ per degree of latitude, the altitude effect accounts for change from -0.2 to -0.6‰ per increase of 100m. The continental effect can be illustrated by the depletion of the precipitation of 7‰ when moving from Irish coast to the Ural mountains (Mook 2000). Finally, the seasonal effect is dependent on the climate at the specific site. For the temperate region of the northern hemisphere the usual pattern is a continuous transition from the most depleted winter precipitation toward the most enriched summer precipitation and back. The physical basis of all these effects is now well understood. The effects could be explained by applying the Rayleigh model on the processes of evaporation and condensation during which the temperature seems to play a crucial role (Gat 1996). A short term variation in stable isotope composition of the precipitation also exists, e.g. it has been shown that during heavy storms rain has more negative δ. This situation is called the amount effect. However, short term trends have not been studied so comprehensively until now.

On balance, the main mechanisms controlling isotopic composition of precipitation have been already understood. The researchers developed models providing predictions which are in satisfactory concordance with the data measured. At that point, it is appropriate to stress the merit of the GNIP sampling program. If it were not for the data acquired and shared in the GNIP program, the patterns and principles of isotopic composition of precipitations would not have been resolved yet.

1.3.2 Isotopic composition of soil water

Isotopic composition of soil water is determined by the interplay between rainfall and evaporation. This process can be briefly described as follows. The rain water recharges the soil and mixes with water already present within the soil profile. Evaporation from the soil surface caused that the water close to the surface becomes

progressively enriched. The enrichment at the surface of bare soil can be predicted by Craig-Gordon model (Eq. 1.9 discussed later). This often fails in reality since many factors may disturb isotopic equilibrium. Eventually, the enrichment is "diluted" by isotopically depleted rain. Therefore, one would anticipate that isotopic signal of water is highly variable since it depends on actual environmental conditions such as precipitation and evaporative demands. Besides this, vegetation cover may also affect the signal by preventing evaporation and reducing precipitation throughfall. Hence, the isotopic composition of soil water usually varies with soil depth, with the layers close to the soil surface being isotopically heavier. For instance, Hsieh et al. (1998) observed isotopically enriched signal at soil depths up to 30cm, with the difference in $\delta^{18}O$ between soil surface and 70cm depth being 1-7‰ in average. The vertical heterogeneity in isotopic composition of soil water has been widely used to distinguish possible water sources for plants, e.g. Ehleringer et al. (1991) investigated the differential utilization of water sources in desert plant community using this method.

Many of studies dealing with soil water isotopic composition have been carried out in arid and semiarid regions (Yepez et al. 2003). In my opinion, there are two reasons for that. First, water availability is a crucial issue in this region and correspondingly attracts attention of many researchers. Second, it is easy to survey since the rain in arid regions is usually restricted to a defined time period and does not unexpectedly interfere with the experiment. Furthermore, intensive evaporation generates steeper gradients and the patterns are better visible. The second group of publications on soil water isotopes focuses on tropical rain forests and usually aims to partition the contributions of soil evaporation and plant transpiration to overall vapour flux (e.g. Moreira et al. 1997). In this case, minimal evaporation from the soil surface may be advantageous for the researchers.

Despite the fact that much less is known on isotopic composition of soil water in temperate region, one would anticipate that it will be analogous with the patterns found in the arid regions and the tropics. However, enhanced variability can be expected owing to a frequent and irregular rainfall which perturbs isotopic equilibrium attained in the soil.

1.3.3 Isotopic composition of plant water

Both root water uptake and water movement through vascular tissues are not associated with the isotopic fractionation. Changes in isotopic composition of plant water arise almost exclusively from evaporation from the leaf. Consequently, the leaf water becomes enriched in heavy isotopes while water in the roots, stems and petioles should retain the isotopic signature of the water taken up from the soil. The fact that there is also the phloem sap containing enriched water from the leaves can be perhaps neglected because of a rather small amount of this water (Barbour et al. 2000).

The isotopic signal of leaf water is worth studying. Leaf water isotopically interacts with O_2 and CO_2, molecules participating in processes of photosynthesis. O_2 is directly derived from chloroplast water and CO_2 exchanges oxygen atoms with water during the hydration of CO_2 according to the reaction:

$$H_2O_{(l)} + CO_{2\,(g)} \leftrightarrow H^+ + [HCO_3^-]_{(aq)} \qquad \text{Eq.1.8}$$

Therefore, O_2 and CO_2 molecules once present in the leaf cell take on the ^{18}O signature of leaf water. This arises an opportunity to trace the fluxes of carbon dioxide and oxygen (Farquhar et al. 1993, Gillon & Yakir 2001). In addition, the isotopic signal of leaf water is transmitted via CO_2 into a plant biomass. Then, the isotopic signals from tree rings can be used in paleoclimatic reconstructions (e.g. Saurer 2003).

Recognising the importance of this issue, a substantial effort has been made to find a model which would reliably predict the isotopic composition of the leaf water. First, the Craig-Gordon model, originally designed for the lake water enrichment, was adapted for leaves (Dongmann et al. 1974, Farquhar et al. 1989):

$$\Delta_c = \varepsilon_k + \varepsilon^* + \left(\Delta_{vapour} - \varepsilon_k\right) \cdot \frac{e_a}{e_i} \qquad \text{Eq.1.9}$$

where Δ_c stands for isotopic enrichment of the water at the evaporating sites above the source water, Δ_{vapour} represents depletion of the air water vapour with respect to source water. ε_k and ε^* is kinetic and equilibrium fractionation, respectively (Table 1.1) . e_a is vapour pressure in the ambient air, and e_i stands for the vapour pressure in the air space of the leaf mesophyll. Assuming that the air in the leaf interior is fully saturated with

water vapour and that the leaf has the same temperature as the surrounding air, the e_a/e_i ratio can be approximated by relative humidity of ambient air.

temp (℃)	oxygen	hydrogen
10	10.6	93.2
15	10.2	87.2
17	10.0	84.9
20	9.7	81.6
22	9.6	79.5
25	9.3	76.4
30	8.9	71.4
35	8.6	66.8

Table 1.1 Temperature-dependent values of equilibrium fractionation (ε*) for oxygen and hydrogen (computed after Majouble 1971; cited in Yepez et al. 2003)

Since the predictions based on the Craig-Gordon model were often found to overestimate the real measured enrichment of bulk leaf water (e.g. Yakir et al. 1990, Flanagan et al. 1993, Gan et al. 2002), several alternative refinements of the Craig-Gordon model have been suggested. Allison et al. (1985) and Leaney et al. (1985) recognised that the leaf, in contrast to the lake, is not a homogenous water body. Therefore, the two-pool mixing model was introduced presuming that two isotopically distinct compartments can be distinguished within the leaf, i.e. 1) the xylem water which is not directly subjected to evaporation and therefore should retain the isotopic signature of the water taken up by roots, and 2) the water at evaporating sites which should be enriched in the heavy isotopes. Owing to the mixing with non-enriched xylem water, the bulk leaf water appears isotopically lighter than predicted by Craig-Gordon model. Another alternative explanation is based on the fact that the leaf might not be in a steady state[4] which is one of the principal assumptions of the leaf-adapted Craig-Gordon model (Eq.1.9). To address this possibility, several non-steady state models have been offered recently (Farquhar & Cernusak 2005, Ogée et al. 2007).

Furthermore, it has been observed that increased transpiration leads to the decrease in enrichment (Walker et al. 1989, Helliker & Ehleringer 2002, Barbour et al. 2004). A possible explanation for this finding could be seen in the interplay between the convective stream of transpirational flux and the back diffusion of heavy water molecules from the sites of evaporation (the so-called Péclet effect). This explanation was suggested by Farquhar and Lloyd (1993) for the first time and since that time the

[4] The steady-state means that the isotopic signal of transpired water should be equal to the isotopic signal of source water, i.e. the transpirational flux is non-fractionating.

significance of the Péclet effect has been recognised several times (e.g. Barbour et al. 2000, Ripullone et al. 2008).

Moreover, the isotopic composition of water extracted from different portions of leaf blade seems to follow a non-random trend: the enrichment progressively increases in both longitudinal (along the leaf midrib) and transversal (perpendicular to the midrib) directions (e.g. Bariac et al. 1994, Wang & Yakir 1995, Gan et al. 2002 & 2003, Šantrůček et al. 2007). It follows that water from the leaf tip is more enriched than water from the leaf base. The same holds for water from the margin of the leaf blade in comparison with water extracted from the middle part of the leaf (avoiding the midrib) at the same longitudinal distance. In addition, this pattern seems to be independent from the leaf size and shape and the organization of vascular network within the leaf. Similar gradient in enrichment was described in hydrology in case of a desert river (Gat & Bowser 1991 referring to work by Fontes & Gonfianitini 1967). As the desert river gradually loses its water due to evaporation, the river is slowly vanishing and the remaining water becomes enriched. Hence, the gradient of increasing enrichment which is alike for river and leaf appears. However, the back diffusion of heavy isotopes can not be neglected in case of leaves because of their smaller size in comparison with the river basin. Therefore, Farquhar and Gan (2003) implemented the longitudinal Péclet effect into the desert river model resulting in the first model which predicts spatial heterogeneity of isotopic composition of leaf water. Recently, even more complex models including the radial Péclet effect has been introduced (Ogée et al. 2007, Cuntz et al. 2007). Nevertheless, these models are restricted only to monocotyledonous leaves with parallel venation and the attempts to understand the mechanisms determining the pattern of isotopic composition of water in dicotyledonous leaves are at the very beginning (Šantrůček et al. 2007).

From what was written above is obvious that predicting the isotopic composition of leaf water is not an easy task. By now the Craig-Gordon equation remains to be the most often used model of leaf water enrichment despite the fact that the predictions are not always accurate. Taking into account that the stable isotope techniques have been more extensively used in geochemistry than in biology, it is not surprising that the knowledge of mechanisms controlling isotopic composition of leaf water is not so comprehensive compared to the precipitation. Approaches combining experimental work with monitoring of natural patterns would be useful for further research in this field. The MIBA data might be really helpful in this regards.

1.4 Carbon isotopes in a plant biomass

It is well-known that plant biomass is always depleted in ^{13}C isotope when compared to the isotopic signal of carbon dioxide which represents the source for plant organic matter. The reasons for that are clear now[5] and can be demonstrated on a model developed for C_3 plants by Farquhar et al. (1982):

$$\Delta^{13}C = a + (b - a) \cdot \frac{c_i}{c_a} \qquad \text{Eq.1.10}$$

where $\Delta^{13}C$ is the predicted discrimination against heavy carbon isotope (δ_{CO_2} - $\delta_{dry\ matter}$), c_i and c_a stand for CO_2 concentration at the sites of carboxylation and in the air, respectively, a refers to fractionation during diffusion in air (4.4‰) and b is the fractionation associated with carboxylation reaction catalyzed by RUBISCO (30‰). Since the fractionations (a and b) are more or less constant, the discrimination is virtually determined by the ratio of CO_2 concentration (or partial pressure) in the leaf to that in ambient atmosphere. It follows that the plant discriminates more when the ratio c_i/c_a is higher (closer to 1).

Under natural conditions, changes in partial pressure of CO_2 at the site of carboxylation (c_i) are usually responsible for changes in the c_i/c_a ratio. Two principal causes leading to the decrease in internal CO_2 concentration can be distinguished. These are i) the limited supply of CO_2 into the leaf and ii) the intensive consumption of CO_2 by the photosynthetic machinery. Many environmental and physiological factors influence the interplay between supply and consumption of CO_2. One can anticipate that the instantaneous discrimination is rather dynamic and variable. It is possible to determine these short-term variations in discrimination using an on-line measurement system during which changes in isotopic composition of CO_2 leaving the gas exchange chamber are monitored. Nevertheless, the information on prevailing growing conditions can be derived from the carbon isotopic composition of plant dry matter. For instance, it has been reported many times that plants experiencing drought stress discriminate less than the well-watered ones due to the fact that the diffusion of CO_2 into the leaf is limited by closed stomata (e.g. Barbour & Farquhar 2000).

[5] However, it took about 40 years to reveal the mechanisms which are responsible for plant biomass being depleted in ^{13}C.

Discrimination of ^{13}C occurs not only during photosynthetic reactions but also in the post-photosynthetic processes. It has been reported that certain isotopic discrimination is associated with assimilate transport, modification and its final incorporation into tissues (Badeck et al. 2005, Jaggi et al. 2002). As a result of post-photosynthetic processes, secondary compounds with different carbon isotopic composition may be generated. It is known that secondary metabolites such as lignin, suberin and lipids are more depleted in ^{13}C than the cellulose (Hobbie & Werner 2004). Therefore, distinct chemical composition of plant material can result in different values of $\delta^{13}C$. Furthermore, respiration is also known to cause changes in $\delta^{13}C$. CO_2 generated by respiratory processes is generally enriched in ^{13}C with a notable exception in root respiration which releases ^{13}C depleted CO_2. A comprehensive review on this issue has been published recently by Bowling et al. (2008).

1.5 Characteristics of sun-exposed and shaded parts of the canopy

Solar radiation represents the primary source of energy for the whole biosphere because it powers the photosynthetic reactions. Leaves of green plants evolved to optimize absorption of incident light[6]. Therefore, availability of photosynthetically active radiation generates differences in biochemistry, physiology and anatomy of leaves growing in different light environments. In turn, it is reasonable to expect that the differences in leaf temperature, gas exchange characteristics and leaf anatomy will be reflected in the isotopic composition of both plant biomass and water. The vertical heterogeneity in irradiance which occurs within the forest canopy provides an ideal opportunity for investigating these acclimations by comparing sun-exposed and shaded leaves of the same tree. Some of the characteristics of sun and shade leaves are described in the following text.

One of the most apparent differences between leaves from contrasting light environments can be found in their anatomy. Sun leaves tend to be thicker (with longer palisade cells often stalked in more layers) whereas shade leaves are usually thin but with larger surface area so that they can effectively absorb the light transmitted through the vegetation (e.g. Gomes-Laranjo et al. 2008).

High values of light saturated rate of CO_2 assimilation and high carboxylation capacity are characteristic for sun leaves, as opposed to shade leaves for which

[6] For the sake of simplicity, the term "light" is used to refer to photosynthetically active radiation (*PhAR*, 400-700nm) in the text.

investments in carbon-assimilating apparatus would not be of much use because they grow in the environment where high photon flux densities of *PhAR* rarely occur. Instead, shade leaves are able to thrive under light conditions close to the light compensation point because of their low dark respiration rate (Lambers et al. 1998). Owing to the intensive photosynthetic uptake of CO_2 by sun leaves, high stomatal conductance is required so that the photosynthesis would not be limited by the substrate. As a result, transpiration rate is usually higher in sun leaves in comparison with shade leaves (eg. Cochard et al. 1999, Nardini et al. 2005). The leaf-internal CO_2 effect on stomatal conductance and transpiration can be enhanced by high absorption of radiation energy and, consequently, increased leaf temperature in sun-exposed leaves. Hydraulic feedback of enhanced transpiration can, in turn, decrease the time-integrated stomatal conductance in sunny leaves. As a result, sunny leaves can suffer from CO_2 defficiency at the carboxylation sites. Thicker and more compacted leaf anatomy in the sunny leaves can also result in higher CO_2 drawdown so the operating CO_2 concentration in chloroplasts may be even lower.

1.6 Working hypotheses and the expected results

Isotopic composition of water in the soil-beech tree system

1/ Bulk leaf water will be the most enriched water compartment in the soil-plant-atmosphere continuum because of evaporation during which heavier isotopes are left behind in the leaf water. Leaf water enrichment is predicted to reach the maximum during summer. This hypothesis is based on the assumptions that *VPd* (vapour pressure difference between leaf and ambient atmosphere), and correspondingly the ratio of water vapour pressure in leaf to water vapour pressure of the ambient air, is expected to be the highest during the whole vegetation season. The importance of this parameter for determining leaf water enrichment is obvious from the leaf-adapted Craig-Gordon model (Eq.1.9).

2/ Sun leaves are expected to become more enriched in heavier water isotopologues than shade leaves. This is due to the fact that *VPd* is expected to be greater in presumably warmer sun leaves.

3/ Stem water should be more depleted than leaf water. Its isotopic composition should generally reflect precipitations and soil water, with the δ-values slightly more negative since the water is predominantly taken up from deep soil layers. Besides, twig water isotopic composition should change minimally in response to actual environmental conditions such as temperature or humidity and correspondingly should not differ between sun and shade samples

4/ The soil samples are taken from the 10 cm depth. We anticipate that the isotopic composition of soil water should be rather variable here. Basically, soil water isotopic signature should arise from isotopic composition of rain water but the influence of evaporation can not be neglected. Therefore, we expected to find the soil water isotopic signal to be similar or slightly enriched than precipitation with the greatest enrichment being found during the drought and warm periods when intensive evaporation occurs.

Carbon isotopes in beech stems and leaves

1/ Leaf dry matter should be more depleted in ^{13}C than twig dry matter. This hypothesis is empirically derived based on results published by other researchers. The reasons for such observations have not been satisfactory explained yet. We hoped to learn more on this issue by investigating potential seasonal changes in the offset between $\delta^{13}C_{leaf}$ and $\delta^{13}C_{stem}$.

2/ Thicker and more compacted leaf anatomy in the sun leaves can result in higher CO_2 drawdown so the operating CO_2 concentration in chloroplasts may be lower than in the shade leaf. Thus, sun-exposed leaves will be more enriched in ^{13}C than their shaded counterparts.

2/Material and methods

2.1 Site description

The study site Brloh (N48°55´ E14°12`) is located in South Bohemia, 20km southwest of České Budějovice, at an elevation of 629 m. The site lies in the center of Blansky Les Nature Reserve at the edge of a deciduous forest (the alliance Luzulo-Fagion). The site experiences a mild temperate climate with annual mean temperature about 7 °C and precipitation of approximately 600mm. The hottest month is usually June while January is the coldest one. Most of the precipitation occurs during the summer months. Courses of monthly mean air temperature and monthly precipitation during years 2005 – 2007 together with long-term normals measured by two field climate stations of the Czech Hydrometeorological Institute (CHMI) situated near the sampling site are shown in Fig. 2.1.

A beech tree (*Fagus sylvatica* L.) growing at the southward exposed edge of forest was chosen for sampling. *Fagus sylvatica* L. represents a dominant species of the forest and would naturally occur at the site according to the map of potential natural vegetation of the Czech Republic (Neuhäuslová et al. 1998). The chosen beech tree was approximately 50 years old and 15 m high. The position at the forest-meadow ecotone and growth form of the tree enabled to sample sun-exposed and shaded leaves and twigs easily from the ground.

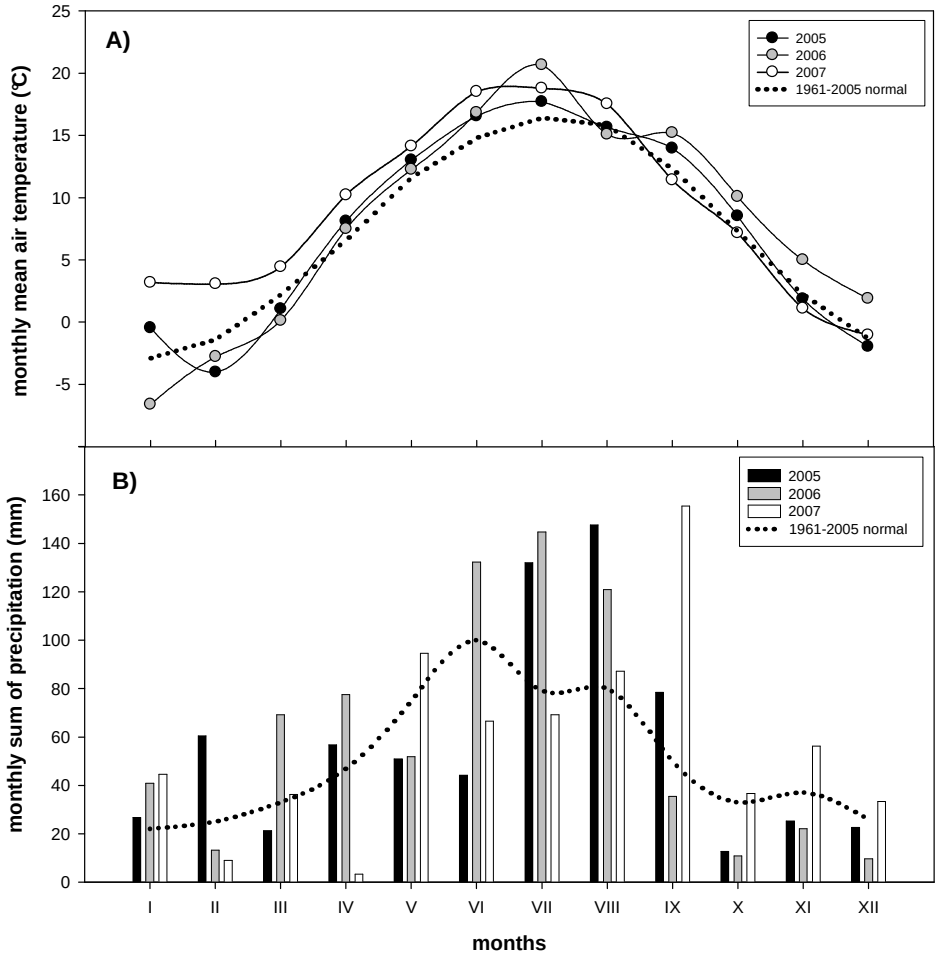


Fig 2.1: Monthly mean air temperature and monthly precipitation. The data courtesy of the CHMI

2.2 Sampling strategy and sample preparation

Field sampling was carried out in approximately two weeks intervals during three subsequent vegetation seasons (2005-2007). Samples of beech stems and leaves as well as soil samples were collected between 11 and 14hour local time. We avoided sampling during rainy days. Small branchlets and adjacent leaves were excised from the tree using a fresh razor blade. Mid veins of the leaves were carefully removed. Three halves of the leaves were then put into a gas tight 12 ml vial (EXETAINER®, Labco, UK). Terminal twigs of less than 0.5 cm in diameter and approximately 8 cm in length were collected into an 'exetainer'. Leaves and twigs were sampled in two replicas from both sun-exposed and shaded parts of the canopy (further referred as sun and shade samples). In addition to those plant samples, two soil samples were taken from 10 cm depth; one from the meadow (in 7 m distance from the tree foot) and the other from the forest floor (2 m from the forest foot). Tightly sealed 'exetainers' containing the

samples were immediately transported to the laboratory and stored in a freezer (at temperature -12 °C) until used for water extraction followed by stable isotope analysis.

sample	treatment	# of samples
soil	meadow	1
	forest	1
stem	sun	2
	shade	2
leaf	sun	2
	shade	2

Table 2.1 Types and number of samples taken during each sampling for water extraction and assessment of isotopic composition of water.

Water was extracted from the samples by cryodistillation using a device principally similar to that described in Šantrůček et al. (2007) slightly modified for larger samples. Open 'exetainers' were placed in duralumin blocks. The openings were quickly covered with 2 ml glass vials. Potential leakiness of the system was avoided using rubber O-rings at glass-metal interfaces. The blocks with 'exetainers' were heated to 90 °C while the bottoms of the vials were cooled down using liquid nitrogen. As a result, the water evaporates from the samples and condensates and consequently turns into ice in the glass vials. The distillation process took 4 days for each sample set (48 samples). On the first, second and third day of the distillation, the heating and cooling had to be ceased overnight and the whole device was turned upside down. This handling was necessary for removing blockage caused by ice in the vial bottleneck. At the end of the third day, the vials were removed and sealed with aluminum seals with PTFE/rubber liner. The cryodistillation device had been tested before used in this study. A piece of cotton saturated with water of known isotopic composition was placed into the 'exetainer'. After the distillation, isotopic composition of water was measured and compared with a control sample which had not undergone distillation. The deviation from the control lies within the measurement uncertainty. Furthermore, recovery of the distillation process was checked for each sample by weighting the 'exetainer' with the sample prior and after the distillation and after a control drying in an oven (80 °C, 3 hours). Extracted water was immediately measured for oxygen and hydrogen isotopic composition or stored in a freezer until the mass spectrometric analysis.

The dry plant material remaining after the water extraction was used for preparation of solid samples for carbon isotope analysis. Properly dried leaves and twigs were grained into a fine powder with a mixer mill (MM200, Retsch, Haan, Germany).

0.7 – 1 mg of the powder was packed into tin capsules which were fed into a mass spectrometer.

2.3 Stable isotope analysis

Stable isotope composition of water and plant dry mass was assessed with an isotope ratio mass spectrometer, IRMS, (DeltaPlus XL, ThermoFinnigan, Bremen, Germany). For water samples, the IRMS was coupled to a high-temperature conversion elemental analyser (TC/EA ThermoFinnigan, Bremen, Germany). A 1µl volume of the individual water sample was injected into the helium carrier stream and pyrolized at 1400°C on a carbon-filled column. CO and H_2 gases produced by pyrolysis were chromatographically separated and consequently ionized. When solid samples were measured, the IRMS interfaced with another elemental analyzer (NC 2100 Soil Analyzer, ThermoQuest Italia S.p.A., Rodano, Italy) where tin capsules containing 0.7-1 mg of powdered sample were combusted at temperature up to 1600 °C. Gases generated during combustion passed through a set of three columns at which end CO_2 molecules entered an ion source and ionized. Accelerated ions entered magnetic field where their trajectories bent according to their weight and charge. The detection was provided by Faradys' collectors.

The $^{18}O/^{16}O$, $^{2}H/^{1}H$ and $^{13}C/^{12}C$ ratios of sample were compared with that in a working standard. This standard was calibrated against the Vienna Standard Mean Ocean Water (V-SMOW) in case of oxygen and hydrogen or against the Vienna Pee-Dee belemnite (VPDB) standard in case of carbon. Each water sample was measured twice and the second value was taken as a true measure of isotopic composition. Despite flushing the sample needle carefully, the first measurement is often influenced by the previous sample (especially when it has very different isotopic composition). Therefore, we used the second measurement to reduce this "memory effect".

Isotopic composition of samples was expressed as $\delta^{18}O$, $\delta^{2}H$ and $\delta^{13}C$

$$\delta^{18}O\left(^{2}H,\ ^{13}C\right)[‰] = \left(\frac{R_{sample}}{R_{s\,\mathrm{tan}\,dard}} - 1\right)\cdot 1000 \qquad \text{Eq.2.1}$$

where R is the $^{18}O/^{16}O$ ($^{2}H/^{1}H$, $^{13}C/^{12}C$) ratio. Leaf water isotope enrichment (Δ_{leaf}) was computed according to

$$\Delta_{leaf}\,{}^{18}O({}^{2}H)[‰] = \left(\frac{\delta_{leaf} - \delta_{stem}}{\delta_{stem} + 1000}\right) \cdot 1000 \qquad \text{Eq2.2}$$

i.e. considering stem water as source water. Mean values of δ_{leaf} and δ_{stem} (n=2) were used to calculate Δ_{leaf}.

2.4 Measurements of chosen environmental characteristics

Environmental conditions were monitored in detail during 2007 sampling season. While collecting the samples, air temperature (t_{air}), air humidity (*RH*) and amount of photosynthetically active radiation (*PhAR*) was measured using portable meteorological dataloggers (Minikin TH, Minikin QT, EMS, Brno). While sampling, the dataloggers were placed first on the open meadow and then in the forest understory (under the beech tree). The measurements took at least 30 min at each position, so that the sensors equilibrated properly with the surrounding environment (important for t_{air} and *RH* readings). The values from the end of the measurements (approximately last 10 minutes) were then averaged. In addition to these measurements, temperature of sun-exposed and shaded leaves (t_{leaf}) was determined. At least three leaves of each type were measured for leaf temperature using a datalogger with external remote temperature sensor (Minikin TV, EMS, Brno) and the average value was computed.

From the data acquired as described above, leaf-to-air vapour pressure difference (*VPd*) was computed according to equation

$$VPd = (e_i - e_a) \qquad \text{Eq.2.3}$$

where, e_i is saturated water vapour pressure in leaf internal space, computed for the given leaf temperature (t_{leaf}, °C) as

$$e_i = 0.6108 \cdot \exp^{\frac{17.27 \cdot t_{leaf}}{t_{leaf} + 237.3}} \qquad \text{Eq.2.4}$$

e_a refers to partial water vapour pressure of the air surrounding the leaf and its value is given by the equation

$$e_a = \left(0.6108 \cdot \exp^{\frac{17.27 \cdot t_{air}}{t_{air}+237.3}} \right) \frac{RH}{100} \qquad \text{Eq.2.5}$$

where *RH* (%) stands for relative humidity and t_{air} (°C) is air temperature. Both e_a and e_i are in kPa.

In general, e_a should be lower than e_i. However, we found an opposite pattern (i.e. $e_a > e_i$) on 6th June which would mean that the water from air will tend to diffuse into the leaf internal air space. We suppose that the sensor which measured t_{air} and *RH* had not equilibrated entirely with the environment which led to this false result. The t_{air} would have probably decreased a bit more if the sensor was left longer at the site and consequently e_a would decrease. In turn, the e_a/e_i ratio would have been lower than 1. Despite being aware of this inaccuracy, we used this suspicious e_a value (and correspondingly $e_a/e_i > 1$ or negative *VPd*) in further analyses presuming that the e_a/e_i would be smaller but still close to unity. We believe that this case was a rare exception. In the rest of observations, the values obtained from the sensors were stable at the end of the measuring period.

Table 2.2 Environmental and physiological variables measured at the sampling site while collecting the samples for isotope analysis. Duration of the measurements, number and types of samples taken or measured are indicated.

variable	treatment	duration
t_{air} (℃)	meadow	>30min
	forest	>30min
RH (%)	meadow	>30min
	forest	>30min
PhAR (μmol m^{-2} s^{-1})	meadow	>30min
	forest	>30min

The symbols stands for t_{air}: air temperature; *RH*: relative humidity; *PhAR*: amount of photosyntetically active radiation; t_{leaf}: leaf temperature; Ψ_{stem}: stem water potential

variable	treatment	# of samples
t_{leaf} (℃)	sun	3
	shade	3
Ψ_{stem} (bar)	sun	5
	shade	5
fresh weight (g)	sun	5
	shade	5
dry weight (g)	sun	5
	shade	5
leaf area (m^2)	sun	5
	shade	5

2.5 Stem water potential, water content

Five sun-exposed and five shaded twigs were taken during each sampling for stem water potential measurements and assessment of water content. Twigs were excised from the tree and put separately into plastic bags. All the bags were then closed into a glass jar.

This procedure should ensure minimal water loss during transportation to the laboratory. In the laboratory, stem water potential (Ψ_{stem}) was measured with Scholander pressure bomb (Model 3000, SoilMoisture Equipment Corp., Santa Barbara). Immediately after removing the twig from the pressure bomb, leaves were cut off from the stem and weighted with an analytical balance (KERN 770, Germany). The weight of stems including petioles was measured subsequently. Leaf area of a leaf set excised from each individual branchlet was determined using a scanner equipped with image analysis software ImageJ (available at: http://rsb.info.nih.gov/ij/). After that, plant material was dried (80 °C, >48 hours) and weighted again. Water content per unit of dry mass (W_{leaf}, W_{stem}) and the specific leaf area (SLA = leaf area/leaf dry weight) were computed.

2.6 Data analysis

The sampling was designed as a long term project. Thus, the possibility to collect replicated samples for time- and money-demanding isotopic analyses was limited. Two repeated measurements for stem and leaf and one single measurement for soil samples enable relatively weak statistical treatment in some cases. The probability of both type I error and type II error is high especially when comparing the treatments (sun versus shade, meadow versus forest)) from a single sampling. However, the differences between sample types and the seasonal variability can be satisfactory analyzed.

Water isotope data showed significant seasonal variability, thus, measurements conducted during one sampling day for sun and shade treatment were regarded as a dependant observation. Differences between the contrasting light treatments were tested for significance using Student's t-test for dependant samples (paired Student's t-test). Relationship between different quantities was modeled by the least-squares linear regression. Multiple linear regression was employed to analyze leaf water isotopic composition.

Differences in $\delta^{13}C$ were tested for significance using factorial ANOVA with treatment (sun and shade) and sample type (leaf and stem) being the categorical fixed-effect factors.

Statistical analysis was carried out using the statistical package STATISTICA (version 6.0, StatSoft, USA). Figures were plotted using graphing software SigmaPlot (version 9.0, Systat Software Inc, USA).

3/ Results

3.1 Environmental conditions

Courses of monthly rainfall and monthly mean air temperature measured near the Brloh sampling site during seasons 2005-2007 together with long-term normals are shown in Fig. 2.1. The total annual precipitation was similar for all the seasons (652, 688, 648 mm for 2005, 2006 and 2007, respectively), while their seasonal distribution was rather variable between the years. The mean annual temperature was 7.5, 7.9 and 9.0 °C in 2005, 2006 and 2007, respectively. There was a relatively warm winter 2006/2007 with average temperature over the 4-months period (Nov-Feb) being 3.3°C in comparison with the same period in 2005/2006 when the mean temperature reached -2.4°C only. Relatively warm and drought conditions prevailed in June and July 2007. On the contrary, extreme precipitation occurred in the first half of September 2007. At that time, 123 mm of water rained out in only 11days (and nearly 80% of the rain fell down in 2 subsequent days). Air temperature (t_{air}), air relative humidity (*RH*), amount of photosynthetically active radiation (*PhAR*) and leaf temperature (t_{leaf}) measured for each treatment during the sampling in year 2007 are depicted in Fig. 3.1. The overall mean values ± standard deviations (n=13) are shown in the table together with significance level (p-value) of the Student's t-test for dependent samples which was used to test the difference between the treatments (Table 3.1). Shade leaves were nearly always cooler then the air, while sun leaves were sometimes heated up above the air temperature. This usually happened when *PhAR* was high. The ratio of water vapour pressure in the air to water vapour pressure in the leaf air space (e_a/e_i) did not significantly differed between the treatments despite the fact that *VPd* ($VPd=e_i-e_a$) differed.

Table 3.1: Means ± standard deviations (n=13) of chosen environmental characteristics measured during the sampling. The differences between meadow and forest (sun and shade) were tested with paired Student's t-test. The data from year 2007 only.

	meadow	forest	*p*		sun	shade	*p*
t_{air} (℃)	21.8 ± 6.0	20.3 ± 5.9	<0.01	t_{leaf} (℃)	22.8 ± 9.3	18.5 ± 5.4	<0.05
RH_{air} (%)	51.8 ± 17.2	54.2 ± 17.2	*NS*	e_i (kPa)	3.17 ± 2.0	2.24 ± 0.9	<0.05
e_a (kPa)	1.36 ± 0.4	1.28 ± 0.4	<0.05	*VPd* (kPa)	1.84 ± 1.9	0.96 ± 0.8	<0.05
PhAR (μmol m^{-2} s^{-1})	1047.8 ± 589.3	28.9 ± 20.4	$<10^{-4}$	e_a/e_i	0.53 ± 0.3	0.61 ± 0.2	*NS*

The symbols stands for t_{air}: air temperature; *RH*: relative humidity; e_a: partial water vapour pressure in the air; *PhAR*: amount of photosyntetically active radiation; t_{leaf} : leaf temperature; e_i: saturated water vapour pressure in the leaf interior; *VPd*: vapour pressure difference; p: significance level, NS for p≥0.05

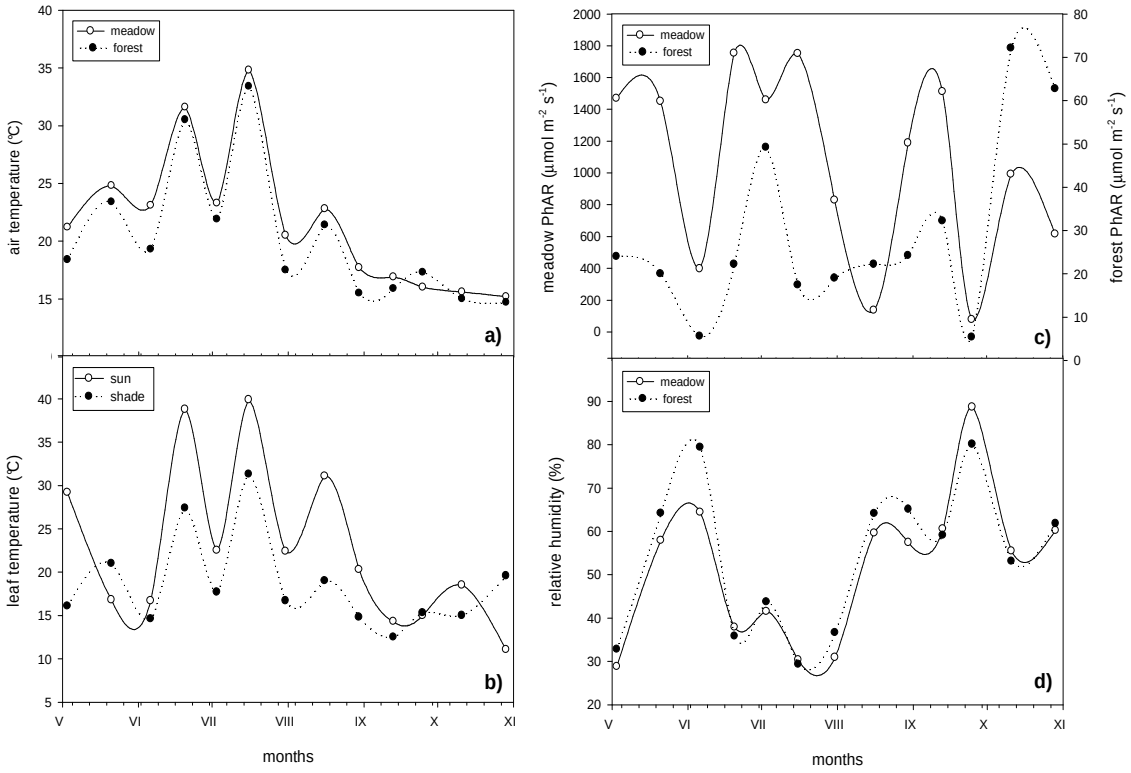


Fig. 3.1: The variation of chosen environmental characteristics measured while collecting the samples for stable isotope analysis. The lines connecting the points are depicted for the sake of clarity and do not account for real course of the variables between the two measurements. The data from year 2007 only.

3.2 Water isotope composition

Means, ranges and other descriptive statistics of $\delta^{18}O$ and $\delta^{2}H$ of water extracted from all the sample types (soil, stem and leaf) are presented in Table 3.2. Not surprisingly, leaf water was the most enriched water compartment with δ ranging from -3.0‰ to 21.2‰ and from -40.1‰ to -2.3‰ for ^{18}O and ^{2}H, respectively. Soil water and water extracted from twigs showed similar isotopic signature in oxygen (from -15.8‰ to -7.2‰) but strikingly differed in hydrogen, with soil water being about 15‰ more enriched than twig water. The span of δ-values was generally wider for $\delta^{2}H$ than for $\delta^{18}O$. Note, that leaves showed the widest range from all the sample types in oxygen while the same was true for twigs when hydrogen data were compared. This difference in ranges arose as an effect of evaporation. The δ-values of soil and twig water were usually less enriched than those of May-to-Oct precipitation, computed with the On-line Isotopes in Precipitation Calculator (available at http://www.waterisotopes.org/) and shown in Table 3.3, but corresponded well with isotopic signal expected for winter and early spring precipitation. Hence, the isotopic

signal of soil and stem water seems to be rather independent from current precipitation. The isotopic composition of soil and stem water was close to that of precipitation only at the end of growing season (in October) and in rare cases when heavy rain occurred a few days prior the sampling. The $\delta^{18}O$ versus $\delta^{2}H$ plot showed that the twig water data spread along the local meteoric water line whereas leaf water data can be approximated by the local evaporation line. These results can be anticipated based on the theory. In contrast, the soil water data lied in an unexpected deuterium enriched region of the plot (Fig. 3.2).

Table 3.2: Descriptive statistics for $\delta^{18}O$ and $\delta^{2}H$ values of water extracted from different types of samples. Data from all three sampling period were used.

	sample	treatment	n	mean	SD	median	minimum	maximum	range
$\delta^{18}O$ (‰)	soil	meadow	30	-10.0	1.9	-9.6	-15.7	-7.3	8.5
		forest	31	-10.3	1.6	-10.5	-15.8	-7.2	8.6
	stem	sun	62	-10.5	1.3	-10.5	-13.9	-8.2	5.6
		shade	57	-10.1	1.3	-10.0	-12.9	-7.4	5.6
	leaf	sun	62	8.4	5.2	8.2	-1.7	21.2	22.9
		shade	59	7.2	4.3	7.6	-3.0	16.7	19.6
	sample	***treatment***	***n***	***mean***	***SD***	***median***	***minimum***	***maximum***	***range***
$\delta^{2}H$ (‰)	soil	meadow	30	-58.6	10.6	-56.5	-88.5	-41.5	47.1
		forest	31	-59.5	9.2	-58.1	-93.4	-44.4	49.0
	stem	sun	62	-76.0	10.0	-73.7	-99.8	-59.3	40.5
		shade	57	-74.0	10.5	-72.2	-97.3	-56.0	41.2
	leaf	sun	62	-17.9	8.6	-16.5	-39.4	-1.5	37.9
		shade	58	-16.2	8.8	-13.2	-40.1	-2.3	37.8

The symbols stand for *n*: number of observations; *SD*: standard deviation; *range*: maximum-minimum

Table 3.3: Isotopic composition of precipitation calculated for the Brloh sampling site (N48° 55`, E14° 12`, 629 m a.s.l.) using the On-line Isotopes in Precipitation Calculator (available at http://www.waterisotopes.org/).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
$\delta^{2}H$ (‰)	-86	-84	-74	-67	-53	-50	-42	-43	-52	-60	-76	-86
$\delta^{18}O$ (‰)	-12	-11.9	-10.4	-9.6	-7.9	-7.6	-6.5	-6.7	-7.8	-9	-11.4	-12

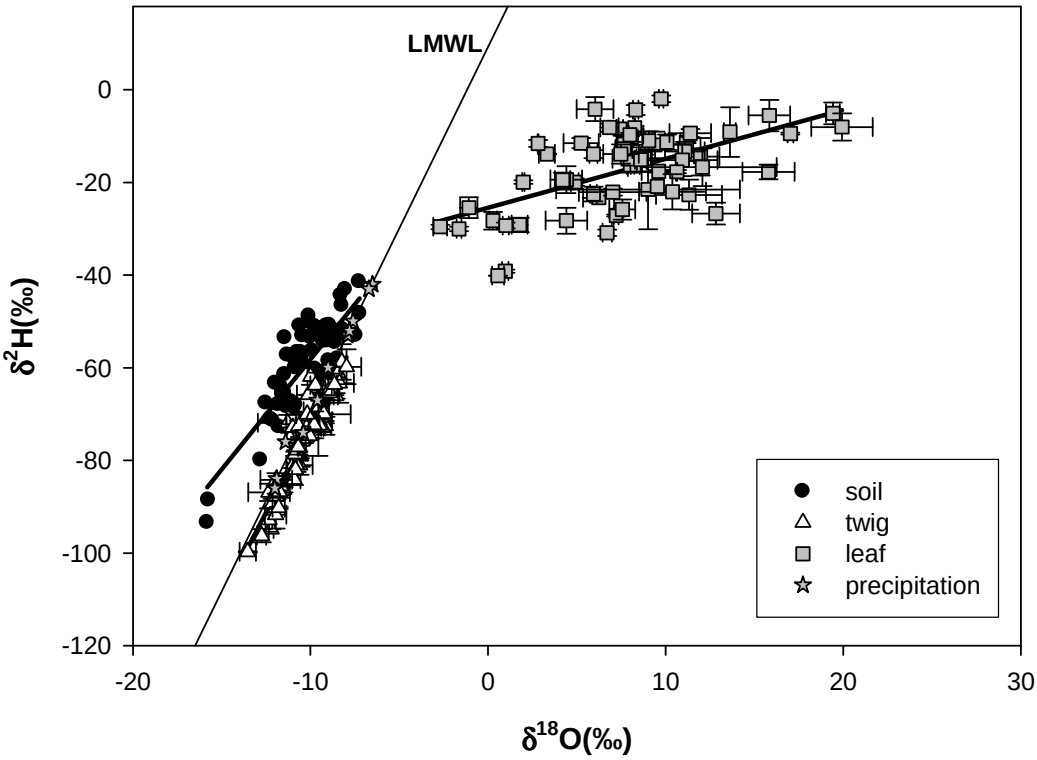


Fig. 3.2: δ^2H versus $\delta^{18}O$ in water from different soil-plant-atmosphere compartments. Data from Brloh sampling site and 2005-2007 seasons are shown.

The general pattern discussed above (i.e. the most enriched leaf water, data range...) seems to be similar for all the seasons observed (Fig. 3.3 to Fig. 3.7). However, each year had its distinct features based on specific environmental conditions. For instance, the δ-values of twig water were slightly higher in 2007 than in the rest two years. When the data from all the years were plotted together seasonal trends in $\delta^{18}O$ and δ^2H of all the water compartments became more obvious (Fig. 3.8). The isotopic signature of leaf water tended to decrease toward less enriched values (Fig. 3.8c & d) whereas the isotopic signals of both soil water and twig water gradually increased toward autumn (Fig.3.8 a & b). The decrease in leaf water isotopes was steeper when Δ_{leaf}, rather than δ_{leaf}, was plotted (Fig. 3.8d). However, these were only general tendencies and the data fluctuated significantly according to the changing environmental conditions at the site. More specifically, the local maxima of twig water isotopic signature seemed to reflect recent heavy rain. The soil water isotopic signal tended to respond to rain in the same manner as twig water although the peaks corresponding to rain were not so obvious in soil samples (especially those collected from the forest).

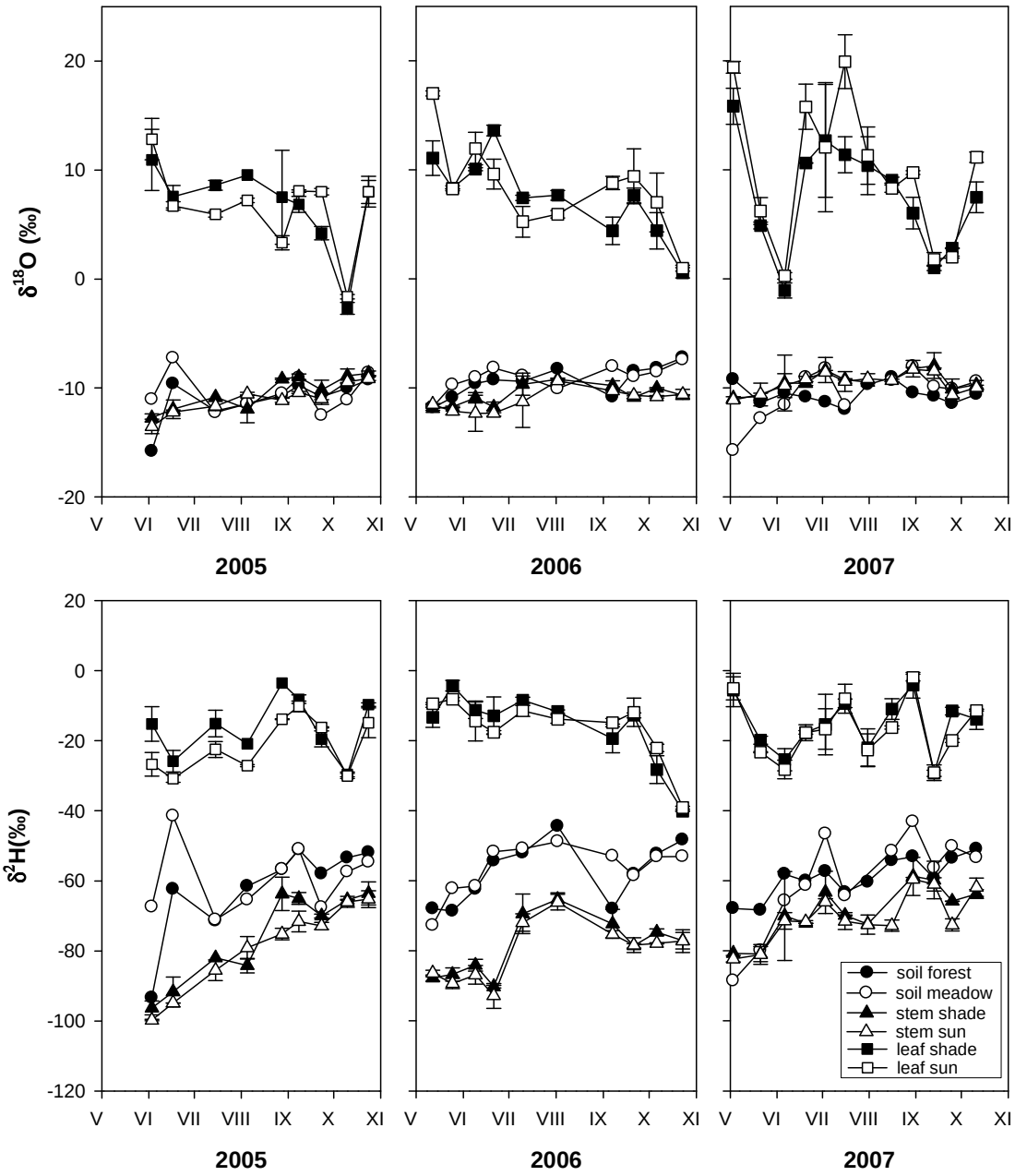


Fig. 3.3: Seasonal courses of $\delta^{18}O$ and $\delta^{2}H$ of water extracted from soil, stems, and leaves. Points and error bars represent mean ± range (*n*=2) in case of stems and leaves or one single measurement for soil samples (dots without error bars).

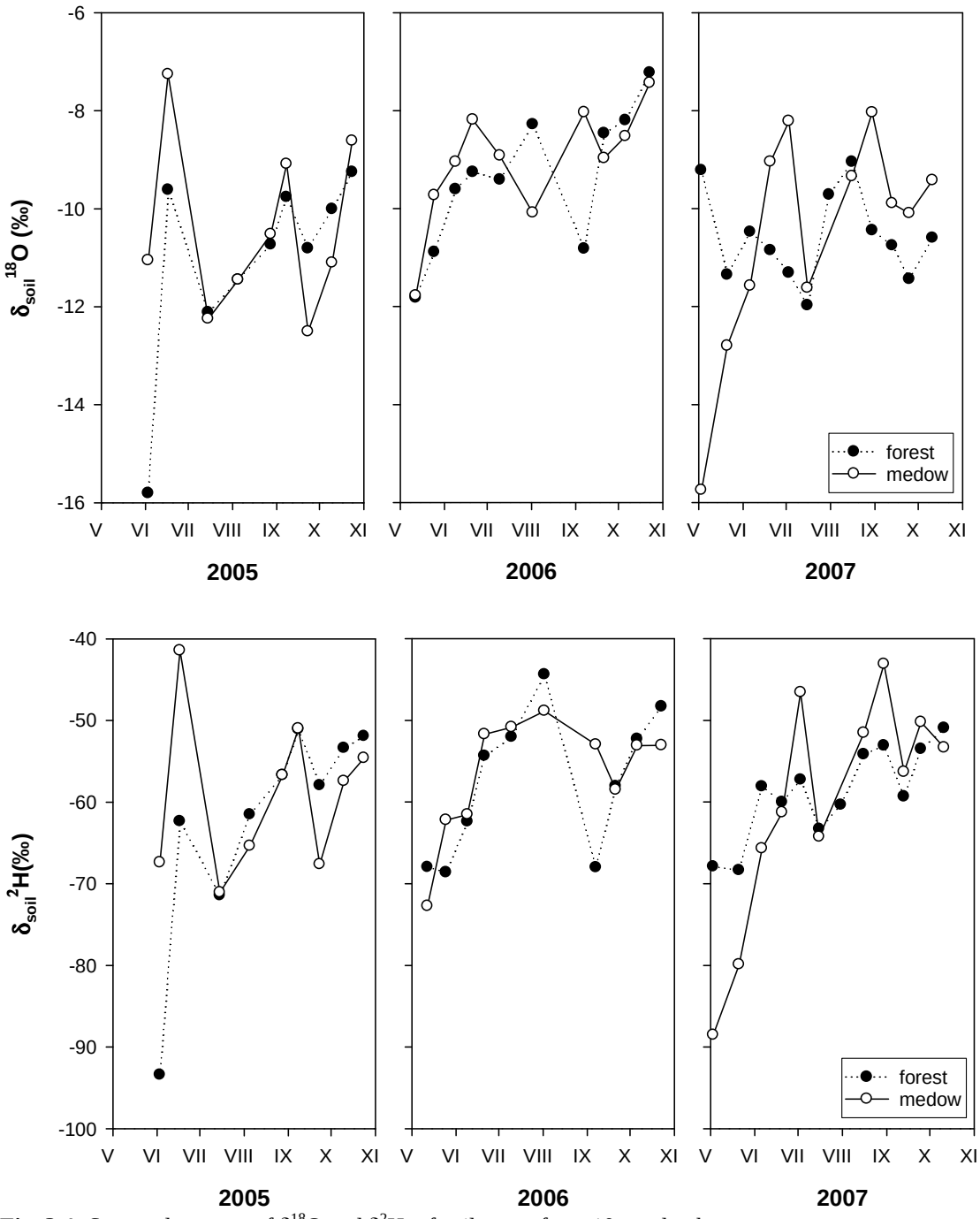


Fig. 3.4: Seasonal courses of $\delta^{18}O$ and $\delta^{2}H$ of soil water from 10 cm depth.

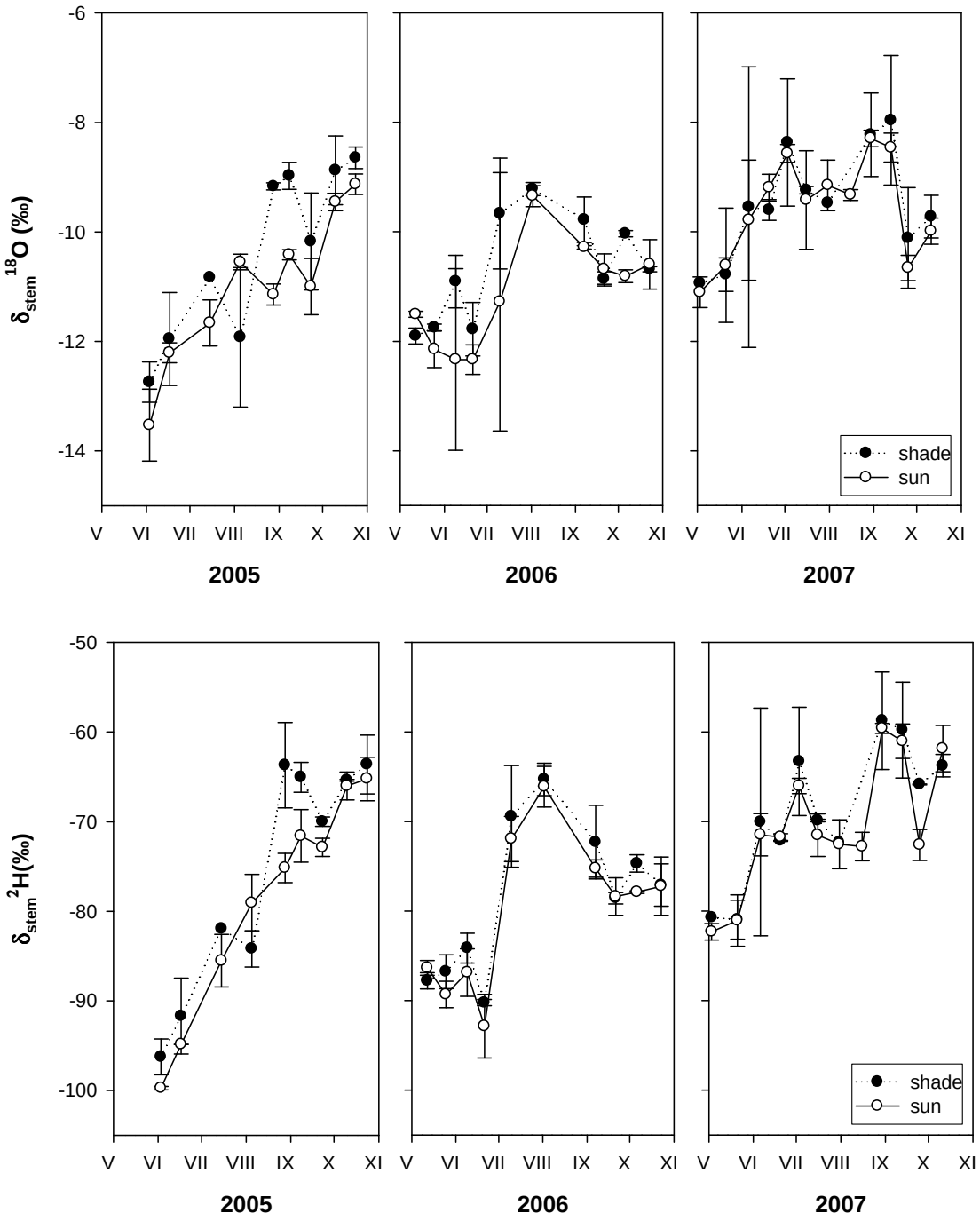


Fig. 3.5: Seasonal courses of $\delta^{18}O$ and $\delta^{2}H$ of stem water. Points and error bars represent mean ± range (*n*=2).

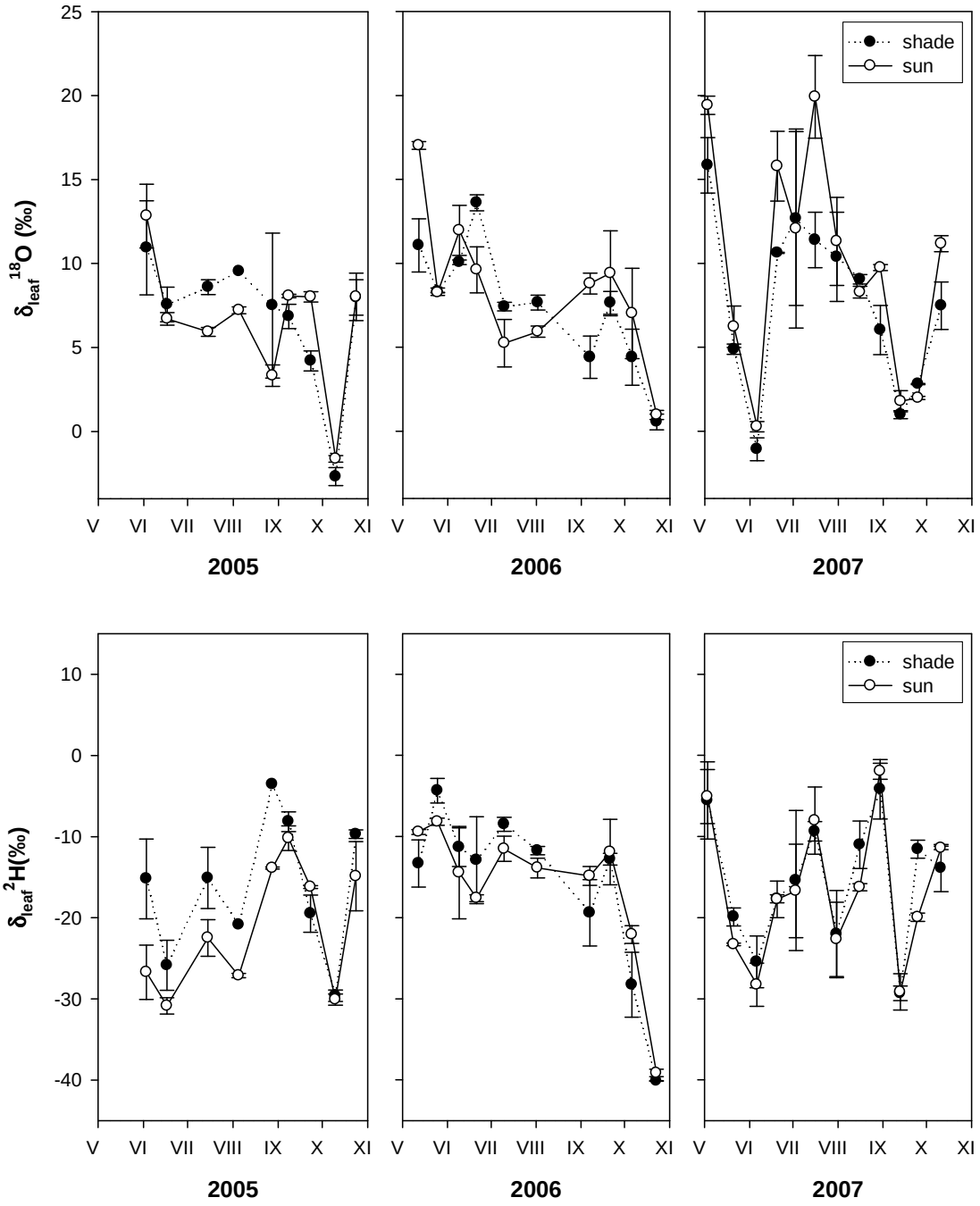


Fig 3.6: Seasonal courses of $\delta^{18}O$ and $\delta^{2}H$ of stem water. Points and error bars represent mean ± range (*n*=2).

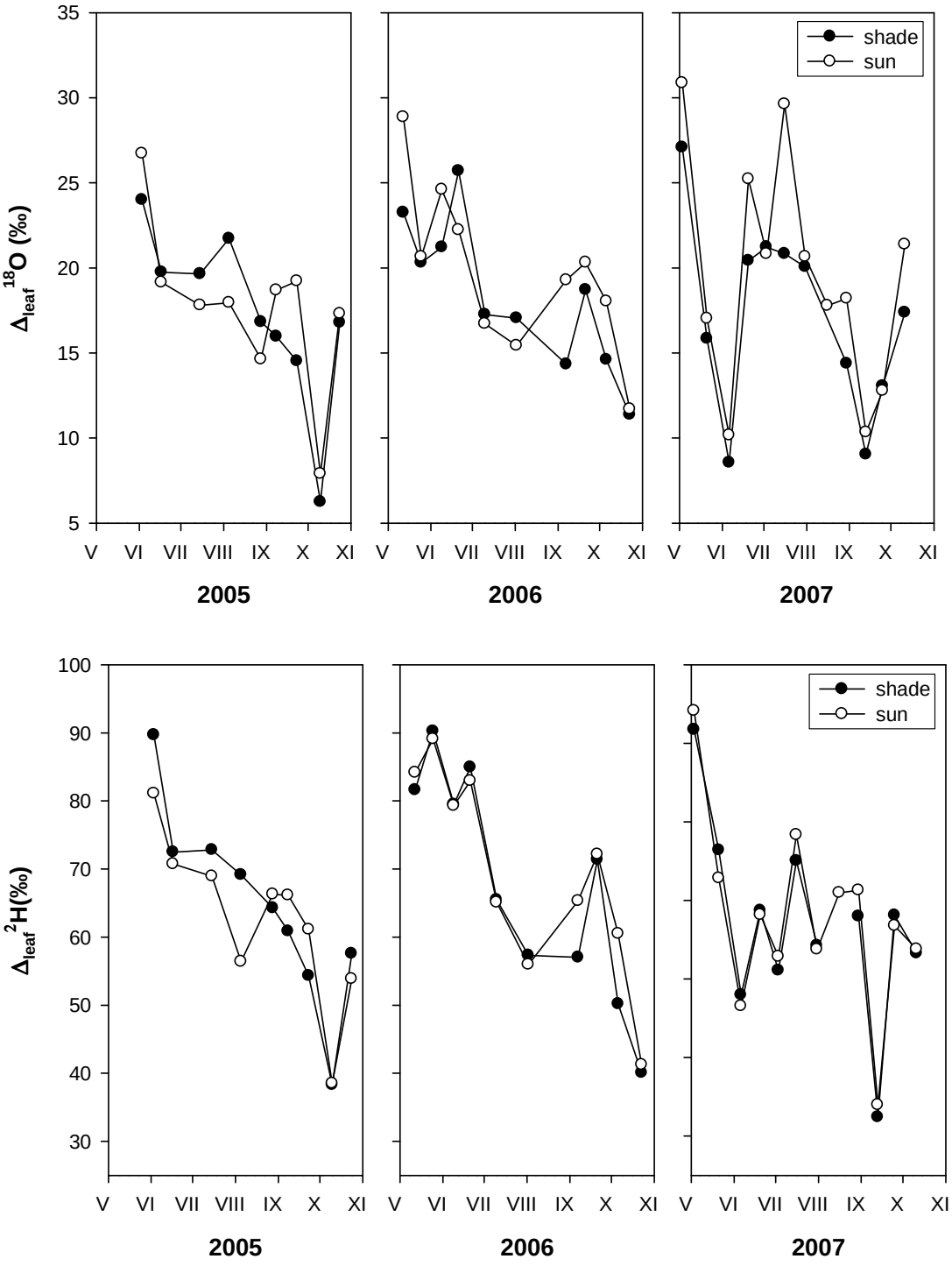


Fig. 3.7: Seasonal courses of leaf water enrichment ($\Delta^{18}O$ and $\Delta^{2}H$). The points represent the value computed according to Eq.2.2 using means of δ_{stem} and δ_{leaf}.

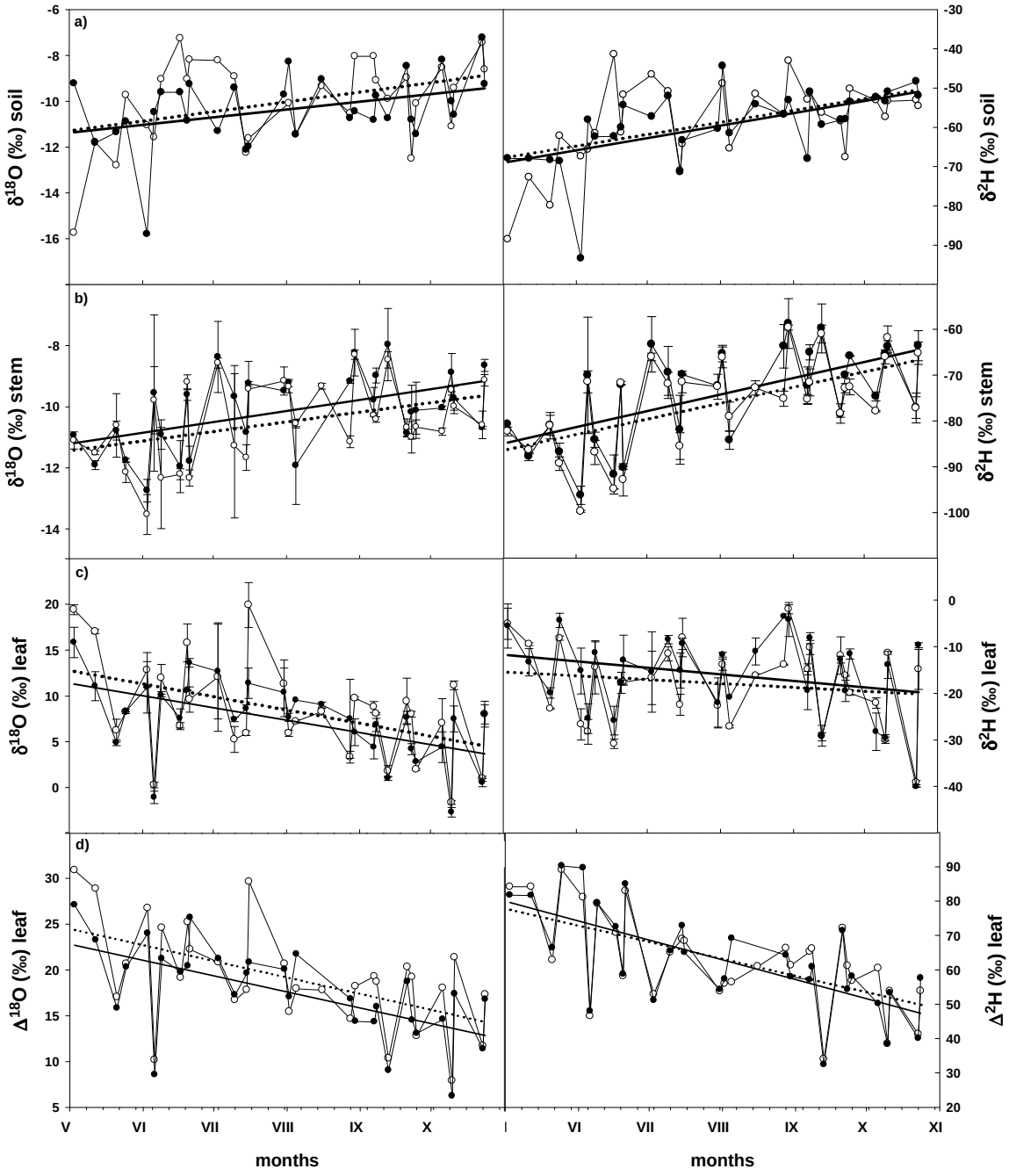


Fig. 3.8: Soil water (**a**) and twig water (**b**) show increase in $\delta^{18}O$ and $\delta^{2}H$ whereas leaf water (**c,d**) tends to become less enriched toward the end of vegetation season. The data from all three years (2005-2007) are plotted together. The open symbols and dotted narrow lines represent sun (or meadow) samples, the closed symbols and solid narrow lines are for shade (or forest) samples. The points and error bars shows mean ± range (n=2) in case of stems and leaves and one single measurement in soil. The narrow lines represent least-squares linear regressions to the data.

Our examination of leaf water enrichment above source (stem) water (Δ_{leaf}), was theoretically based on the Craig-Gordon formula (Eq. 1.9). In case of oxygen, nearly 77% of variability in ^{18}O enrichment was explained by the ratio of water vapour pressure in the ambient air to that in the leaf interior (e_a/e_i) (Fig. 3.9a).The regression was much worse, but still significant, when hydrogen data were analyzed (Fig. 3.9b). A multiple linear regression model using e_a/e_i and δ_{stem} as predictors explained 81% and 55% of variability of Δ_{leaf} for oxygen and hydrogen data, respectively (Table 3.4). The results indicates that δ_{stem} is much better predictor of Δ_{leaf}(^2H) than the e_a/e_i ratio (see standardized regression coefficients, β, in Table 3.4). Third possible predictor would be the isotopic composition of atmospheric water vapour, δ_{vapour}. Unfortunately, δ_{vapour} was not measured directly at the site. Measurements done on samples collected from the rooftop of the university building in České Budějovice (20 km far of the Brloh sampling site) during the Jun-Nov 2006 period gave δ_{vapour} ^{18}O(^2H) of 19.5 ± 2.7‰ (129.4 ± 22.5‰, n=6) and did not show any consistent seasonal trend. δ_{vapour} was computed from measured leaf water enrichment according to Eq.A1 & 2, see Appendix 3, (n=19, 4 deviating values were excluded) gave slightly lower values (-11.3 ± 3.9‰ and -123.3 ± 19.3‰ for oxygen and hydrogen, respectively). The sensitivity analysis (Appendix 3) performed for relevant data range showed that high values of δ_{vapour} could also suppress the effect of e_a/e_i and that this happens in oxygen to much lower extent.

Table 3.4: Results of multiple regression analysis for Δ_{leaf} ^{18}O and ^2H as a dependent variable and e_a/e_i and δ_{stem} ^{18}O(^2H) as predictors.

	regression equation	R^2	p	β	
^{18}O	Δ_{leaf} (‰)= 16.2 - 21 e_a/e_i - 1.5δ_{stem}	0.81	$<10^{-4}$	e_a/e_i	-0.86
				δ_{stem}(‰)	-0.22
^2H	Δ_{leaf} (‰) = -0.1 – 15 e_a/e_i -1δ_{stem}	0.55	0.001	e_a/e_i	-0.33
				δ_{stem}(‰)	-0.58

The symbols stand for Δ_{leaf}: leaf water enrichment above stem water; δ_{stem}: isotopic composition of stem water; R^2: regression coefficient of multiple linear regression, p: level of significance for H_0 that the variables are independent; β: standardized regression beta coefficient (for data standardized to a mean of 0 and a standard deviation of 1); e_a: partial air water vapour pressure (kPa); e_i: saturated water vapour pressure at leaf temperature.

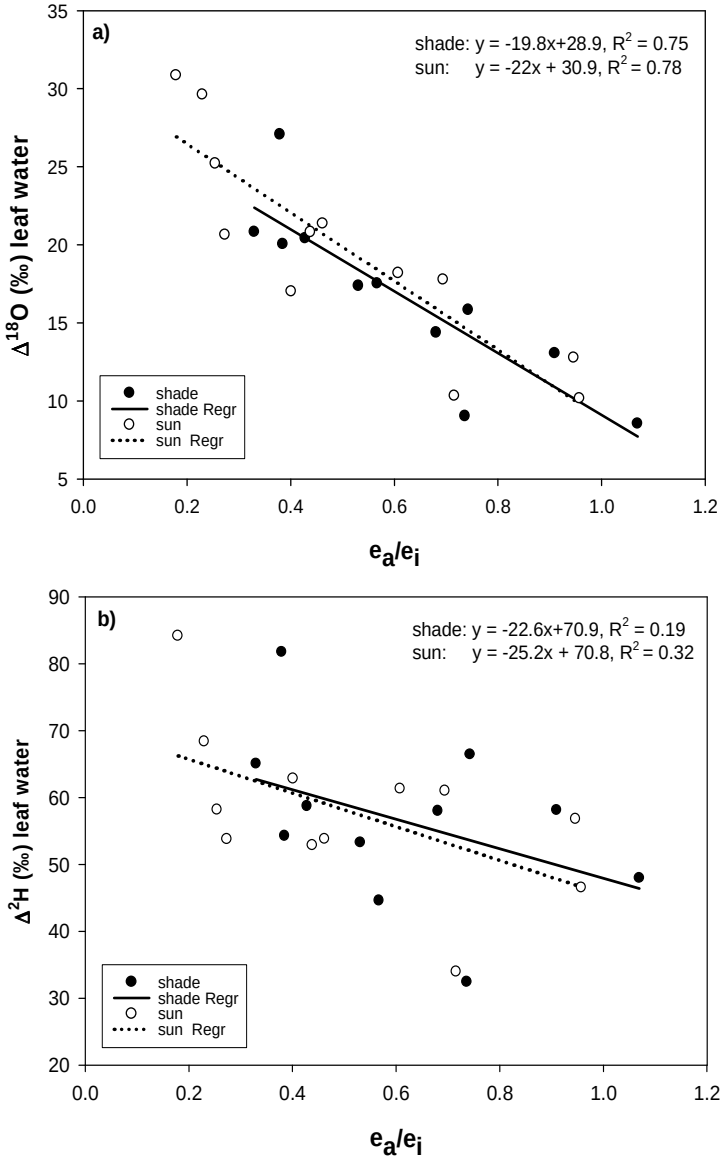


Fig. 3.9: Relationship between the ratio of water vapour pressure in the ambient air to water vapour pressure in the leaf interior (e_a/e_i) and leaf water enrichment ($\Delta_{leaf}{}^{18}O$, $\Delta_{leaf}{}^{2}H$). The lines represent least-squares linear regressions (their equations and regression coefficients are indicated in the figure, all were significant at p=0.05). Data from year 2007 only.

The Craig-Gordon equation can be expressed also in δ-notation, so we performed the regression analyses using δ_{leaf} instead of Δ_{leaf}. Again, a strong relationship between e_a/e_i and δ_{leaf} was found in case of oxygen (y= -20.4x – 22.3, R^2=0.78, $p<10^{-4}$, *df*=23). The regression was not significant for hydrogen (p<0.05, df=22). δ_{leaf} and δ_{stem} were not correlated for neither oxygen nor hydrogen.

Further, we tested weather the e_a/e_i ratio can be approximated by relative humidity (*RH*, expressed on a scale 0 to 1). Generally, e_a/e_i should equal *RH* when t_{leaf} is the same as t_{air}. When values form sun and shade environment were analyzed together,

the two variables were correlated (R^2=0.84, $p<10^{-4}$, *df*=23) and the slope differed form unit (slope = 0.63). The correlation was stronger and a bit closer to the expected 1:1 relationship when e_a/e_i and *RH* from shade were used apart (R^2=0.93, $p<10^{-4}$, slope = 0.75, *df*=11). e_a/e_i differed from *RH* more when the values were higher (i.e. closer to 1), with *RH* being lower than e_a/e_i. Correspondingly to those results, *RH* used as a predictor of $\Delta_{leaf}(^{18}O)$ explained significant part of the variability (R^2=0.66, $p<10^{-4}$, *df*=22). In addition, its explanatory strength was higher for shade leaves (R^2=0.71, p<0.01, *df*=10). Nevertheless, *RH* was worse predictor then e_a/e_i. For $\Delta_{leaf}(^2H)$ the regression was not significant ($p\geq0.05$, *df*=22).

The difference in isotopic composition of water extracted from sun and shade samples was tested using paired Student's t-test. The means were compared when two sample replicas were available (i.e. in case of δ_{stem} and δ_{leaf}). The differences between the treatments were significant for δ_{stem} and δ_{leaf} in both oxygen and hydrogen. Δ_{leaf} was significantly different in hydrogen only (Table 3.5). Despite the statistical significance, we doubt the practical significance. As apparent from the figures (Fig. 3.3 to 3.7), the differences between light treatments were neither large nor consistent within and between the seasons.

Table 3.5: Results of paired Student's t-test testing differences in isotopic composition between sun (or meadow) and shade (or forest) samples. *NS* for $p\geq0.05$

	treatment	***n***	***^{18}O (‰) (mean ± SD)***	***p***	***^{2}H (‰) (mean ± SD)***	***p***
δ_{soil}	meadow	30	-10.0 ± 1.9	*NS*	-58.6 ± 10.6	*NS*
	forest		-10.3 ± 1.6		-59.5 ± 9.2	
δ_{twig}	sun	30	-10.5 ± 1.3	<0.01	-76.0 ± 10.0	<0.01
	shade		-10.1 ± 1.3		-74.0 ± 10.5	
δ_{leaf}	sun	31	8.4 ± 5.2	<0.05	-17.9 ± 8.6	<0.05
	shade		7.2 ± 4.3		-16.2 ± 8.8	
Δ_{leaf}	sun	30	19.1 ± 5.6	<0.01	63.0 ± 13.8	*NS*
	shade		17.6 ± 4.9		62.8 ± 14.7	

3.3 Carbon isotope composition

Carbon isotope composition of dry mass differed significantly between both the sample types and treatments ($p<10^{-4}$). Moreover, the differences were in the opposite direction when comparing the δ-values of leaves and twigs between the treatments (as specified below), i.e. the interaction was significant ($p<10^{-4}$). Shade leaves and twigs contained less ^{13}C (i.e. more discriminated the heavier carbon) in comparison with samples collected from sun exposed parts of the canopy. $\delta^{13}C$ values measured in sun

samples (leaves and twigs together) were -27.3 ± 0.9‰ (*n* = 40) on average, while shade samples were more depleted, averaging -30.1 ± 0.8‰ (*n* = 40). Interestingly, twigs were more enriched in ^{13}C than leaves, with the mean difference 1.3‰, in sun samples while the opposite was true for shade samples (with the difference being -1.2‰). The latter pattern, i.e. leaves being less depleted than other plant tissues, has been rarely reported by other researchers. The carbon isotopic composition of dry matter seemed to be relatively stable over the vegetation season, with increased variability in shade samples found especially at the beginning of vegetation season (May and first half of June) (Fig. 3.10) when the $\delta^{13}C$ was slightly higher than for the rest of the season. In addition, slow gradual increase in $\delta^{13}C$ of shade leaves in June and July is noteworthy since it probably indicates water stress.

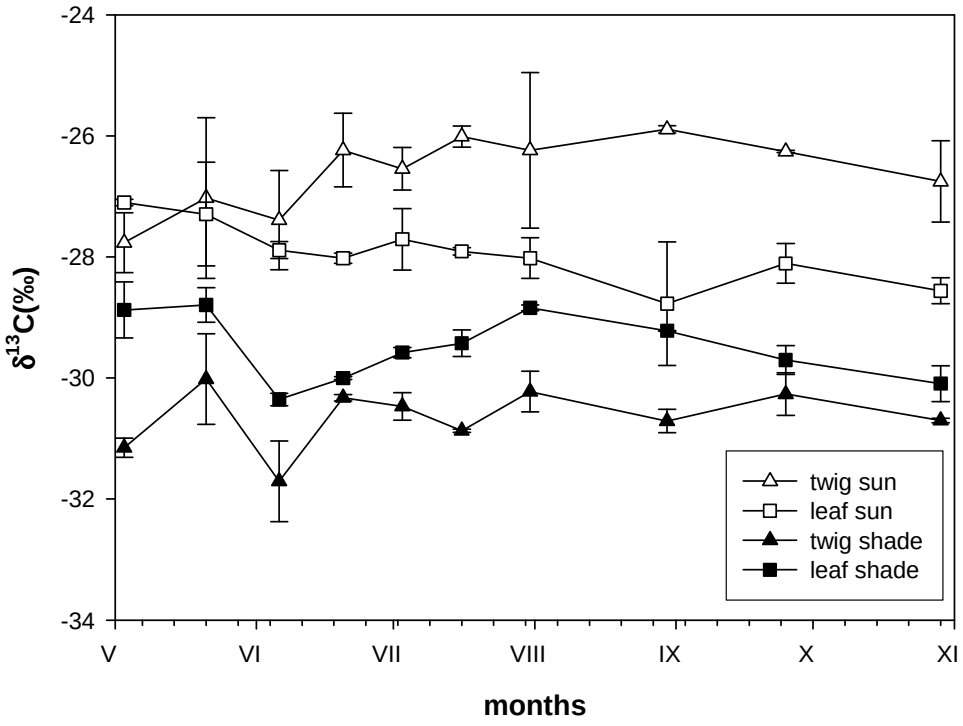


Fig. 3.10: Seasonal courses of the carbon isotopic composition of dry mass ($\delta^{13}C$) of twigs and leaves from sun-exposed and shaded parts of the beech tree. Data from year 2007 only.

3.4 Leaf and stem water content, stem water potential

Leaves and twigs experiencing high irradiance differed in their consistence and appearance from those growing in the deep shade. Sun leaves were thicker, stiffer and

contained less water per unit of dry weight (further referred as leaf water content, W_{leaf}) in comparison with shade leaves (Fig. 3.11a). W_{leaf} of shade leaves was relatively stable during the season, with the average of 1.52 g water per 1 g of dry mass from mid-June to end-September and slightly higher values at the beginning and at the end of the growing season. W_{leaf} of sun leaves was generally of 35% lower and varied more within the season. The decrease of about 50% (from 1.71 to 0.86) was detected in the period from May to June. In October W_{leaf} increased again. A local maximum in W_{leaf} in both sun and shade leaves reaching its peak on 31st July is noteworthy as it corresponded to minimal water potential (i.e. the most negative) measured during the whole season. Specific leaf area (*SLA*=leaf area/dry mass) was lower in sun leaves. Once leaves had fully developed (by the first half of June), *SLA* remained stable until the end of growing season when it slightly increased again (Fig. 3.11c).

Sun and shade twigs differed as well. Sun twigs had bigger diameter and rough surface while shade twigs were thinner and their bark was smooth. Twig water content (W_{stem}) was generally similar (about 1.1 g water per 1 g dry mass) for both sun and shade samples. Nevertheless, the shade twigs tented to contain slightly more water than sun-exposed ones in spring and summer. The increase in W_{stem} toward 31st July is much steeper than that observed in W_{leaf} (Fig. 3.11b). Furthermore, W_{stem} decreased at the end of growing season whereas the opposite was observed in W_{leaf}.

Sun twigs usually showed more negative values of water potential (Ψ_{stem}). In late June Ψ_{stem} dropped to more negative values (about -20 and -15 bar in sun and shade twigs, respectively. Ψ_{stem} maintained such low until the end of July (Fig. 3.12a). Further in the season, Ψ_{stem} were about -11 and -9 bar for sun and shade samples, respectively, except for 25th September when the least negative values in the whole season (-4 and -2.9 bar for sun and shade stems, respectively) were detected.

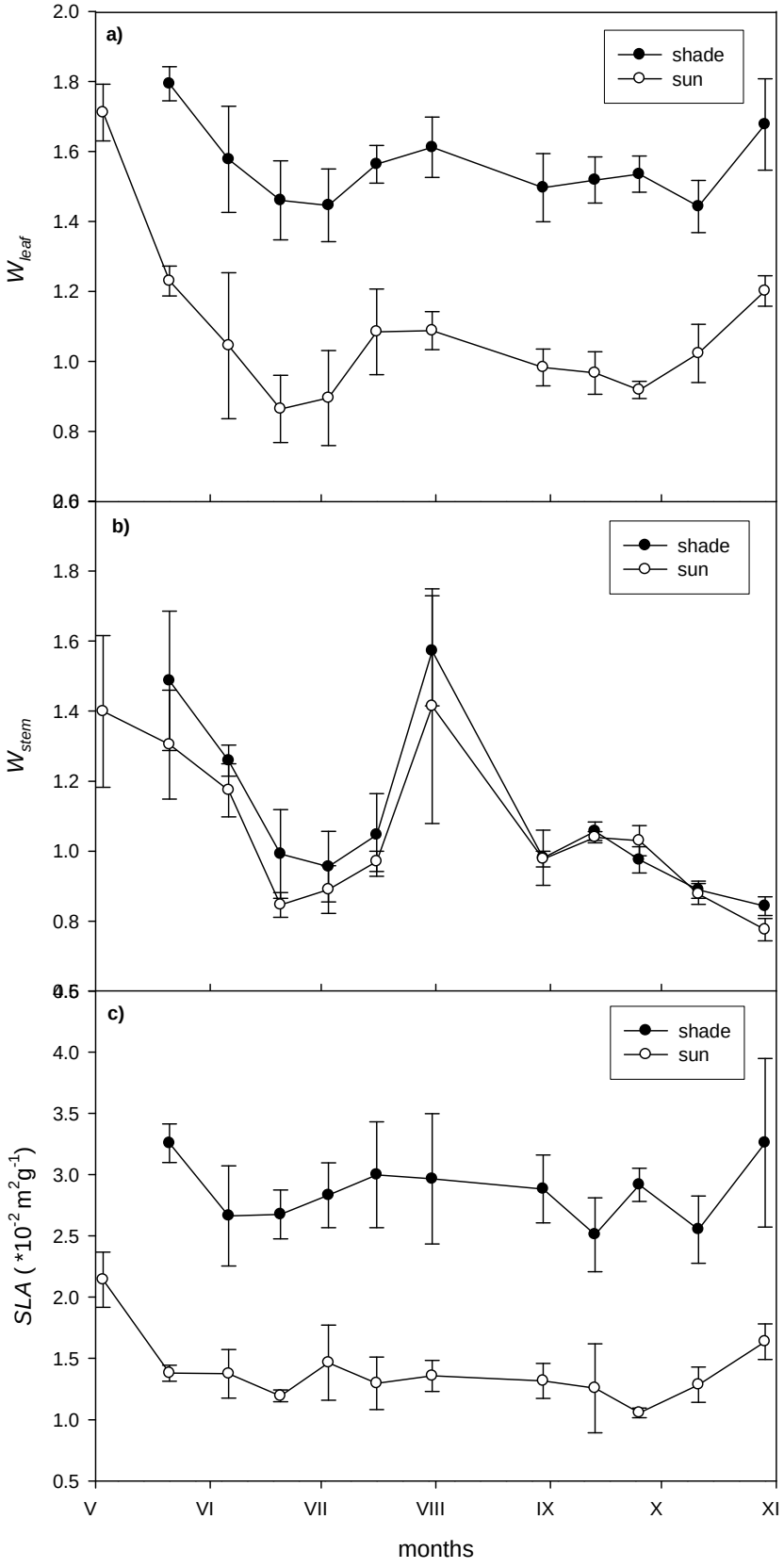


Fig. 3.11: Seasonal course of leaf and twig water content (W_{leaf} and W_{stem}, respectively) and specific leaf area (*SLA*). *W* = (fresh weight-dry weight)/dry weight; *SLA* = leaf area/dry weight. Data from year 2007 only. The dots and error bars represent menas ± SD (*n*=5).

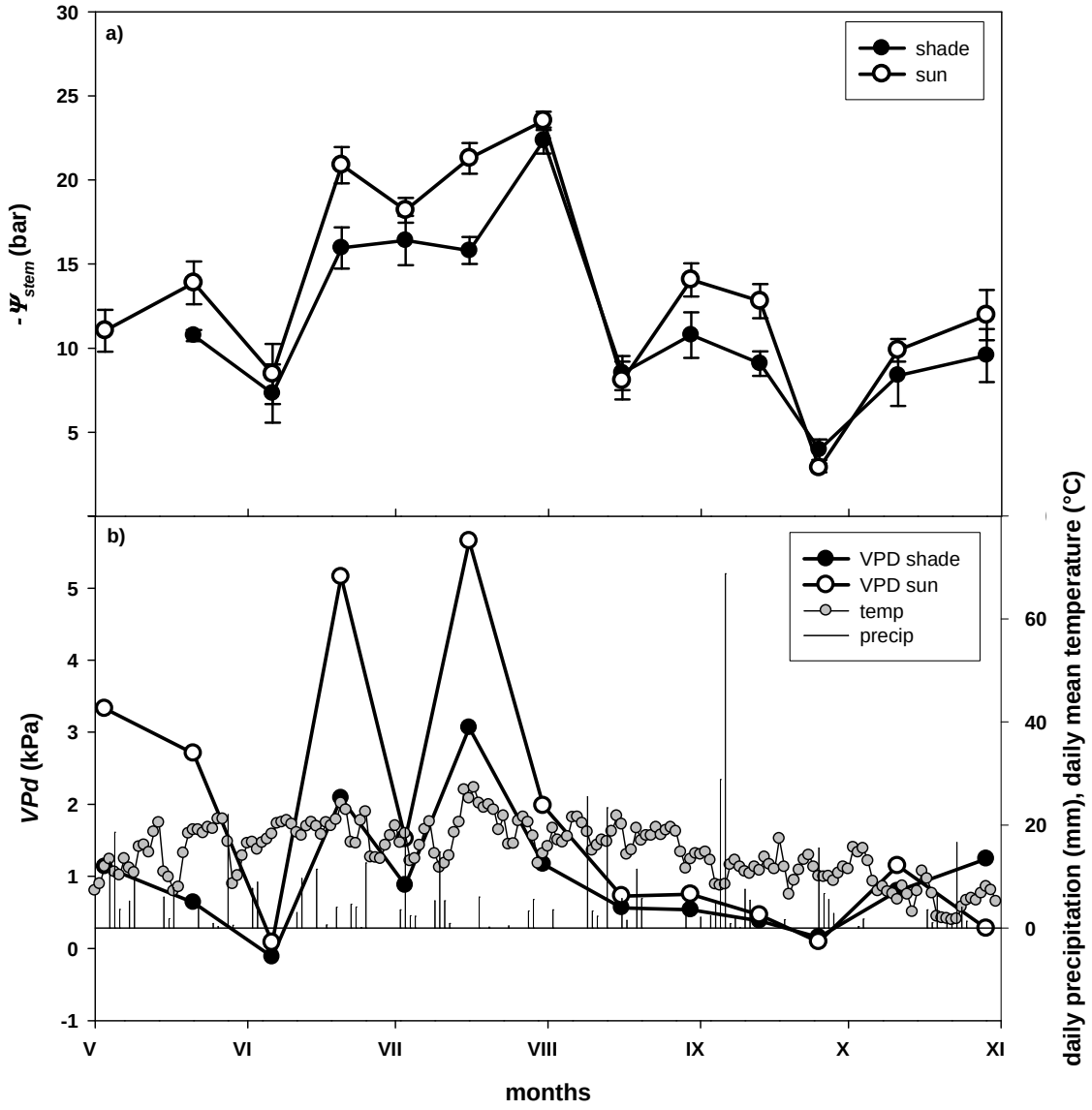


Fig.3.12: Seasonal course of A) twig water potential, Ψ_{stem}, (mean±SD, n=5) and B) vapour pressure difference (*VPd*), precipitation and daily mean temperature. The B) illustrates environmental conditions in terms of water supply (precipitation) and potential water loss (temperature, *VPd*). Data from year 2007 only.

4/ Discussion

Isotopic composition of leaf water is a useful tracer of processes occurring in the biosphere since it affects isotopic signal of atmospheric gases emitted by green plants. Using stable isotope techniques, proportion of molecular oxygen, carbon dioxide and water vapour produced by terrestrial vegetation can be estimated (Farquhar et al. 1993, Gillon & Yakir 2001). However, to provide reasonable predictions of global budgets, we have to start at lower levels. Better understanding of factors determining leaf water isotopic composition is desirable. Several elaborate models of leaf water enrichment based on strictly controlled laboratory measurements and using advanced mathematical tools have been worked out (Cuntz et al. 2007, Ogée et al. 2007). However, complicated models are sometime useless in reality. The results presented in the thesis complement the effort from the other site, by investigating actual variability in the isotopic signal of leaf water and related water compartments (soil and stem) under natural conditions. As far as we know, such an extensive data set on biospheric water isotopes, gathered at one sampling site during three growing seasons, has not been published yet. In the following text, I discuss the results which we obtained.

4.1 Isotopic composition of water

Isotopic composition of soil, stem and leaf water was rather variable during vegetation season as expected. However, several general patterns and trends were similar for all three seasons. Leaf water was the most enriched water compartment measured which was anticipated. In contrast, stem and soil water isotopic signals were surprising. The $\delta^{18}O$ and $\delta^{2}H$ values of soil and twig water were expected to mirror the isotopic signal of current precipitation. However, both water fractions were more depleted in heavy isotopes, especially in spring and summer. The δ-values corresponded well with values computed for winter precipitation. We assume that the beech tree took up water from deep soil layers or directly from the groundwater where the more negative $\delta^{18}O$ and $\delta^{2}H$ values are expected since the groundwater recharges mainly from winter precipitation and therefore inherits its isotopic signature. The convergence of isotopic signals of stem water and precipitation which occurred in autumn can be attributed to either a starting replenishment of groundwater with current rain or a switch

in source water which was newly taken from the layers closer to the soil surface. Such negative δ-values are even more striking in soil water since an evaporative enrichment was expected to increase the δ-values above the isotopic signal of precipitation. Instead, our findings indicate that the soil water extracted from 10 cm depth below the soil surface may originate from winter precipitation even during the spring and summer. It is not so surprising for spring samples when soil profile is fully saturated by water from winter precipitation since the water loss by evapotranspiration is relatively small in winter and spring. However, it is unlikely that the soil in 10 cm depth remains water saturated until the late summer. Besides, it is improbable that the groundwater rose so high by capillary elevation in such amount to dissolve the isotopic signal of incident precipitation. A possible physiological mechanism which may explain our observations is a so-called hydraulic lift which can be described as the nocturnal water movement from moist to dry soil via plant roots (Richards & Caldwell 1987, Caldwell and al. 1998). The hydraulic lift has been reported mainly from arid and semiarid regions. However, there is evidence that it occurs even in mesic environments where the lifted water may help to compensate the soil water deficit in periods of drought (Dawson 1996).

Another peculiarity of soil water isotopic data emerged comparing $\delta^{18}O$ and $\delta^{2}H$ values. Oxygen isotopic composition of soil and twig water was generally similar. However, twig water appeared more depleted than soil water in hydrogen. It is generally accepted that root water uptake is not associated with any isotopic fractionation, however, several studies showed that isotopic fractionation leading to more depleted xylem water may occur. Such observations were often made at saline or xeric habitats (Lin & Sternberg 1993, Ellsworth & Williams 2006) which is not a case of our study site. Moreover, our stem water data nearly exactly followed the local meteoric water line which indicates that they were directly derived from meteoric water (i.e. precipitation) and were probably not subjected to evaporation or another fractionation process. Therefore, we assume that the isotopic signal of soil water, rather than stem water, was modified. However, the question still is weather the oxygen or hydrogen signal changed. One possible process which may change the signal is the isotopic exchange between water and other chemical compounds such as carbon dioxide. The quantity of water involved in the exchange should be significantly greater than the amount of CO_2. Therefore, CO_2 should take on soil water signature while no apparent change should be detected in the water isotope abundance. However, the concentration of carbon dioxide

can reach up to tens percent near plant roots (Šantrůčková *personal communication*) which may be already significant in this regards. The other possible source of fractionation of soil water may be associated with condensation. Water is known to move within the soil also in the form of water vapour which eventually condensates when the temperature drops below the dew point (e.g. during night). In addition, horizontal precipitation (dew) may also provide a substantial source of soil moisture. On balance, we do not know what shifted the isotopic signal of soil water. Soil water isotopes integrate isotopically distinct water sources (precipitation, groundwater, dew...) and there are several processes accompanied by fractionation (evaporation, condensation) which all may modify the signal. Nevertheless, groundwater transported by the beech-mediated hydraulic lift seems to be the main source of water in 10 cm depth. Since the unusual behavior of soil δ^2H is consistent within the seasons and treatments, and since we thoroughly checked for completeness of soil water distillation gravimetrically, we believe that it is not an artifact caused by water extraction or IRMS measurement.

A possible weak point can be seen in the fact that we did not measure the isotopic composition of precipitation directly. Instead, we used the On-line Isotopes in Precipitation Calculator which is based on rigorous scientific research. We believe that the computed data are reliable. Indirect evidence in support of our conclusion that the current precipitation was more enriched than the soil and stem water can be given by the increase in isotopic signal in both soil and stem water detected when heavy rain occurred shortly before sampling.

Leaf water was always enriched above source water. The enrichment was generally higher at the beginning of vegetation period and decreased toward autumn. Relatively weak correlation of $\delta_{leaf}{}^{18}O$ and $\delta_{leaf}{}^{2}H$ of leaf water is noteworthy (Fig.3.2) In contrast to our observations, Twining et al. (2006) found the strongest correlation of oxygen and hydrogen data for leaf water than for any other water compartment (soil and stem water). Twining et al. used leaf and stem water data gathered during the 1 day period only and they found no significant variation in stem water. As indicated by our results, changes in the isotopic composition of source water may worsen the reproducibility of leaf water data as is explained further.

Owing to different equilibrium fractionation (ε^*) for hydrogen and oxygen, with ε^* being approximately 8 times higher in hydrogen than oxygen, evaporation results in relatively greater change in $\delta^{18}O$ than δ^2H (note the word ‘relatively’, which refers to a

comparison with non-evaporating water compartments, the range in absolute values is naturally greater for hydrogen). This can be illustrated by our observations on leaf water and stem water (see Fig.3.2 and compare the slopes of the lines for the different water samples). Therefore, the effect of evaporation will be strongly imprinted in oxygen rather than hydrogen isotopic signature. The term e_a/e_i in the leaf-adapted Craig-Gordon model (Eq.1.9) represents the effect of evaporation. We showed that the e_a/e_i ratio is a satisfactory predictor of Δ_{leaf} and δ_{leaf} in oxygen, however, its explanatory potential is much lower in case of hydrogen (regression coefficient for Δ_{leaf} ^{2}H was very low and regression for δ_{leaf} ^{2}H was even insignificant). A theoretical analysis of robustness of Δ_{leaf} against variation in values of e_a/e_i, δ_{stem} and δ_{vapour} provides deeper insight into these findings (Appendix 3). The analysis revealed that shift in δ_{stem} toward more depleted δ-values as well as shift in δ_{vapour} toward more enriched values goes against the effect of e_a/e_i (Fig.A3.2 & 3). This is pronounced especially under humid conditions (i.e. when e_a/e_i is close to unit). In addition, under conditions of high evaporative demands which usually occur when temperature is high, temperature-dependent decrease in ε* slows down the increase or even causes decrease in Δ_{leaf} in oxygen and hydrogen, respectively, which contradicts the effect of e_a/e_i. Most importantly, all these effects are more pronounced in hydrogen than oxygen. Therefore, the relative importance of change in δ_{stem} and δ_{vapour} and the effect of temperature-dependant change in ε* is greater for hydrogen.

In conclusion, when isotopic signal of source water and air water vapour is variable, leaf water isotopic signal can be satisfactory predicted by the Craig-Gordon model only for oxygen. In addition, air relative humidity seems to be a sufficiently accurate approximation of the e_a/e_i ratio which is promising taking into account that relative humidity is routinely measured by an extensive network of climate stations. In case of hydrogen, we should be aware that we can not neglect the changes in δ_{stem} and δ_{vapour} which may mask the effect of e_a/e_i. Thus, we point out the greater practical potential of oxygen isotopes for global modeling and other applications on ecosystem level.

The majority of biological studies using stable isotopes have focused on sunny environments because biological processes (photosynthesis, evaporation...) are usually more intensive here and isotopic imprints are often weighted by photosynthesis or transpiration rates. The generalization of results gained under such conditions may be doubtful. Therefore, a comparative analysis of isotopic composition of samples

originating from sun-exposed and shaded habitats was desirable. Our results showed that no large differences in isotopic composition of water occurred between samples from contrasting light conditions at least in the range which can be naturally observed in forest ecosystems. It indicates that isotopic signals are quite homogenous in this regard and that the natural contrasts in local environmental conditions do not modify the signals significantly. Therefore, we conclude that results obtained on sun samples can be extrapolated upon shade samples and vice versa at least for our sampling site in temperate deciduous forest. If that pattern holds for other plant communities and habitats, it would be pleasing information for global modelling because it would mean the measurements conducted on any leaf (sun or shade) might be upscaled without introducing a significant error.

4.2 $\delta^{13}C$ of stems and leaves

The $\delta^{13}C$ values measured in the dry mass of sun and shade twigs and leaves fell within the range from -25‰ to -32‰ which are the values typical for C_3 plant. Samples of different type and treatment showed distinct carbon isotopic composition. More specifically, sun-exposed plant material contained more ^{13}C than shaded one. Similar observations have been already made in tropical and temperate forests (Schlesser 1990, Martinelli et al. 1998, Pate & Arthur 1998)

Since the majority of organic matter is created in leaves, I will focus on the leaf isotopic composition first. Presuming that the isotopic composition of source CO_2 was similar for sun and shade leaves which should be the case in freely mixing atmosphere, the observed difference between sun and shade leaves should be attributed to photosynthetic discrimination. The discrimination can stem from either diffusional limitation due to closed stomata or limitation caused by the steep drawdown in the CO_2 concentration as a result of intensive carboxylation and the leaf internal diffusional limitation. More negative $\delta^{13}C$ (i.e. large discrimination) is usually interpreted as evidence of stress accompanied by stomatal closure (e.g. Skomarkova et al. 2006). However, we assume that this is not the case of sun leaves investigated here. Despite the possible depression in transpiration during the periods of substantial drought, overall average stomatal conductance should be higher in sun leaves. The measurements carried out by other researchers (Cochard et al. 1999, Lichtenthaler et al. 2007) have confirmed that this assumption holds for beech (*Fagus sylvatica* L.) as well. High photosynthetic

capacity and intensive fixation of CO_2 can be expected in sun leaves (e.g. Warren et al. 2007). We suggest that the intensive consumption of CO_2 in sun leaves together with anatomy of the thick sun-exposed leaf generates low discrimination of heavy carbon isotope. A corollary of this explanation is that sun leaves were carbon limited.

We are aware of the possible effect of post-photosynthetic discrimination associated with metabolite transport and modification as well as respiration and photorespiration which may significantly perturb the carbon isotopic signal although the isotopic fractionation associated with respiratory processes is believed to be negligible (Farquhar et al. 1989). Ghashghaie et al. (2003) has recently reviewed this issue and arrived to the conclusions that on average the CO_2 released by the dark respiration is enriched of about 6‰ in comparison with the substrate. Even though the exact value can be doubtful, it is generally accepted that respiration favours heavy substrate and consequently makes the plant biomass isotopically lighter. Root respiration producing isotopically lighter CO_2 is the only exception (Bowling et al. 2008). Moreover, newly assimilated sugars are probably not the only substrates for respiration. A rapid mixing of new and old C pool has been reported for beech (Keel et al. 2007) which may further change the signal. It follows that the fractionation during respiration is highly variable and depends on many factors including environmental conditions, period of growing season, tissue-specific metabolism (e.g. prevailing compound being oxidized) etc. Furthermore, a change in isotopic composition of biomass caused by respiration depends on relative proportions of respiration to photosynthesis which is thought to be more or less the same for sun and shade leaves (Lambers et al. 1998). Thus, the fractionation connected with respiration should be similar for both treatments and should not account for the differences observed.

The differences between leaf and stem $\delta^{13}C$ are discussed below. The carbon isotopic composition is known to differ between plant organs. Badeck et al. (2005) analyzed so far published data and found that in more than 80% of cases (specifically, in 333 out of 410 observations) leaves are isotopically lighter than the other plant organs (namely stems and roots). The difference of about 2.5‰, with stems being more enriched than leaves, has been independently determined in beech at least twice (Damesin & Lelarge 2003, Nogués et al. 2006). The results showing that woody stem tissues contain less ^{13}C than the leaves are truly exceptional. We found the usual pattern (i.e. isotopically lighter leaves) in sun-exposed plant material while the opposite was true for shaded samples. By all means, the majority of carbon in the stem organic matter

originates from CO_2 which is fixed during the photosynthetic processes in leaves. Sucrose is the predominant form in which assimilated carbon is transported. Sap containing sucrose and other solute flows from source to sink plant organs via sieve elements (the phloem). The sucrose synthesis, translocation and processes during which sucrose is metabolized in the sink to final product may be associated with isotopic fractionation (Brandes et al. 2006) which may influence the final isotopic composition of stem dry matter. However, the knowledge of these processes is too scarce to enable rigorous analysis of the differences between sun and shade samples from this point of view. In addition, respiration, as discussed above, may be another factor perturbing the isotopic signal of stem tissue.

Moreover, the isotopic signal of dry matter of both leaves and stems seems to be rather invariant during vegetation season with the biggest fluctuations observed at the beginning of vegetation season when carbon used for growth is not generated exclusively by photosynthesis but originates from stored reserves as well. Relatively higher $\delta^{13}C$ values found in shade leaves in May can reflect the use of different carbon pools shortly after budburst and later in the season. The spring isotopic signal of shade leaves came closer to that of sun leaves. This may indicate that the material used for early growth of shade leaves originated from sun leaves (most probably, shade buds contained organic material which had been synthesized in sun leaves in the previous growing season).

4.3 Water content, water potential

Plant water status is an important factor influencing the intensity of biological processes in plants. The water shortage usually leads to stomatal closure which consequently suppresses photosynthesis and transpiration. Thus, such an action should be reflected in isotopic composition of both plant water and biomass. The effect of drought is better understood for carbon isotopes and lower discrimination (i.e. higher values of $\delta^{13}C$ in plant biomass) have been used as an indicator of water deficit in many ecological studies. In contrast, the influence of water availability on leaf water isotopic composition has not been satisfactory explained so far. There are many possible direct and indirect repercussions of reduced transpiration which may affect the isotopic signal often in contradicting ways (Farquhar et al. 2007). For instance, presumably increased temperature of leaf will result in increase of *VPd* and thus should favour increase in

enrichment. Furthermore, slower water flux through plant probably leads to an elongation of time required to reach isotopic steady-state and shifting of the balance between advection of unfractionated source water and back diffusion of heavy isotopes from the sites of evaporation (Flanagan 1993, Farquhar & Cernusak 2005). In addition, Yakir (1998) pointed out the importance of leaf water volume and predicted changes in leaf water enrichment due to changes in leaf volume in desiccating leaves. Our research was not primary aimed to investigate impacts of drought on isotopes, however, the measurements of stem water potential (Ψ_{stem}) and plant water content (W) together with meteorological data enable to take a glance at this issue.

Low values of Ψ_{stem} observed during the second half of June to end-July 2007 indicate that the beech experienced relatively dry environmental conditions. This is confirmed also by small amount of precipitation and high air temperature during that time. Leaf temperature of sun leaves increased up to nearly 40 °C and *VPd* reached more than 5 kPa. Furthermore, the change in water content was detected in both leaves and twigs. The evidence that stomata closed during that time can be found in the increase of $\delta^{13}C$ of shade leaves. This was not observed in sun leaves probably due to the fact that the stomatal conductance did not represent the main limitation and the discrimination was low during the whole season as a result of intensive photosynthetic CO_2 uptake (as discussed above). The isotopic composition of water did not show any unexpected marks of drought stress. Leaf water enrichment reached its maximum on 16th July which corresponded well with extremely high *VPd* (and correspondingly low e_a/e_i ratio). Neither soil nor stem water behaved in a strange way. The 'suspicious' increase of δ-values of both sample types detected on 3rd July may be attributed to rain which occurred the day before the sampling. Hence, we conclude that the mild drought did not affect the water isotope signal significantly despite the fact that it was accompanied by stomatal closure and change in water content. Those parameters were anticipated to be capable of perturbing isotopic signal of leaf water.

Nevertheless, the data on water content and stem water potential are worth further comment because they provide several interesting hints on water balance of the beech tree. Leaves and twigs growing under contrasting light conditions differ in their consistence. Sun leaves were thicker and contained lower proportion of water in they fresh weight in comparison with their shade counterparts which is a well known phenomenon. In contrast, W_{stem} did not differ so much between sun and shade treatment and also the fluctuations within the season had similar course and range (Fig 3.11).

Since W represents a relative measure of water amount, two different processes may lead to a change in its value, i.e. W changes due directly to change in amount of water or indirectly to change in dry matter content. We assume that both principles played a role in case of beech. W_{leaf} and W_{stem} seem to reflect both ontogenesis of the organ and plant water status. In early spring, sun leaves were juicier and they promptly sclerophyllized as a result of acclimation to high light. In autumn, the relative water content increased again probably due to the allocation of non-structural carbohydrates to storage tissues. Therefore, the increase in W_{leaf} observed in spring and autumn can be attributed to the decrease in dry matter content. In contrast, increase in water amount itself is probably responsible for the rising W_{leaf} values detected at the end of July. At the same time, Ψ_{stem} dropped toward the lowest values indicating water shortage which seems to be inconsistent with the highest plant water content measured (see Fig. 3.11 & 12). We assume that the explanation can be found in stomatal closure which prevented excessive water loss and finally resulted in the increase in leaf and stem water content. The change in dry matter amount did not occurred during this period as obvious from stable SLA values. Seasonal course of W_{stem} can be interpreted in similar manner. W_{stem} appeared to decrease in the spring owing to the increase in dry matter content as twig grew and lignified. The increase in W_{stem}, culminating on 31st July, probably stemmed from reduction of water loss due to stomatal closure as explained above. However, the increase was steeper and a bit delayed in comparison with W_{leaf}. Twigs may have a relatively high water storage capacity and they recharge slower, therefore, W_{stem} saturated later but to a greater extent. Furthermore, leaves lose the water more intensively than twigs. In spite the fact that stomata are closed, cuticular transpiration from large leaf surface may represent significant water loss (Burghardt & Riederer 2003). Thus, the increase in W_{leaf} is limited. In contrast to leaves, W_{stem} was getting lower toward the autumn. This may be attributed to a possible increment in biomass of twigs due to the organic material allocated from leaves. For instance, Damesin & Lelarge (2003) described that starch content in beech twigs increased of 2.3% during September. However, this increment would mean only small decrease in W_{stem} which can fully explain the difference we observed. Nevertheless, other compounds are probably transported apart form starch.

Twig water potential Ψ_{stem} seems to reflect a balance between water supply and loss. More negative values of Ψ_{stem} found in sun twigs correspond with higher evaporative demands (expressed as VPd) and faster transpiration rate generally observed

in sun leaves (e.g. Cochard et al. 1999, Nardini et al. 2005). However, the lowest Ψ_{stem} values were not achieved while *VPd* was the highest. We assume that problems with water supply rather than excessive evaporative demands were the reason for the lowest Ψ_{stem} observed on 31st July. The fact that Ψ_{stem} of shade samples equals that of sun samples at that day supports this hypothesis.

As already mentioned above, from the second half of June until the end of July the beech suffered from drought stress. The water potential was very low and stomata started to close to reduce water loss. As a result, the water content began to rise, however, neither did Ψ_{stem}. This indicates that soil water potential was probably very low during this period and the beech may lowered its water potential by synthesis of osmotically active compounds (the so-called osmotic adjustment) to withdrawn water from the soil. Unfortunately, plant osmotic potential was not assessed.

5/ Conclusions

Seasonal variability in the stable isotope composition of soil, stem and leaf water was investigated. Apart from this main goal of the project, several other measurements providing insight into ecophysiology of beech were carried out. The measurements were conducted and the samples were taken at a single study site during three subsequent growing seasons. A beech tree being the dominant species at the study site was chosen as an experimental plant. The need for better understanding of leaf water isotopic signature and endeavour to quantify its variability motivated this research. The results presented here are useful for global modelling as well as for ecological studies at a lower scale.

The most important conclusions outcoming from the research are listed:

1/ The variability in leaf water enrichment has to be interpreted separately for oxygen and hydrogen data. In oxygen, differences in evaporative demands were the main source of variability of leaf water signal. The ratio of water vapour pressure in the air to water vapour pressure in the leaf interior (e_a/e_i) accounted for a significant amount of variability. Air relative humidity may be also used as a rough predictor of $\Delta_{leaf}(^{18}O)$ when more detailed measurements are not available. In contrast to oxygen data, the variability in $\Delta_{leaf}(^{2}H)$ was poorly explained by environmental conditions. Instead, isotopic composition of source water was the best predictor. Therefore, when we do not know isotopic composition of source water or significant variability in it exists, we should not rely on the Craig-Gordon-model-based estimate of $\delta_{leaf}(^{2}H)$. On the contrary, $\delta_{leaf}(^{18}O)$ seems to be quite robust against changes in the isotopic composition of source water.

2/ Isotopic signal of twig water indicates that the beech utilized groundwater or water from deep soil layers originating from winter precipitation as a dominant water source.

3/ Water at 10 cm soil depth surprisingly showed more negative δ-values than those computed for current precipitation. This indicates that the groundwater may have been transported towards the soil surface by hydraulic lift via beech roots.

4/ The isotopic signal of all the types of water samples (soil, stem, and leaf) did not differ between sun-exposed and shaded treatment to a large extent which shows that the isotopic composition is probably rather homogenous (at least in terms of light conditions) and the extrapolation of measurements done on one part of the tree upon higher levels is justifiable.

5/ Sun leaves discriminate against heavy carbon less than their shade counterparts. Assimilation rate of sun leaves was probably so high that the RUBISCO was CO_2 limited even when the stomata were fully open.

6/ Other plant organs (stems, roots) are usually less depleted in ^{13}C than leaves. In contrast to this general pattern, shade twigs were more depleted than leaves in this study. The explanation for this observation is not clear.

7/ Leaf and twig water content culminated while stem water potential reached its most negative values. The increase in water content is probably a consequence of stomatal closure. Osmotically active compounds were probably synthesized by the beech to maintain such low water potential while water content was high.

For further research, I would suggest:

1/ to conduct controlled laboratory experiments manipulating isotopic composition of source water and investigate the response of $\delta^{18}O$ and $\delta^{2}H$ in leaf water under different humidity treatments

2/ to carry out more extensive sampling of soil water at Brloh site (measurements in different soil depths and in different distances from the tree foot) to test the hydraulic lift hypothesis

3/ to sample precipitation water at the sampling site and compare measured δ-values with that calculated with On-line Isotopes in Precipitation Calculator

4/ to take samples of atmospheric water vapour and carbon dioxide at the sampling site to confirm our assumption that δ-values did not differ for sun and shade treatment

5/ to make gas exchange measurements on the beech to characterize stomatal conductance, photosynthetic rate, respiration rate of sun and shade leaves

6/ References:

Allison GB, Gat JR, Leaney FWJ (1985): The relationship between deuterium and oxygen-18 delta values in leaf water. *Chemical Geology* 58: 145-156.

Barbour MM, Schurr U, Henry BK, Wong SC, Farquhar GD (2000): Variation in the oxygen isotope ratio of phloem sap sucrose from castor bean. Evidence in support of the Péclet effect. *Plant Physiology* 123:671-679.

Barbour M & Farquhar GD (2000): Relative humidity- and ABA-induced variation in carbon and oxygen isotope ratios of cotton leaves. *Plant, Cell & Environment* 23: 473-485.

Barbour MM, Fischer RA, Sayre KD, Farquhar GD (2004): Oxygen isotope ratio of leaf and grain material correlates with stomatal conductance and grain yield in irrigated wheat. *Australian Journal of Plant Physiology* 27: 625-637.

Badeck FW, Tcherkez G, Nogués S, Piel V, Ghashghaie J (2005): Post-photosynthetic fractionation of stable carbon isotopes between plant organs – a widespread phenomenon. *Rapid Communication in Mass Spectrometry* 19: 1381-1391.

Bariac T, Gonzalez-Dunia J, Tardieu F, Tessier D, Mariotti A (1994): Spatial variation of the isotopic composition of water (18O, 2H) in organs of aerophytic plants.1. Assessment under laboratory conditions. *Chemical Geology* 115: 307-315.

Bowling DR, Pataki DE, Renderson JT (2008): Carbon isotope in terrestrial ecosystem pools and CO2 fluxes. *New Phytologist* 178: 24-40.

Brandes E, Kodama N, Whittaker K, Weston C, Rennenberg H, Keitel C, Adams MA, Gessler A (2006): Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stems of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. *Global Change Biology* 12: 1922-1939.

Burghardt M & Riederer M (2003): Ecophysiological relevance of cuticular transpiration of deciduous and evergreen plants in relation to stomatal closure and leaf water potential. *Journal of Experimental Botany* 54: 1941-1949.

Caldwell MM, Dawson TE, Richards JH (1998): Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* 113:151-161.

Cochard H, Lemoine D, Dreyer E (1999): The effects of acclimation to sunlight on the xylem vulnerability to embolism in *Fagus sylvatica* L. *Plant, Cell and Environment* 22: 101-108.

Craig H (1961): Isotopic variation in meteoric water. Science 133:1702-1703.

Cuntz M, Ogée J, Farquhar GD, Peylin P, Cernusak LA (2007): Modelling advection and diffusion of water isotopologues in leaves. *Plant, Cell & Environment* 30: 892-909.

Damesin C & Lelarge C (2003): Carbon isotope composition of current-years shoots from *Fagus sylavatica* in relation to growth, respiration and use of reserves. *Plant, Cell & Environment* 26: 207-219.

Dawson TE (1996): Determining water use by trees and forests form isotopic, energy balance and transpiration analyses: the role of tree size and hydraulic lift. *Tree Physiology* 16:263-272.

Dongmann G, Nürnberg HW, Förstel H, Wagener K (1974): On the enrichment of $H_2{}^{18}O$ in the leaves of transpiring plants. *Radiation and Environmental Biophysics* 11:41-52.

Ehleringer JH, Hall AE, Farquhar GD, eds, (1993): Stable Isotopes and Plant Carbon-Water Relations. Academic Press, New York, pp 19-70.

Ellsworth PZ & Williams DG (2006): Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant & Soil* 291:93-107.

Farquhar GD, O'Leary MH, Berry JA (1982): On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* 9:121-137.

Farquhar GD, Hubick KT, Condon AG, Richards RA (1989): Carbon isotope fractionation and plant water-use efficiency. *In* PW Rundel, JR Ehleringer, KA Nagy, eds, Stable Isotopes in Ecological Research. Springer Verlag, New York, pp 21-40.

Farquhar GD, Ehleringer JR, Hubick KT (1989): Carbon isotope discrimination and photosynthesis. *Annual Reviews of Plant Physiology & Plant Molecular Biology* 40: 503-37.

Farquhar GD, Lloyd J, Taylor JA, Flanagan LB, Syvertsen JP, Hubick KT, Wong SC, Ehleringer JR (1993): Vegetation effects on the isotopic composition of oxygen in atmospheric CO_2. *Nature* 363: 439-442.

Farquhar GD & Gan SK (2003): On the progressive enrichment of the oxygen isotopic composition of water along leaves. *Plant, Cell & Environment* 26:1579-1597.

Farquhar GD & Cernusak LA (2005): On the isotopic composition of leaf water in the non-steady state. *Functional Plant Biology* 32: 293-303.

Farquhar GD, Cernusak LA, Barnes B (2007): Heavy water fractionation during transpiration. *Plant Physiology* 143: 11-18.

Flanagan LB (1993): Environmental and Biological Influences on Stable Oxygen and Hydrogen Isotopic Composition of Leaf Water. *In* JH Ehleringer, AE Hall, GD Farquhar, eds, Stable Isotopes and Plant Carbon-Water Relations. Academic Press, New York, pp 71-90.

Fontes JC & Gonfiantini R (1967): Comportement isotopique au cours de l´evaporation de deux bassins sahariens. *Earth & Planetary Science Letters* 3:258-266.

Gan KS, Wong SC, Yong JWH, Farquhar GD (2002): ^{18}O spatial patterns of vein xylem water, leaf water and dry matter in cotton leaves. *Plant Physiology* 130: 1008-1021.

Gan KS, Wong SC, Yong JWH, Farquhar GD (2003): Evaluation of models of leaf water 18O enrichment using measurements of spatial patterns of vein xylem water and dry matter in maize leaves. *Plant, Cell & Environment* 26: 1479-1495.

Gat JR & Bowser C (1991): The heavy isotope enrichment of water in coupled evaporative systems. *In* HP Taylor, JR O'Neil & IR Kaplan, eds, Stable Isotope Geochemistry: a Tribute to Samuel Epstein. The Geochemical Society, St Louis, MO, USA, pp.159-168.

Gat JR (1996): Oxygen and hydrogen Isotopes in the hydrological cycle. *Annual Reviews in Earth Planetary Science* 24: 225-262.

Ghashghaie J, Badeck FW, Lanigan G, Nogués S, Tcherkez G, Deléens E, Cornic G, Griffiths H (2003): Carbon isotope fractionation during dark respiration and photorespiration in C_3 plants. *Phytochemistry Reviews* 2: 145-161.

Gillon J & Yakir D (2001): Influence of carbonic anhydrase activity in terrestrial vegetation on the ^{18}O content of atmospheric CO_2. *Science* 291: 2584-2587.

Gomes-Laranjo GJ, Coutinho JP, Galhano V, Ferreira-Cardoso JV (2008): Differences in photosynthetic apparatus of leaves from different sites of the chestnut canopy. *Photosynthetica* 46: 63-72.

Helliker BR & Ehleringer JR (2002): Grass blades as tree rings: environmentally induced changes in the oxygen isotope ratio of cellulose along the length of grass blades. *New Phytologist* 155: 417-424.

Hobbie EA & Werner RA (2004): Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis. *New Phytologist* 161: 371-385.

Hsieh JCC, Chadwick OA, Kelly EF, Savin SM (1998): Oxygen isotopic composition of soil water: Quantifying evaporation and transpiration. *Geoderma* 82: 269-293.

Jaggi M, Saurer M, Fuhrer J, Siegwolf RTW (2002): The relationship between the stable carbon isotope composition of needle bulk material, starch, and tree rings in *Picea abies*. *Oecologia* 131: 325-332.

Keel SG, Siegwolf RTW, Jäggi M, Körner C (2007): Rapid mixing between old and new C pools in the canopy of mature forest tree. *Plant, Cell & Environment* 30: 963-972.

Lambers H, Chapin FS, Pons TJ (1998): Plant Physiological Ecology. Springer-Verlag New York Inc., USA, pp 34.

Leaney FWJ, Osmond C, Allison GB, Ziegler H (1985): Hydrogen-isotope composition of leaf water in C3 and C4 plants: its relationship to the hydrogen-isotope composition of dry matter. *Planta* 164: 215-220.

Lepš J (1996): Biostatistika. University of South Bohemia in České Budějovice. pp 1-166.

Lichtenthaler HK, Ač A, Marek MV, Kalina J, Urban O (2007): Difference in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. *Plant Physiology & Biochemistry* 45: 577-588.

Lin G & Sternberg LdaSL (1993): *In* J.H. Ehleringer, A.E. Hall, G.D. Farquhar, eds, Stable Isotopes and Plant Carbon-Water Relations. Academic Press, New York, pp 497-510.

Schlesser GH (1990): Investigations of the δ13C pattern in leaves of Fagus sylvatica L. *Journal of Experimental Botany* 41:565-572.

Majouble M (1971): Fractionnement en oxygene-18 et en deuterium entre l'eau et sa vapaeur. *Journal of Chemical Physics* 10: 1423-36.

Martinelli LA, Almeida S, Brown IF, Moreira MZ, Victoria RL, Sternberg LdaSL, Ferreira CAC, Thomas WW (1998): Stable carbon isotope ratio of tree leaves, boles and fine litter in a tropical forest in Rondônia, Brazil. *Oecologia* 114: 170-179.

Mook WG, eds, (2000): Environmental Isotopes in the Hydrological Cycle Principles and Applications. Available on-line at: http://www.iaea.org/programmes/ripc/ih/ volumes/volumes.htm.

Moreira MZ, Sternberg LdaSL, Martinelli LA, Victoria RL, Barbosa EM, Bonates LA, Nepstad DC (1997): Contribution of transpiration to forest ambient vapour based on isotopic measurements. *Global Change Biology* 3: 439-450.

Nardini A, Gortan E, Salleo S (2005): Hydraulic efficiency of the leaf venation system in sun- and shade-adapted species. *Functional Plant Biology* 32: 953-961.

Neuhäslová Z et al. (1998): Map of Potential Natural Vegetation of the Czech Republic. Academia, Praha, pp:1-341.

Nisbet E (2007): Earth monitoring: Cinderella science. *Nature* 450: 789-790.

Nogués S, Demesin C, Tcherkez G, Maunoury F, Cornic G, Ghashghaie J (2006): 13C/12C isotope labelling to study leaf carbon respiration and allocation in twigs of field-grown beech trees. *Rapid Communication in Mass Spectrometry* 20: 219-226.

Ogée J, Cuntz M, Peylin P, Bariac T (2007): Non-steady state, non-uniform transpiration rate and leaf anatomy effects on the progressive stable isotope enrichment of leaf water along monocot leaves. *Plant, Cell & Environment* 30:367-387.

Pate J & Arthur D: δ13C analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signature of foliage and trunk wood of *Eucalyptus globulus*. *Oecologia* 117: 301-311.

Saurer M (2003): The influence of climate on the oxygen isotopes in tree rings. *Isotopes in Environmental & Health Studies* 39:102-105.

Skomarkova MV, Vaganov EA, Mund M, Knohl A, Linke P, Boerner A, Schulze ED (2006): Inter-annual and seasonal variability of radial growth, wood density and carbon isotope ratio in tree rings of beech (*Fagus sylvatica*) growing in Germany and Italy. *Trees* 20: 571-586.

Smith AF & Freeman KH (2006): Influence of physiology and climate on δD of leaf wax n-alkanes from C_3 and C_4 grasses. *Geochemica et Cosmochemica Acta* 70: 1172-1187.

Šantrůček J, Květoň J, Šetlík J, Bulíčková L (2007): Spatial variation of deuterium enrichment in bulk water of snowgum leaves. *Plant Physiology* 143: 88-97.

Richards JH & Caldwell MM (1987): Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridantata* roots. *Oecologia* 73: 486:489.

Ripullone F, Matsuo N, Stuart-Williams H, Wong CS, Borghetti M, Tani M, Farquhar G (2008): Environmental Effects on Oxygen Isotope Enrichment of Leaf Water in Cotton Leaves. *Plant Physiology* 146: 729-736.

Twining J, Stone D, Tadros C, Henderson-Sellers A, Williams A (2006): Moisture Isotopes in the Biosphere and Atmosphere (MIBA) in Australia: A priori estimates and preliminary observations of stable water isotopes in soil, plant and vapour for Tumbarumba Field Campaign. *Global & Planetary Change* 51: 59-72.

Walker CD, Leaney FWJ, Dighton JC, Allison GB (1989): The influence of transpiration on the equilibrium of leaf water with atmospheric water vapour. *Plant, Cell & Environment* 12: 1377-1385.

Wang XF & Yakir D (1995): Temporal and spatial variation in the oxygen-18 content of leaf eater in different plant species. *Plant, Cell & Environment* 18: 1377-1385.

Warren CR, Löw M, Matyssek R, Tausz M (2007): Internal conductance to CO_2 transfer of adult *Fagus sylvatica*: Variation between sun and shade leaves and due to free-air ozone fumigation. *Environmental and Experimental Botany* 59: 130-138.

Yakir D (1998): Oxygen-18 of leaf water: a crossroad for plant-associated isotopic signals. *In* H Griffiths, eds, Stable Isotopes integration of biological, ecological and geochemical processes. BIOS Scientific Publishers Limited, Oxford, pp 147-168.

Yakir D, DeNiro MJ, Gat JR (1990): Natural deuterium and oxygen-18 enrichment in leaf water of cotton plants grown under wet and dry conditions: evidence for water compartmentation and its dynamics. *Plant, Cell & Environment* 13: 49-56.

Yakir D & Sternberg LdaSL (2000): The use of stable isotopes to study ecosystem gas exchange. *Oecologia* 123: 297-311.

Yepez EA, Williams DG, Scott RL, Lin G (2003): Partitioning overstory and understory evapotranspiration in a semiarid savanna woodland from the isotopic composition of water vapor. *Agricultural & Forest Meteorology* 119: 53-68.

Appendix 1

List of symbols and abbreviations:

a	fractionation during diffusion of CO_2 in air (‰)
b	fractionation during carboxylation catalyzed by RUBISCO (‰)
c_a	CO_2 concentration in the ambient air
c_i	CO_2 concentration at the sites of carboxylation
df	degrees of freedom
e_a	water vapour pressure in the ambient air (kPa)
e_i	water vapour pressure in the leaf air space (i.e. saturated water vapour pressure at a given leaf temperature) (kPa)
GMWL	global meteoric water line
LMWL	local meteoric water line
n	number of repeats
NS	non-significant (at 0.05 significance level)
p	level of significance
PhAR	photosynthetically active radiation (μmol m^{-2} s^{-1})
R	isotope ratio (heavy-to-light isotope)
RH	air relative humidity (%)
RUBISCO	Ribulose 1,5-bisphospate carboxylase/oxygenase
SD	standard deviation
SLA	specific leaf area, i.e. leaf area/leaf dry mass (m^2g^{-1})
t_{air}	ambient air temperature (°C)
t_{leaf}	leaf temperature (°C)
VPd	leaf-to-air vapour pressure difference ($=e_i-e_a$) (kPa)
V-PDB	Vienna Pee Dee Belemnite
V-SMOW	Vienna Standard Mean Oceanic Water
W_{leaf}	leaf water content per unit of dry mass
W_{stem}	stem water content per unit of dry mass
α_k	kinetic fractionation factor
α^*	equilibrium water-vapour fractionation factor
β	standardized regression beta coefficient (for data standardized to a mean of 0 and a standard deviation of 1)
δ_{leaf}	difference in isotope ratios of leaf water and V-SMOW relative to V-SMOW (‰)
δ_{stem}	difference in isotope ratios of stem water and V-SMOW relative to V-SMOW (‰)
δ_{vapour}	difference in isotope ratios of air water vapour and V-SMOW relative to V-SMOW (‰)
$\delta^{13}C$	difference in isotope ratios plant dry matter and V-PDB relative to V-PDB (‰)
Δ_c	estimate of leaf water isotopic enrichment based on Craig-Gordon equation (‰)
Δ_{leaf}	deviation in isotopic composition of leaf water (δ_{leaf}) and source water (stem, δ_{stem}) (‰) - 'leaf water enrichment'
Δ_{vapour}	isotope ratio of ambient water vapour relative to source (stem) water (‰)
$\Delta^{13}C$	isotope ratio of plant dry matter to atmospheric CO2 (‰)
ε^*	equilibrium water-vapour fractionation (‰)
ε_k	kinetic fractionation (‰)
Ψ_{stem}	stem water potential (bar)

Appendix 2

Fig.A3.1: Brloh sampling site.

Fig.A3.2: The beech tree at Brloh sampling site.

Appendix 3

Sensitivity analysis

The analysis aimed to reveal why the e_a/e_i ratio was only a poor predictor of leaf water enrichment in hydrogen in comparison with oxygen. Responses of leaf water enrichment to changes in parameters of the Craig-Gordon equation (Eq.A.1) were modelled. Oxygen and hydrogen data were compared. $\Delta_C{}^{18}O$ and $\Delta_C{}^{2}H$ was computed according to Eq.A.1.

$$\Delta_C = \varepsilon_k + \varepsilon^* + \left(\Delta_{vapour} - \varepsilon_k\right) \cdot \frac{e_a}{e_i} \qquad \text{Eq.A.1}$$

where ε_k and ε^* are kinetic and equilibrium fractionation, respectively, e_a/e_i represents the ratio of water vapour pressure in the air to water vapour pressure in the leaf interior and Δ_{vapour} stands for the depletion of water vapour in heavy isotopes with respect to source water. Thus,

$$\Delta_{vapour} = \left(\frac{\delta_{vapour} - \delta_{source}}{\delta_{source} + 1000} \right) \cdot 1000 \qquad \text{Eq.A.2}$$

where

$$\delta[‰] = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \cdot 1000 \qquad \text{Eq.A.3}$$

where $R = [^{18}O]/[^{16}O]$ and $[^{2}H]/[^{1}H]$ for oxygen and hydrogen, respectively.

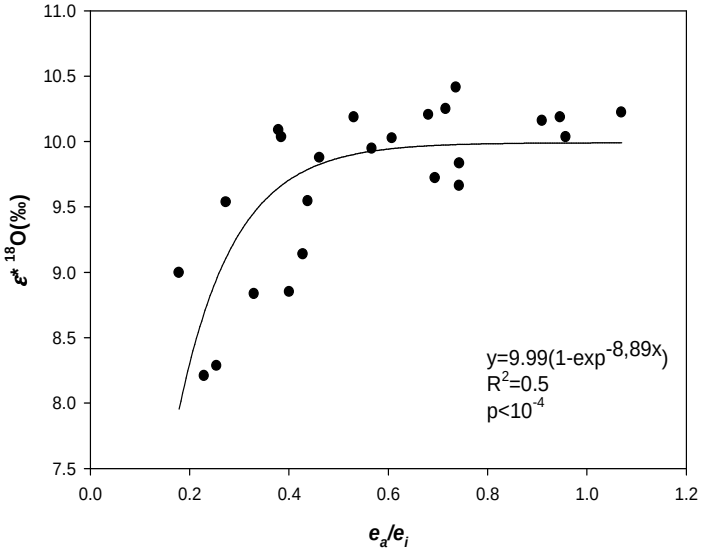
At each step, one of the three parameters (δ_{source}, δ_{vapour} or e_a/e_i) was held constant while the rest two parameters varied in the range which is anticipated to be relevant for the Brloh study site (Tab.A). The values of δ_{source} and e_a/e_i were chosen to cover the variability of real data measured during the Brloh sampling campaign. δ_{vapour} values were derived based on real data sampled in České Budějovice, 20 km far of Brloh. Finally, slightly less negative δ_{vapour} values than the measured average were used for the analysis because δ_{vapour} computed according to Eq.A.1 & A.2 for Brloh sampling site using observed leaf water enrichment were slightly higher than those obtain in České Budějovice. Equilibrium fractionation (ε^*) was variable with respect to e_a/e_i as explained below. ε^* is known to decrease linearly with increasing temperature (Table 1.1). Since e_a/e_i ratio is also significantly influenced by the temperature relationship between e_a/e_i may be anticipated. Regression analysis confirmed this assumption and the e_a/e_i versus ε^* relationship was fitted by exponential rise curve (Fig. A3.1). The regression equations were than used to compute ε^* for different values of e_a/e_i. Kinetic fractionation was assumed to be constant (ε_k = 18.9‰ and 17‰ for oxygen and hydrogen, respectively).

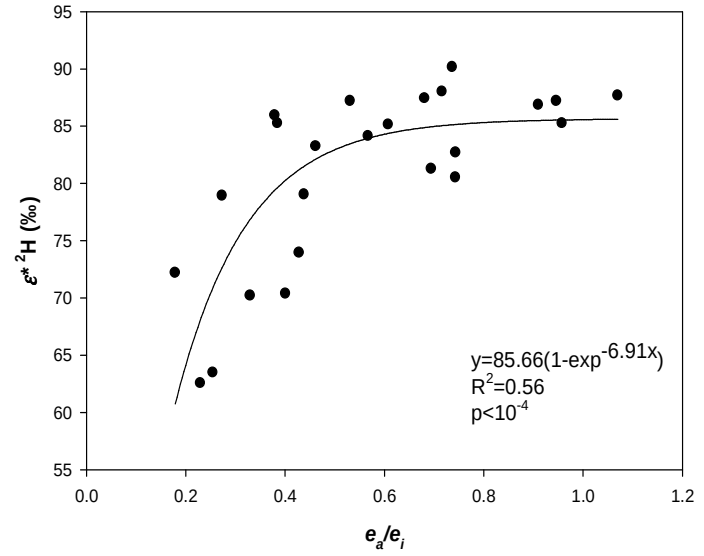
Table A.1: Range of parameters used in the modelling.

	δ_{source} (‰)		δ_{vapour} (‰)		ε^* (‰)		ε_k (‰)	e_a/e_i	
	min	*max*	*min*	*max*	*min*	*max*		*min*	*max*
^{18}O	-13.9	-7.4	-19.6	-13.5	8	10	18.9		
^{2}H	-99	-56	-130	-80	60.8	85.6	17	0.18	0.96

The symbols stand for δ_{source} isotopic composition of source water (i.e. stem water); δ_{vapour}: isotopic composition of air water vapour; e_a: partial water vapour pressure in ambient air; e_i: saturated water vapour pressure in the leaf interior

Results:





FigA3.1: Relationship between equilibrium fractionation ε^* and the ratio of water vapour pressure in the ambient air to water vapour pressure in leaf air space (e_a/e_i). The variables are related indirectly via temperature. ε^* was computed after Majouble (1971), e_a/e_i was measured at Brloh, 2007. The regression equations are indicated.

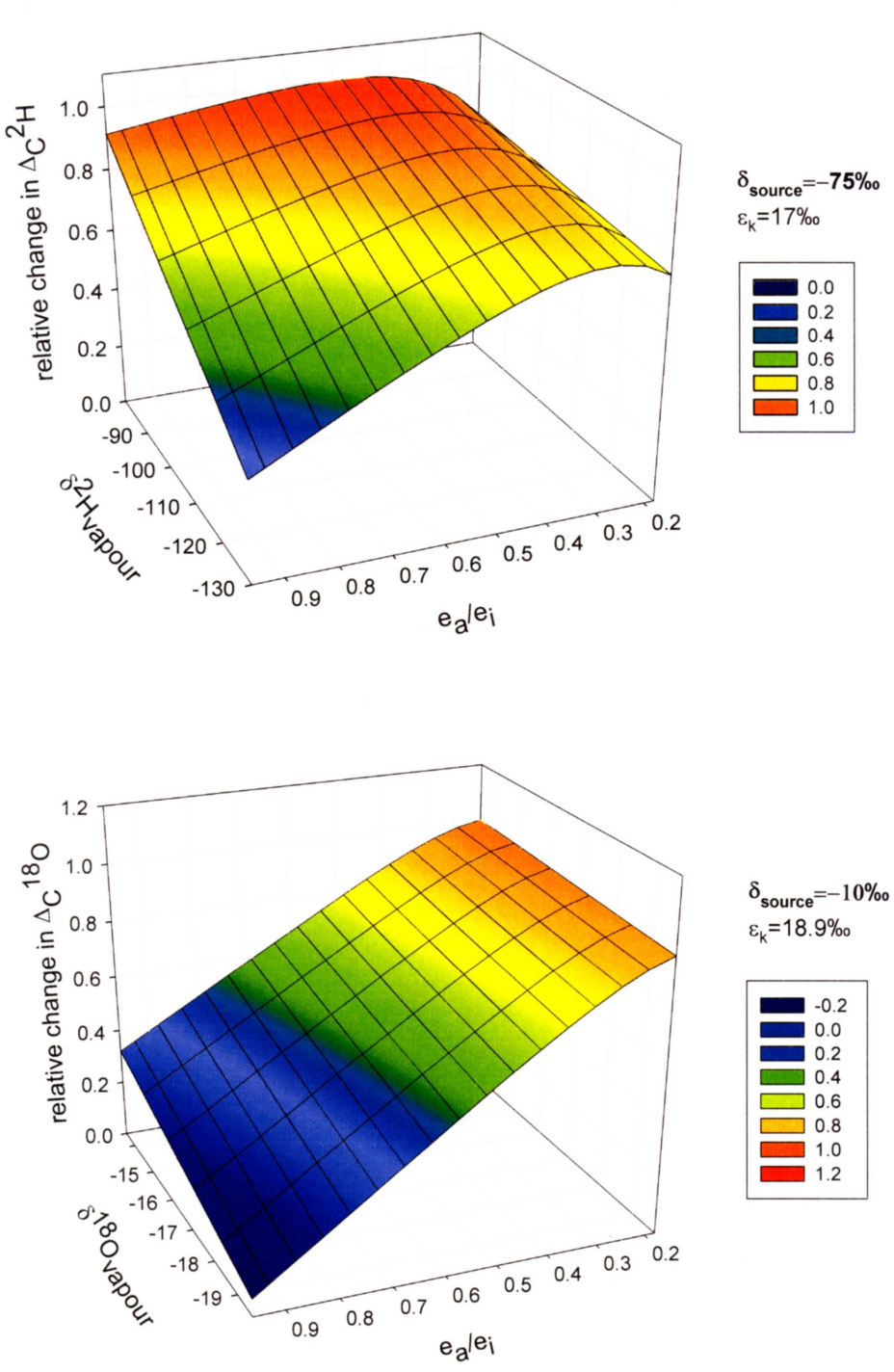


Fig A3.2 Relative change in Δ_C as a function of isotopic composition of air water vapour (δ_{vapour}) and ratio of water vapour pressure in ambient air to that in leaf interior (e_a/e_i). Source water isotopic composition was held constant (δ_{source}= -75‰ and -10‰ for hydrogen and oxygen, respectively).

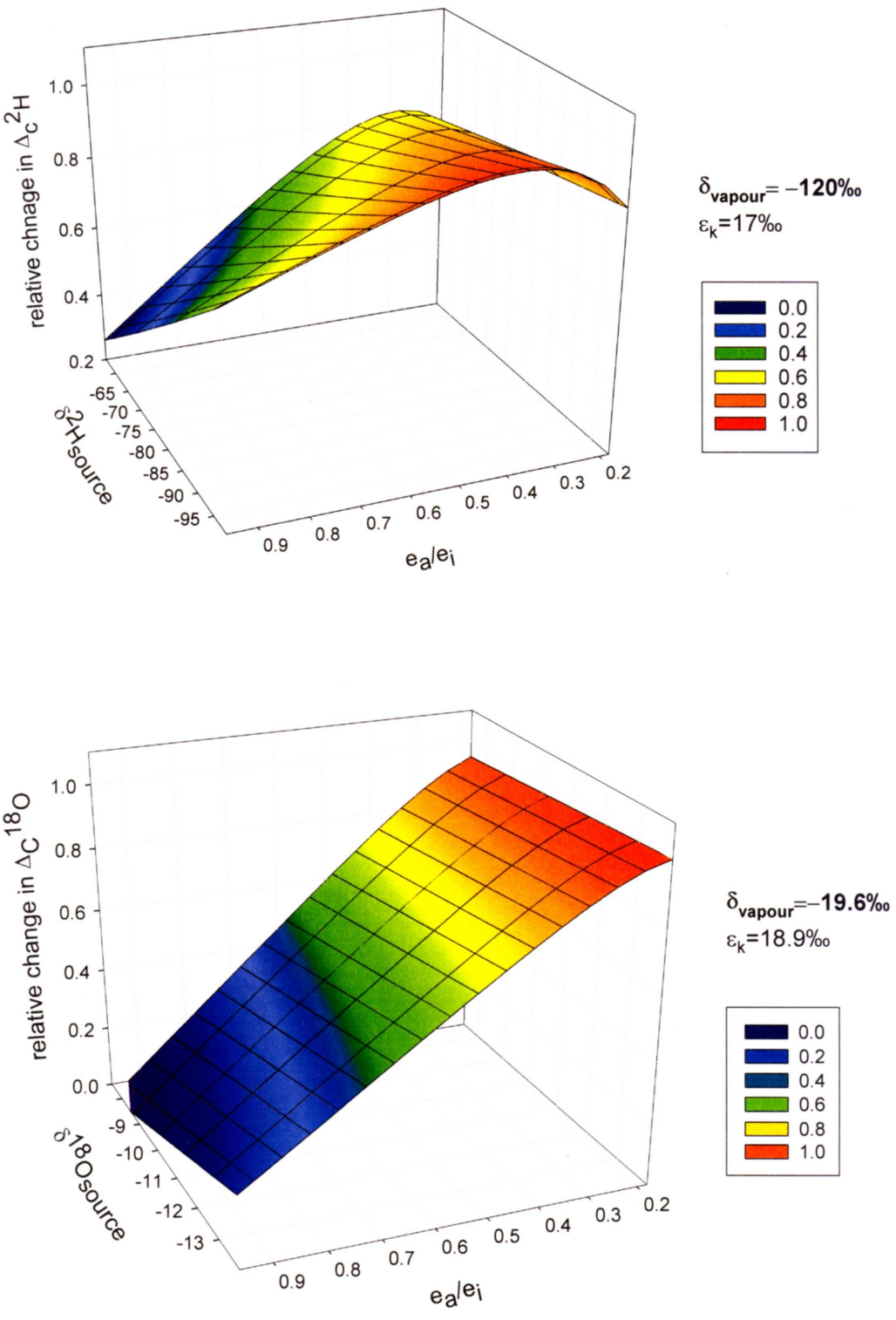


Fig A3.3 Relative change in Δ_C as a function of isotopic composition of source water (δ_{source}) and ratio of water vapour pressure in ambient air to that in leaf interior (e_a/e_i). Air water vapour isotopic composition was held constant (δ_{vapour}= -120‰ and -19.6‰ for hydrogen and oxygen, respectively).

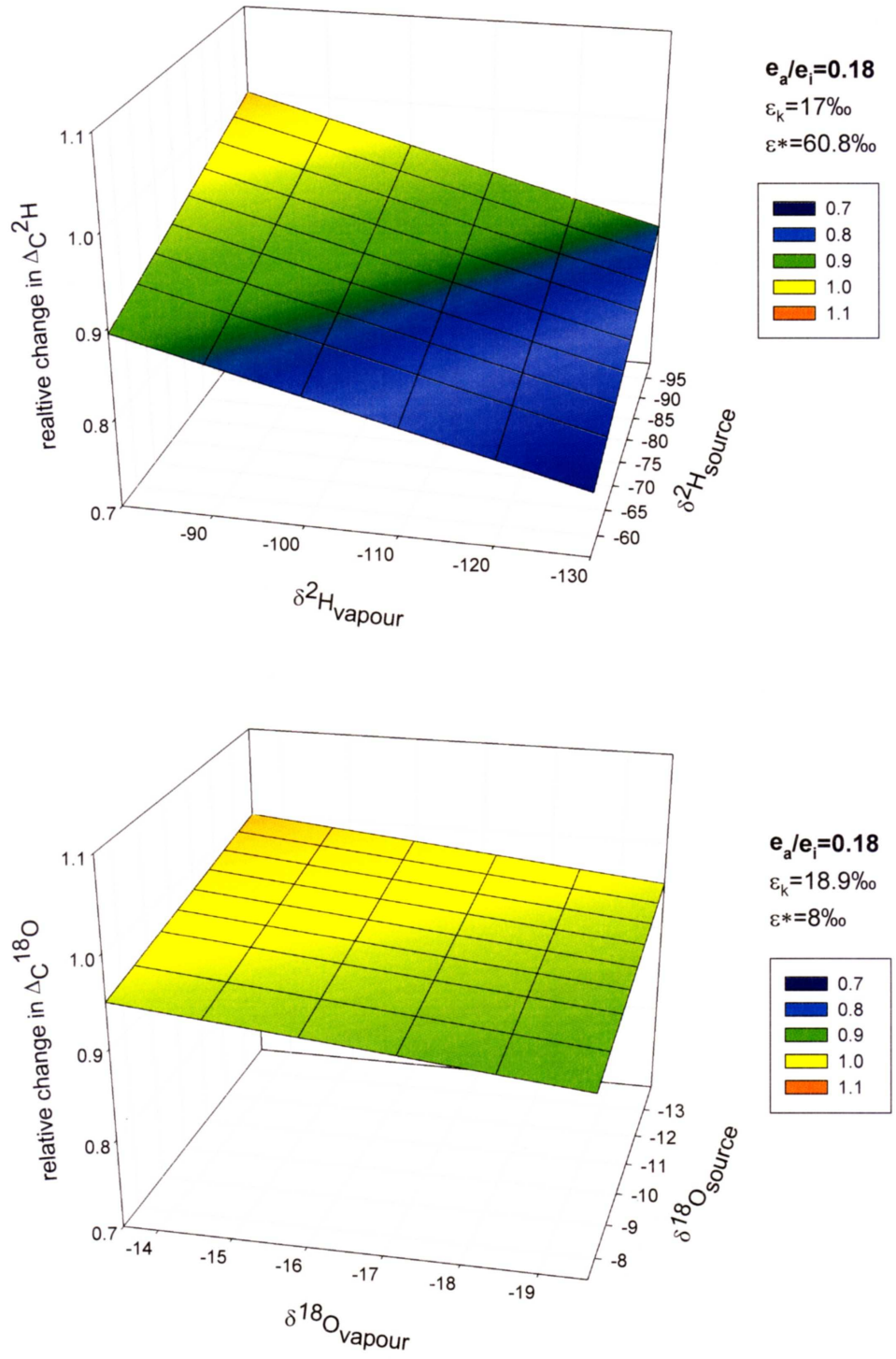


Fig A3.4 Relative change in Δ_C as a function of isotopic composition of air water vapour (δ_{vapour}) and isotopic composition of source water (δ_{source}). Ratio of water vapour pressure in ambient air to that in leaf interior (e_a/e_i) was held constant at 0.18 (i.e. high evaporative demands).

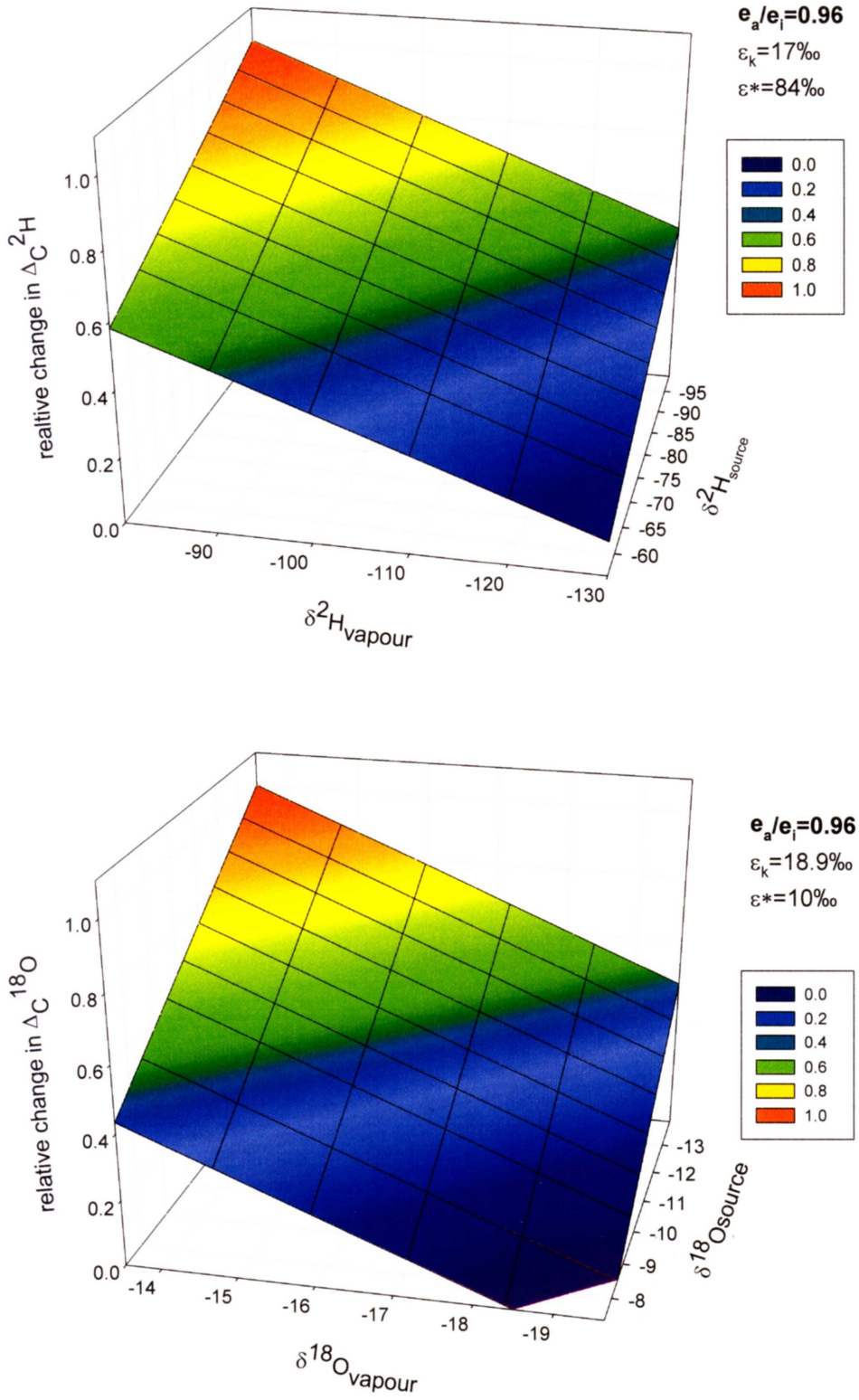


Fig A3.5 Relative change in Δ_C as a function of isotopic composition of air water vapour (δ_{vapour}) and isotopic composition of source water (δ_{source}). Ratio of water vapour pressure in ambient air to that in leaf interior (e_a/e_i) was held constant at 0.96 (i.e. low evaporative demands).

Conclusion:
The effect of e_a/e_i ratio on $\Delta_C{}^2H$ was suppressed when δ_{source} was more negative or δ_{vapour} was less negative. Moreover, change in ε^*, which is intercorrelated with e_a/e_i, caused $\Delta_C{}^2H$ to decrease or at least to increase slower with decreasing e_a/e_i. Neither of the above mentioned phenomena were such strong in oxygen to mask effectively the effect of e_a/e_i. On the whole, leaf water enrichment in oxygen is determined mostly by e_a/e_i ratio while hydrogen signal is more vulnerable to other factors which may suppress the effect of e_a/e_i.