

# **Compound-specific hydrogen isotope ratios of sedimentary n-alkanes: a new palaeoclimate proxy**

## **Dissertation**

Zur Erlangung des akademischen Grades doctor rerum naturalium

(Dr. rer. nat.)

vorgelegt dem Rat der Chemisch-Geowissenschaftlichen Fakultät der Friedrich-Schiller-  
Universität Jena

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Geboren am 24.09.1974 in Halle/Saale

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Datum der öffentlichen Verteidigung: 29. Juni 2005

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## 1. Introduction

The reconstruction of past climate variability can provide valuable information on the causes, timing and magnitude of climate change. Since direct observational evidence for climatic changes is available only for the past 100 years, so-called palaeoclimate proxies (e.g. isotope ratios, pollen data, palaeontology, sedimentological methods etc.) can provide us with a better understanding of natural climate variability and its forcing mechanisms. Analysis of ice-cores from the polar regions and sediments have provided valuable information on the natural climate variability throughout the geological time. The obtained data are used to evaluate climate models, which are needed to predict possible scenarios of anthropogenic climate change. However, even with the improved knowledge of the climate system components obtained over the last years, it is evident that the interactions between the biosphere, atmosphere, lithosphere and especially the hydrosphere are, despite their generally accepted importance, still poorly understood. Research on dynamics of the water cycle in the recent geological past usually focused on the oceans, because of the availability of complete sedimentary archives. Important factors influencing terrestrial ecosystems, such as atmospheric circulation, precipitation and evapotranspiration, could not be reconstructed directly because of a lack of direct proxies. Through the use of indirect proxies, such as sedimentological evidence, fossil plants, etc., some information became available, but remains scarce.

It was recognised long ago that the stable isotopes of water (protium/deuterium, oxygen16/oxygen18) can be used to follow fluxes of water in the water cycle. The ratio of these isotopes in water (expressed as relative deviations from “standard mean ocean water”, SMOW, in  $\delta D$  and  $\delta^{18}O$  values) holds information on the water source region, temperature and evaporation (CRAIG, 1961; GAT, 1996). Tropical ice-cores are doubtlessly the best terrestrial archives for water cycle reconstruction yet, because  $\delta D$  and  $\delta^{18}O$  values can be determined directly on the ice. Data from ice-cores extended the knowledge about atmospheric dynamics in the recent geological past (THOMPSON et al., 2003). However, ice-cores are limited to the high-altitude regions, such as the Andes, the Himalayas and partly Africa (Kilimanjaro). With the global retreat of glaciers as a first effect of Global Warming (OERLEMANS, 2005), these valuable climate archives will be severely damaged and eventually be lost.  $\delta^{18}O$  values determined on carbonate sediments and/or carbonate shells from lake sediments have been shown to record the source water  $\delta^{18}O$  value and therefore variations in climate (VON GRAFENSTEIN et al., 1999). However, interpretation of these records is not straightforward, since the fractionation of oxygen isotopes during incorporation into the shells is dependent on

species, water temperature and the salt content of the water, which can only be estimated from other proxies for the geological past.

The development of compound-specific isotope ratio measurement on individual organic compounds, first for carbon (HAYES et al., 1990) and in the late 1990ies for hydrogen (BURGOYNE and HAYES, 1998; HILKERT et al., 1999) opened new possibilities in tracing the carbon and hydrogen sources of individual biomarker compounds. The  $\delta D$  values of cellulose and also bulk plant lipids from biomass were already proven to record the source water isotopic composition (ESTEP and HOERING, 1980; NORTHFELT et al., 1981; STERNBERG, 1988).

However, cellulose is not stable over geological timescales and  $\delta D$  values of bulk lipids vary due to different biochemical pathways of lipid biosynthesis and associated distinct isotopic fractionations. n-Alkanes belong to one biochemical group of compounds, contain only carbon-bound hydrogen, which is non-exchangeable for temperatures up to 150°C (SCHIMMELMANN et al., 1999) and hold information on their biological source. n-Alkanes are abundant in sediments from the geological past and routinely used for palaeo-ecosystem reconstructions. These compounds are of particular interest since different classes of these hydrocarbons can be used to distinguish terrestrial (i.e. higher plant) from aquatic (i.e. phytoplankton) production. Sessions et al. (1999) were the first to suggest, that different lipid classes have different hydrogen isotopic fractionations between lipid and source water ( $\epsilon$ ), explaining the observed variability in the bulk lipid fraction. Although only 3 different organisms were investigated an  $\epsilon$  value of -160‰ was observed for n-alkanes in laboratory studies. It was hypothesised that  $\epsilon$  is independent of environmental parameters and only dependant on the biochemical pathway used for lipid synthesis. If this were the case, sedimentary n-alkane  $\delta D$  values could be used to reconstruct the isotopic composition of the organisms' source water, and therefore variations in climate. In the following years applications of this new proxy for palaeoclimate reconstruction became already available with promising results over various geological timescales (ANDERSEN et al., 2001; HUANG et al., 2002; SACHSE et al., 2004a; XIE et al., 2000). However, a systematic study investigating the relationships between source water  $\delta D$  values and n-alkane  $\delta D$  values in natural ecosystems is still missing.

## **1.1. Objectives**

The aim of this thesis was to present a systematic study, comparing modern sedimentary and biomass n-alkane  $\delta D$  values with climate, to offer a solid base for the application of this new and promising palaeoclimate proxy. Therefore sediments from 12 lakes throughout Europe following a gradient in meteoric water  $\delta D$  values from -120‰ in Northern Finland to -30‰ in Southern Italy, were recovered and analysed for n-alkane  $\delta D$  values.

To understand the origin of the terrestrial biomarker record, the dominating biomass surrounding these lakes was also sampled. It is well known, that the leaf water in higher terrestrial plants, which is the source water plants use for biosynthesis, is enriched by evaporation processes in the plants leaves by 20-80‰ relative to the meteoric water (DAWSON and EHLERINGER, 1993; LEANEY et al., 1985; ZIEGLER, 1989). In temperate climates, as in Europe, the plant's leaves will be deposited within lakes in autumn. So, if n-alkanes are produced *de novo* year-round in the leaf waxes, the autumn signal would be preserved. It is known, that the chemical composition, e.g. the relative abundance of different compounds, of leaf waxes is changing over the year (GULZ, 1994), however, no such data exist for compound-specific isotope values.

Consequently, in this thesis the following questions, will be addressed:

- Do n-alkane  $\delta D$  values from lake sediments record the modern climate variability?
- Is the isotopic fractionation of hydrogen during lipid biosynthesis ( $\epsilon$ ) in natural systems independent of environmental parameters?
- Is the isotopic enrichment observed in the leaf water of plants also represented in the leaf wax n-alkanes and in sedimentary n-alkanes of terrestrial origin?
- Do  $\delta D$  values of leaf wax n-alkanes show seasonal variations?

These issues culminate in the question:

How robust are sedimentary n-alkane  $\delta D$  values as a proxy for changes in the water cycle over geological timescales?

## **1.2. Thesis Organisation**

The thesis is organized into 9 chapters. The following chapter 2 explains the common methodologies used in all studies (measurement of  $\delta D$  values on individual organic compounds) as well as the sampling sites. Chapters 3 to 5 are peer-reviewed published (Chapter 3 was published in *Geochimica et Cosmochimica Acta* (SACHSE et al., 2004b)) or submitted (Chapter 4 and 5) articles.

Chapter 3 describes the sedimentary n-alkane  $\delta D$  values of 12 European lakes, chapter 4 deals with the associated plant biomass n-alkanes from the lake catchments, whereas seasonal variation in leaf wax n-alkane  $\delta D$  values over the 2004 growing season are discussed in Chapter 5. The thesis ends with a concluding discussion (Chapter 6), where results are synthesised, a summary (Chapter 7 and 8, in German) and the references (Chapter 9).



## 2. Methods/Strategies

### 2.1. Sample Sites and Field Sampling

All sampled lakes (Figure 1) are small, groundwater fed lakes with a relatively small catchment area (Table 1) and low human impact except Pääjärvi Lake in Finland, which is significantly bigger and Lago di Massaciucoli, which is subject to temporal salt-water influx from the Mediterranean Sea. Six lakes are oligotrophic, 4 lakes eutrophic, one lake is mesotrophic and one is oligo/mesotrophic. For an overview of basic limnological parameters see Table 2. The sites cover a mean annual temperature range from  $-2.0\text{ }^{\circ}\text{C}$  (Naimakka, northern Sweden) to  $13.7\text{ }^{\circ}\text{C}$  (Monticchio, southern Italy). Temperature and evaporation are the main factors controlling the  $\delta\text{D}$  value of the meteoric water, source water for the lakes (GONFIANTINI, 1986). The resulting mean annual  $\delta\text{D}$  value of meteoric water on the sites cover a range from  $-119.0\text{ }_{\text{‰}}$  vs. VSMOW in northern Sweden/Finland (mean annual  $\delta\text{D}$  from 1992-1995 in Naimakka,

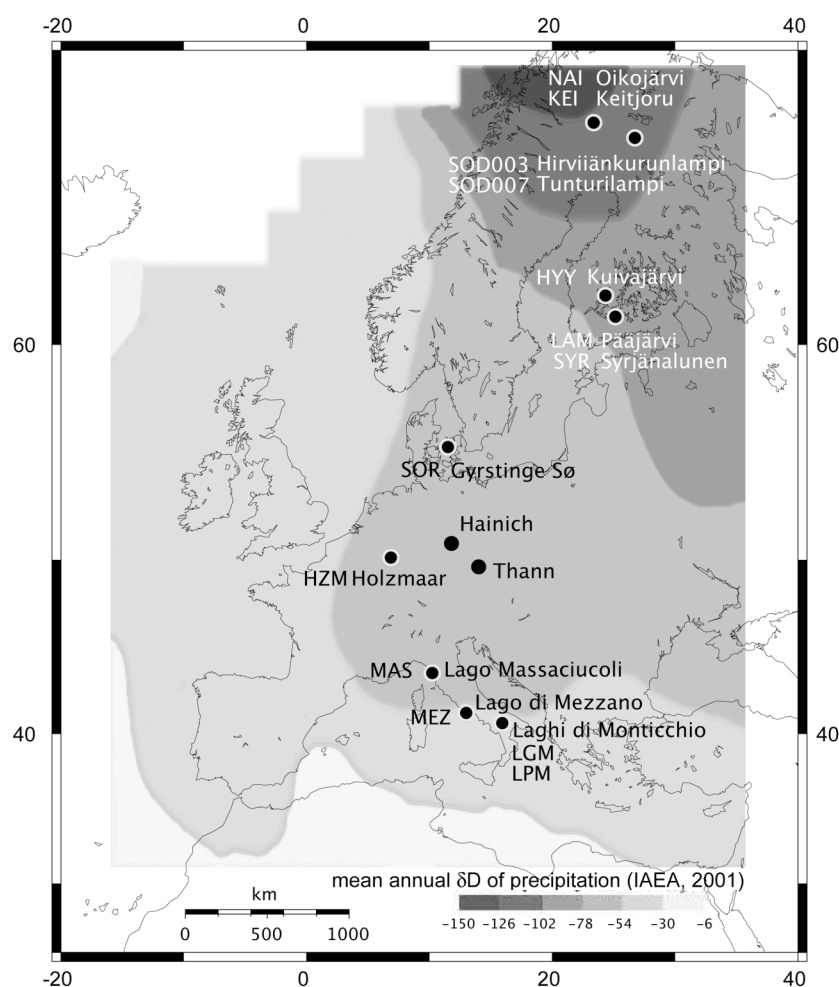


Figure 1: Location of the sampling sites and mean annual  $\delta\text{D}$  values of meteoric water over Western Europe (IAEA, 2001)

Sweden) to  $-36.6\text{‰}$  vs. VSMOW in southern Italy (mean annual  $\delta\text{D}$  from 2000 in Bari) (IAEA, 2001) (Figure 1).

Sediments were sampled in August and September 2002 using a gravity corer (HTH-Teknik, Luleå, Sweden) operated from a dismountable raft. The new sediment covering roughly the last year (usually the upper 1 to 2 cm), was collected up to 4 times in the deepest part of the lake, determined from depth maps and echo-sounding. In Lake Pääjärvi, the eastern basin, whose maximum depth is 44 m, was sampled. Water temperature-, pH-, oxygen saturation and redox profiles were determined on-site using a Multiprobe (YSI Inc., Yellow Springs, Ohio, U.S.A.), ensuring comparability of the lakes. Water samples were collected using a water sampler (Hydro-Bios Apparatebau GmbH, Kiel, Germany) and a pump from the high productivity zone of the lake (usually 1-2 m). Additionally the in- and outflow of the lake, if existent, was sampled. All water samples (11) were filtered through a  $0.45\ \mu\text{m}$  GF/F filter. The GF/F filters were frozen and later used for chlorophyll *a* concentration measurement (Table 2). For this purpose filters were ground and dissolved in acetone, the extinction of the extract was measured using a UV/VIS spectrometer (Helios gamma, ThermoSpectronic, Madison, WI, U.S.A.) following the procedure from Jeffrey and Humphrey (1975).

Phosphorus concentration was determined on-site using a field photometer (PhotoLab S12, WTW GmbH & Co. KG, Weilheim, Germany).

Pooled leaf samples (about 20 shaded leaves from 2 m height) of the 2-3 dominating deciduous tree species were collected from the shores of the lakes, except for lake Grystinge (site SOR). Additionally, two forest sites in northern Finland (SOD004 and FIN002) and one in Italy (ITA001) were sampled for leaves and stream water (representing meteoric water) if available. At the Scandinavian lakes mosses, *Sphagnum* species and *Cladonia* species make up significant portions of the vegetation, and were therefore also sampled.

Since most of the lakes are situated close to a permanent research station, basic meteorological data such as temperature and precipitation data exist for the last 10 years. Long-term precipitation isotope data are taken from the IAEA GNIP database (IAEA, 2001) accessible on the internet. Additionally the Online Isotope Precipitation Calculator (OIPC, accessible at <http://www.waterisotopes.org/>) using the IAEA database and interpolation algorithms described in Bowen and Revenaugh (2003) was used for sites where no  $\delta\text{D}$  values exist. Calculated values were compared with available data and showed virtually no differences (Figure 3).

## Methods/Strategies

Table 1: Location and basic geographic and meteorological data of the sample sites.

Mean annual temperature data and mean annual precipitation data from: NAI, KEI: personal communication D. Hammarlund; SOD: Finnish Meteorological Institute (personal communication T. Laurilla); HYY: Helsinki University (personal communication T. Vesala); LAM: Lammi Biological Station (personal communication L. Arvola); SOR: wetter.com for Kopenhagen; HZM: Manderscheid station, Deutscher Wetterdienst; MAS: worldclimate.com for Pisa; MEZ: data from Valentano (RAMRATH, 1997).; LGM, LPM: Watts et al. (1996). Longitude, latitude and altitude (map datum WGS 84) were determined on-site using a handheld GPS. Water depth (e.g. of the sampling site) was determined using an echosounder; mean annual precipitation  $\delta D$  values were taken from the OIPC and the IAEA database (see text).

sample code	Lake or location	Geographic location	Altitude [m asl]	Mean annual temperature [°C]	Mean annual precipitation [mm]	mean annual $\delta D$ precipitation	sampled biomass
FIN002	near village of Kilpisjärvi (FIN)	69°00'16"N 20°53'52"E	496	-	-	-115.7	<i>Betula pubescens</i>
NAI	Oikojärvi (FIN)	68°50'55"N 21°10'50"E	463	-2.0	450	-114.9 -114.9 -114.9	<i>Betula pendula</i> Moss <i>Sphagnum</i>
KEI	Keitjoru (SWE)	68°40'7"N 21°30'58"E	428	-2.0	450	-114.1 -114.1	<i>Betula pubescens</i> <i>Cladonia</i>
SOD003	Hirviänkurunlampi (FIN)	67°22'44"N 26°51'3"E	178	-0.3	529	-103.9 -103.9 -103.9	<i>Betula pubescens</i> <i>Cladonia</i> <i>Fagus sylvatica</i>
SOD007	Tunturilampi (FIN)	67°21'23"N 27°10'6"E	293	-0.3	529	-103.9 -103.9 -103.9	<i>Betula pendula</i> <i>Cladonia</i> <i>Sphagnum</i>
SOD004	near village of Luosto (FIN)	67°10'20"N 26°51'35"E	300	-	-	-103.9 -103.9	<i>Betula pendula</i> Moss
HYY	Kiuvajärvi (FIN)	61°50'55"N 24°16'46"E	149	3.5	640	-92.9 -92.9 -92.9	<i>Betula pendula</i> Moss <i>Sphagnum</i>
SYR	Syrjänalunen (FIN)	61°11'37"N 25°8'29"E	156	-	-	-91.4 -91.4 -91.4	<i>Myrte</i> <i>Betula pendula</i> Moss
LAM	Pääjärvi (FIN)	61°3'36"N 25°5'55"E	151	3.6	619	-91.3 -91.3	<i>Betula pendula</i> <i>Cladonia</i>
SOR	Grystinge (DK)	55°33'11"N 11°41'42"E	30	8.6	636	-67.1	-
HZM	Holzmaar (GER)	50°7'10"N 6°52'44"E	436	10.5	1042	-60.1 -60.1	<i>Betula pendula</i> <i>Fagus sylvatica</i>
MAS	Lago di Massachiucoli (ITA)	43°50'N 10°18'49"E	18	14.1	906	-41.6 -41.6 -41.6	<i>Quercus variabilis</i> <i>Alnus incana</i> <i>Quercus cerris</i>
ITA001	near village of Castigliano (ITA)	42°45'14"N 10°55'22"E	11	-	-	-38.9	<i>Quercus robur</i>
MEZ	Lago di Mezzano (ITA)	42°36'46"N 11°46'9"E	466	13.1	1030	-45.7 -45.7	<i>Quercus petraea</i> <i>Carpinus betulus</i>
LGM	Lago Grande di Monticchio (ITA)	40°55'58"N 15°36'10"E	674	13.7	815	-47.3	<i>Fagus sylvatica</i>
LPM	Lago Piccolo di Monticchio (ITA)	40°55'55"N 15°36'48"E	685	13.7	815	-47.3	

Table 2: Limnological data of the sampled lakes as determined on-site.

T – temperature; DO – dissolved oxygen, TDS – total dissolved solids; chl a – chlorophyll a; PO4-P: phosphorous (as PO<sub>4</sub> species)

sample code	Water depth [m]	Lake area [km <sup>2</sup> ]	Catchement area [km <sup>2</sup> ]	Secchi depth [m]	water T surface [°C]	DO saturation below thermocline [%]	TDS surface [μS]	pH surface	chl a conc. above thermocline [μg/l]	PO4-P [μg/l]	trophic state
NAI	8.4	1.5	4.5	3.65	16.5	73	27	6.82	3.6	<50	oligotrophic
KEI	2.0	0.01	1.5	2.00	15.0	93	14	6.25	0.3	<50	oligotrophic
SOD003	4.7	0.011	> 50	3.87	15.7	92	31	6.86	2.9	<50	oligotrophic
SOD007	10.7	0.36	8.4	2.95	16.3	50	15	7.28	0.7	<50	oligotrophic
HYY	13.3	0.84	30	1.56	21.1	44	28.7	6.57	5	<50	mesotrophic
SYR	8.5	0.3	n.d.	4.35	20.6	92	51	7.67	0.7	<50	oligotrophic
LAM	44.9	13.42	244	1.77	23.3	50	93	7.18	3.4	n.d.	mesotrophic
SOR	8.2	2.64	n.d.	0.70	22.1	92	420	8.64	63.4	60	eutrophic
HZM	24.0	0.058	2	1.40	20.3	10	246	8.5	4.9	50	eutrophic
MAS	2.0	7	n.d.	0.30	25.5	n.d.	4250	8.3	7.3	<50	eutrophic
MEZ	30.4	0.445	0.907	9.96	22.7	88	197	7.77	0.1	<50	oligotrophic
LGM	36.7	0.405	2.37	1.40	22.5	20	440	7.25	6.8	<50	eutrophic
LPM	36.0	0.08	2.37	3.50	20.6	n.d.	350	7.72	2.5	<50	oligo/meso

## 2.2. Analysis of water samples for $\delta^{18}\text{O}$ and $\delta\text{D}$

The isotope ratios on water were measured by on-line high-temperature (1300°C) reduction in a modified glassy carbon reactor of a high-temperature elemental analyser (TC/EA) coupled to an IRMS (Delta<sup>plus</sup>XL, Finnigan MAT, Bremen, Germany). The average standard deviation ( $2\sigma$ ) was 0.5 ‰ for  $\delta\text{D}$  values and below 0.1 ‰ for  $\delta^{18}\text{O}$  values. A detailed description of the methodology is given in Gehre *et al.* (2004).

## 2.3. Sample preparation, biomarker identification and quantification

Sediment samples were ground and freeze dried. Soluble organic matter was extracted using an accelerated solvent extractor (ASE200, Dionex Corp., Sunnyvale, U.S.A.) with dichloromethane/methanol mixture (10:1) at 100°C and 2000 psi (138 bar) for 15 min in 2 cycles. Depending on the amount of organic C in the sediment samples 2-7 g of sample were used for extraction. Since biomass samples contain much higher amounts of extractable hydrocarbons than sediment samples, 1g of leaf material was sufficient for extraction. Where sufficient amount of sediment was available, the extraction procedure was performed on 2-3 independent samples.

The total extract was separated on a chromatographic column made of glass (ca. 20 cm height, 2.5 cm diameter) (QVF Labortechnik GmbH, Ilmenau, Germany) filled with ca. 25 cm<sup>3</sup>

activated silica gel (0.040 – 0.063 mm mesh size; Merck KGaA, Darmstadt, Germany) into 3 fractions: aliphatic compounds were eluted from the column with 60 ml hexane, aromatics with 40 ml chloroform and other compounds with 40 ml methanol. The chloroform and methanol fractions were archived. Compounds of the aliphatic fraction were identified and quantified using a GC-FID (TraceGC, ThermoElectron, Rodano, Italy) by comparison to an external n-alkane standard mixture (n-C<sub>10</sub> to n-C<sub>32</sub>). The GC was equipped with a DB5ms column (30 m, ID:0.32 mm, film thickness: 0.5 µm, Agilent, Palo Alto, U.S.A.). The PTV injector was operated in constant temperature mode at 280°C with a split ratio of 1:10. The oven was held for 2 min at 80°C then heated at 8°C/min to 320°, where the final temperature was held for 5 min. The column flow was constant at 1.8 ml/min throughout the run.

#### ***2.4. Gas Chromatography Temperature Conversion Isotope Ratio Monitoring Mass Spectrometry (GC-TC-IRMS) for analysis of δD on the n-alkanes***

1 µl of the hexane dissolved aliphatic fraction was injected into a HP5890 GC (Agilent Technologies, Palo Alto, U.S.A.), equipped with a DB5ms column (30 m, ID:0.32 mm, film thickness: 0.5 µm, Agilent). The injector was operated at 280°C in splitless mode. The oven was maintained for 2 min at 60°C then heated at 6°C/min to 320°C and held for 10 min at the final temperature. The column flow was held constant at 1.7 ml/min throughout the run. One fraction of the separated compounds was transferred to an ion-trap mass spectrometer (GCQ, ThermoElectron, San Jose, U.S.A.) to monitor possible co-elution of the n-alkanes with other substances. If co-elution was evident in the MS spectrum, the δD value was not used for interpretation. The other fraction was transferred to a high-temperature conversion oven operated at 1425°C (BURGOYNE and HAYES, 1998; HILKERT et al., 1999) and quantitatively converted to H<sub>2</sub>, which was introduced into an isotope ratio mass spectrometer (IRMS) (Delta<sup>plus</sup>XL, Finnigan MAT, Bremen, Germany) for compound-specific analysis of δD values. Each sample was independently measured 3 times. All δD values were normalized to the VSMOW scale using a mixture of n-alkanes (n-C<sub>10</sub> to n-C<sub>32</sub>). The δD values of the n-alkanes in the standard mixture were calibrated against international reference substances (NBS-22, IAEA-OH22). Individual n-alkanes were analysed for their δD values using the offline TC/EA (Thermal Conversion/Elemental Analyser) technique, a detailed description of the calibration and standardization procedure can be found in Sachse (2002). After the measurement of no more than 2 samples (6 GC runs), the standard mixture was measured independently 3 times. If necessary a drift correction was applied. The reaction of H<sub>2</sub><sup>+</sup>

ions with H<sub>2</sub> molecules in the ion source of the IRMS results in the formation of H<sub>3</sub><sup>+</sup> ions, which also contribute to the measured mass 3. Since the amount of H<sub>3</sub><sup>+</sup> ions formed in the source is a function of the gas pressure, a correction can be applied by determining the so-called H<sub>3</sub><sup>+</sup> factor (WERNER and BRAND, 2001). A constant H<sub>3</sub><sup>+</sup> factor therefore indicates stable ion source conditions. The H<sub>3</sub><sup>+</sup> factor is usually determined once a day. Achieved precision can be expressed as the average standard deviation for all measured samples and standards (3 individual measurements per sample or standard) and is given in Table 3 for the 3 measurement campaigns. The H<sub>3</sub><sup>+</sup> factor was constant during the measurement periods (Table 3).

Table 3: Standard deviation (2σ stdv) and H<sub>3</sub><sup>+</sup> factor of all measured samples and standard mixtures.

	Standard mixture stdv	Total no. of peaks	No. of runs	No. of peaks per run	Samples stdv	Total no. of peaks	No. of runs	No. of peaks per run	H <sub>3</sub> <sup>+</sup> factor	No.	stdv
Campaign 1 (Chapter 3)	4‰	3520	160	22	7‰	2700	180	15	5.9	23	0.3
Campaign 2 (Chapter 4)	3‰	437	23	19	4‰	114	60	1-3	3.9	11	0.1
Campaign 3 (Chapter 5)	4‰	1620	90	18	4‰	303	93	1-8	4.0	14	0.1

The same instrumental setup as for δD measurement was used for carbon isotope analysis on the leaf lipids (Chapter 5). Here the GC was coupled to the IRMS via a GC Combustion III interface, with the oxidation oven operated at 960°C. The δ<sup>13</sup>C values were normalized to the VPDB scale using the same n-alkane mixture as for hydrogen, with predetermined TC/EA δ<sup>13</sup>C values. Average standard deviation for all peaks was 0.3‰ for the samples (n=114, 60 runs with 1-3 peaks each, depending on sample), 0.4‰ for the standard mixtures (n=323, 17 runs with 9 peaks each, n-C<sub>14</sub> to n-C<sub>22</sub>).

## 2.5. Calculation of the isotopic fractionation ε

The isotopic difference between the δD value of the lake water and the δD value of the n-alkanes was calculated using equation 1.

$$\epsilon_{alkane/water} = 1000 \cdot \left( \frac{\delta D_{alkane} + 1000}{\delta D_{water} + 1000} - 1 \right) \quad \text{Equation 1}$$

### 3. Hydrogen isotope ratios of lacustrine sedimentary n-alkanes record modern climate variability

#### 3.1. Introduction

Compound specific hydrogen isotope ratios of organic compounds are emerging as a new palaeoclimatic and palaeohydrological proxy (ANDERSEN et al., 2001; HUANG et al., 2002; SACHSE et al., 2004a; SAUER et al., 2001; YANG and HUANG, 2003). The use of this technique on compounds originating from specific groups of organisms, so called biomarkers, opens new perspectives to trace changes in various compartments of the water cycle and hence climate variability in the geological past. The hydrogen isotope composition of environmental water is dependent on climatic conditions such as temperature, evaporation, precipitation and others (CRAIG, 1961; CRAIG and GORDON, 1965; GAT, 1996; GONFIANTINI, 1986). Current knowledge suggests that the fractionation of H isotopes in biosynthesis is constant and mostly controlled by the biochemical pathway used (SESSIONS et al., 1999). Therefore  $\delta D$  ratios of biomarkers have the potential to record changes in the isotopic composition of the H source. This is well known for  $\delta D$  values from cellulose of tree rings (EPSTEIN et al., 1976; FENG and EPSTEIN, 1995; STERNBERG, 1988; YAPP and EPSTEIN, 1982) and also for bulk plant lipids (STERNBERG, 1988). For n-alkanes from algae a fractionation ( $\epsilon_{n\text{-alkane/water}}$ ) of  $-160\text{‰}$  and for sterols  $-201\text{‰}$  has been reported (SAUER et al., 2001; SESSIONS et al., 1999). Moreover, n-alkanes from terrestrial higher plants are more enriched in deuterium, resulting in an  $\epsilon_{n\text{-alkane/water}}$  value of about  $-117\text{‰}$  for C3 plants (CHIKARAISHI and NARAOKA, 2003; ESTEP and HOERING, 1980; STERNBERG et al., 1984), since leaf water in plants, the H source for biosynthesis, is usually enriched by 20 to 80 ‰ relative to soil water (ZIEGLER, 1989).

Lake sediments are excellent climate archives (e.g. ALLEN et al., 1999), recording regional climate changes on the continents and are therefore the prime target for biomarker  $\delta D$  analysis. Evidentially, palmitic acid extracted from recent lake sediments throughout North America records the  $\delta D$  of the source water (HUANG et al., 2002). Other more specific biomarkers such as several sterols also record the  $\delta D$  value of the source water (SAUER et al., 2001). However, as carboxylic acids are only minor compounds in older sediments, those biomarkers are not always suitable for palaeoclimatic reconstruction. Therefore, n-alkanes, which are present in considerable amounts even in palaeozoic sediments, do not contain exchangeable H (SCHIMMELMANN et al., 1999) and are relatively easy to extract and purify, should be reliable compounds in terms of  $\delta D$  analysis.

The n-alkanes from recent sediments cover a wider range of  $\delta D$  values (SAUER et al., 2001), due to different biological sources. The occurrence of the short chain even-carbon numbered n-

C<sub>12</sub> to n-C<sub>22</sub> alkanes in sediments from different environments is generally attributed to bacteria (GRIMALT and ALBAIGES, 1987; HAN and CALVIN, 1969). Short-chained n-alkanes, especially n-C<sub>17</sub>, are generally considered as indicators for input by algae and photosynthetic bacteria (CRANWELL et al., 1987; HAN and CALVIN, 1969; HAN et al., 1968; MEYERS, 2003), n-alkanes with 21, 23 and/or 25 carbon atoms are derived from submerged aquatic plants (FICKEN et al., 2000), whereas the n-C<sub>25</sub> to n-C<sub>31</sub> alkanes originate from the leaf waxes of land plants (CRANWELL et al., 1987; EGLINTON and HAMILTON, 1967). The n-C<sub>23</sub>, n-C<sub>25</sub> and n-C<sub>31</sub> alkanes can also be derived from *sphagnum* species (BAAS et al., 2000). Consequently, the  $\delta D$  values of different n-alkanes should yield information on the origin of hydrogen in the bacteria, algae and plants.

First reconstructions of palaeohydrological conditions using  $\delta D$  values from sedimentary n-alkanes of various ages up to the Permian show promising results (ANDERSEN et al., 2001; SACHSE et al., 2004a; SAUER et al., 2001; XIE et al., 2000).

So far a systematic approach comparing recent sedimentary n-alkane  $\delta D$  values with climate data is still lacking. Therefore, we sampled surface sediments from 12 lakes along a N-S climatic gradient from Northern Finland to Southern Italy to test if the climatic gradient is recorded in n-alkane  $\delta D$  values and to present a solid base for the application of n-alkane  $\delta D$  values as a new palaeohydrological proxy.

## 3.2. Results and Discussion

### 3.2.1. n-alkane concentrations

In general, the total concentration of extractable n-alkanes is slightly higher in the lakes from southern Europe (up to 0.5 mg/g TOC) compared to the northern lakes (0.2 mg/g TOC as a minimum), indicating a higher organic input in the south (Figure 2). The concentration of the short-chain n-alkanes n-C<sub>17</sub> and n-C<sub>19</sub> of aquatic origin is highest in the eutrophic lakes (up to 0.4 mg/g TOC for n-C<sub>17</sub> in HZM). Most prominent n-alkanes in nearly all lake sediments are the long chain n-alkanes n-C<sub>25</sub> to n-C<sub>31</sub> of terrestrial origin, expressed through the high average chain length (ACL) values varying between 21.2 and 27.6 (Figure 2). A clear odd over even carbon number predominance was found in all sediments with carbon predominance indices (CPI) between 1.4 and 6.6 (Figure 2), typical for a biological origin. The CPI increases slightly from north to south, suggesting together with the longer vegetation period, higher input of organic material and higher productivity in the southern lakes, although dilution with other sedimentary components and different preservation regimes may also influence the TOC contents.



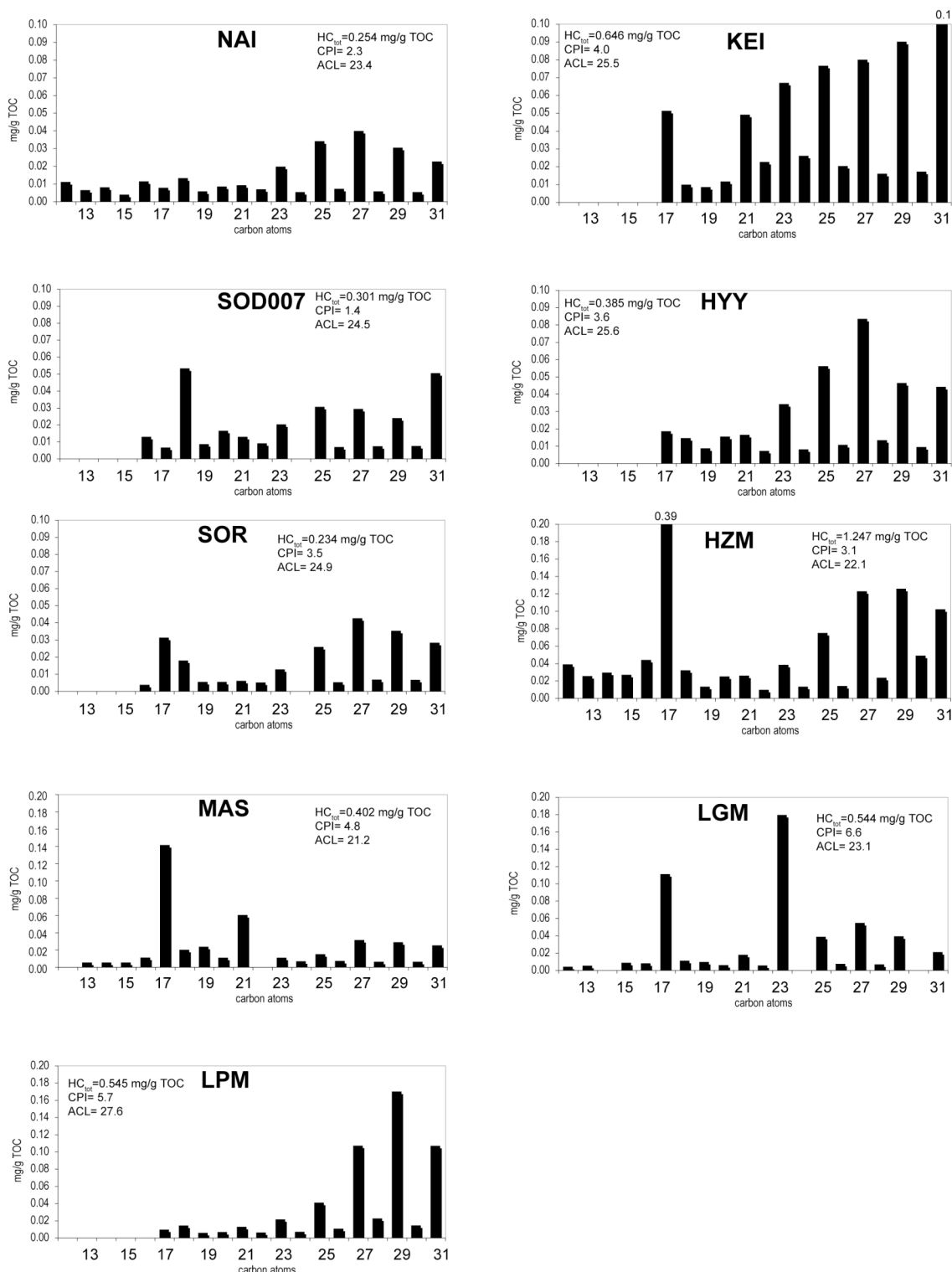


Figure 2: Distribution, CPI (Carbon Preference Index =  $\sum_{odd} C_n / \sum_{even} C_n$ ) and ACL (Average Chain Length =  $(\sum C_n * n) / \sum C_n$ ) indices and total concentration ( $HC_{tot}$ ) of the n-alkanes from 9 of the sampled lakes. Note that the scale of the y-axis (concentration) is doubled for the southern lakes (HZM, MAS, LGM, LPM) because of higher concentration of n-alkanes.

### 3.2.3. $\delta D$ and $\delta^{18}O$ values of water

The  $\delta D$  and  $\delta^{18}O$  values of the lake inflow water, if an inflow is present, are up to 14 ‰ heavier for  $\delta D$  and up to 1.5 ‰ heavier for  $\delta^{18}O$  relative to the calculated OIPC values (Fig. 3).

Considering the confidence interval of the calculated OIPC data, the inflow water  $\delta$  values are within the 95% limit or slightly heavier. Due to evaporation and temperature effects a heavier summer  $\delta$  value would be expected. The northernmost lake NAI represents a notable exception, since measured  $\delta D$  values are 21 ‰ and  $\delta^{18}O$  values 3.4‰ heavier than the calculated meteoric water. Since there is no dense vegetation cover around the small stream and summer 2002 was exceptionally warm and dry in Scandinavia, evaporation already affects the contributing stream. However, the isotopic data for the inflowing waters from the 7 lakes where those data exist, suggest, that the main water source is the meteoric water. Evaporation only seems to be of minor influence.

Table 4: Isotopic composition of meteoric water at the lake sites, taken from the IAEA GNIP database (IAEA, 2001) for the following sites:

1)Naimakka (1992-1995) about 1km from KEI site, 2)Taastrup (1965-1971), 3)Koblenz (1974-1975) 4)Pisa (1992-1995), 5)Ancona (2000), 6)Bari (9-12/2000). Note: except for Naimakka (KEI, NAI site), the IAEA sites are not directly at the sampling site, the differences in IAEA and OIPC data are due to the different geography (altitude, distance to the sea etc.).

OIPC  $\delta D$  and  $\delta^{18}O$  meteoric water data and 95% confidence interval (CI) were calculated using the Online Isotopes in Precipitation Calculator (OIPC) using the interpolation algorithms described in Bowen and Revenaugh (2003).

Lake and inflow water  $\delta D$  and  $\delta^{18}O$  values were measured. All  $\delta$  values given in ‰ vs. VSMOW.

sample code	$\delta D$ meteoric water (IAEA)	$\delta D$ meteoric water (OIPC)	95% CI	$\delta^{18}O$ meteoric water (OIPC)	95% CI	$\delta D$ inflow	$\delta^{18}O$ inflow	$\delta D$ lake water	$\delta^{18}O$ lake water	stdv (2 $\sigma$ )	stdv (2 $\sigma$ )	stdv (2 $\sigma$ )	stdv (2 $\sigma$ )	
NAI	-119.0	1)	-114.9	9.2	-15.43	1.12	-93.7	0.4	-12.04	0.16	-94.9	0.6	-12.5	0.0
KEI	-119.0	1)	-114.1	8.6	-15.32	1.06	-100.1	0.1	-13.75	0.07	-96.6	0.6	-13.62	0.04
SOD003	n.a.		-103.9	8.0	-14.04	1.17	-109.6	1.0	-14.55	0.01	-103.7	0.2	-13.92	0.02
SOD007	n.a.		-105.2	8.1	-14.23	1.19	-108.8	0.4	-14.98	0.05	n.d.	n.d.	-12.29	0.05
HYY	n.a.		-92.9	7.9	-12.85	1.12	-	-	-	-	-81.1	0.6	-10.59	0.00
SYR	n.a.		-91.4	7.0	-12.38	1.02	-	-	-	-	-86.5	0.3	-11.95	0.06
LAM	n.a.		-91.3	7.0	-12.36	1.02	-	-	-	-	-73.7	0.0	-9.55	0.14
SOR	-70.7	2)	-67.1	4.5	-9.45	0.61	-55.2	0.5	-7.79	0.05	-46.4	0.7	-6.40	0.08
HZM	-52.1	3)	-60.1	3.1	-8.67	0.51	-54.5	0.7	-8.48	0.09	-47.1	0.6	-6.44	0.07
MAS	-47.0	4)	-41.6	6.4	-6.47	0.71	-	-	-	-	-6.3	0.6	-0.24	0.05
MEZ	-40.1	5)	-45.7	6.3	-7.04	0.69	-36.8	0.3	-6.29	0.17	-3.5	0.6	0.65	0.11
LGM	-36.6	6)	-47.3	5.2	-7.23	0.56	-	-	-	-	-13.5	0.2	-0.92	0.10
LPM	-36.6	6)	-47.3	5.2	-7.23	0.56	-	-	-	-	-32.6	0.6	-3.99	0.09

$\delta D$  and  $\delta^{18}O$  values of the lake water from the high productivity zone (in general at 1-2m depth) are up to 20 ‰ heavier for hydrogen and up to 3 ‰ heavier for oxygen than the calculated OIPC values, except the three Italian lakes MAS, MEZ and LGM (Fig. 3). Nearly half of the variation still lies within the 95% confidence interval of the calculated OIPC data (Table 4). Furthermore, it must be acknowledged that the isotope ratios of the lake water are only a one-point measurement in summer, when evaporation very likely will enrich deuterium in the water.

However, since the organisms in the water column will incorporate hydrogen throughout the growing season and probably show most productivity in spring and late summer, the summer  $\delta D$  water value will be heavier than the average  $\delta D$  water value for the growing season. We therefore assume the OIPC data to be a mean annual value also valid for the lake water. For the Italian lakes MEZ and LGM a stronger evaporation effect is visible. The lake water is 33 to 35 ‰ heavier in  $\delta D$  than the calculated precipitation value (Fig. 3). Lake MAS, also in Italy, is a special case, because it lies within several kilometres of the Mediterranean coast and temporal influx from isotopically heavier seawater occurs. The isotopic ratios of these three Italian lakes do not plot on the Global Meteoric Water Line, but on an evaporation line, whereas all other lake waters do. In the case of the three Italian lakes an underestimation of the isotopic fractionation  $\epsilon$  would be the case, if we rely on the OIPC data. Despite this deviation in 3 southern lakes, the OIPC data have been used as the hypothetical mean annual  $\delta D$  composition of the lake water.

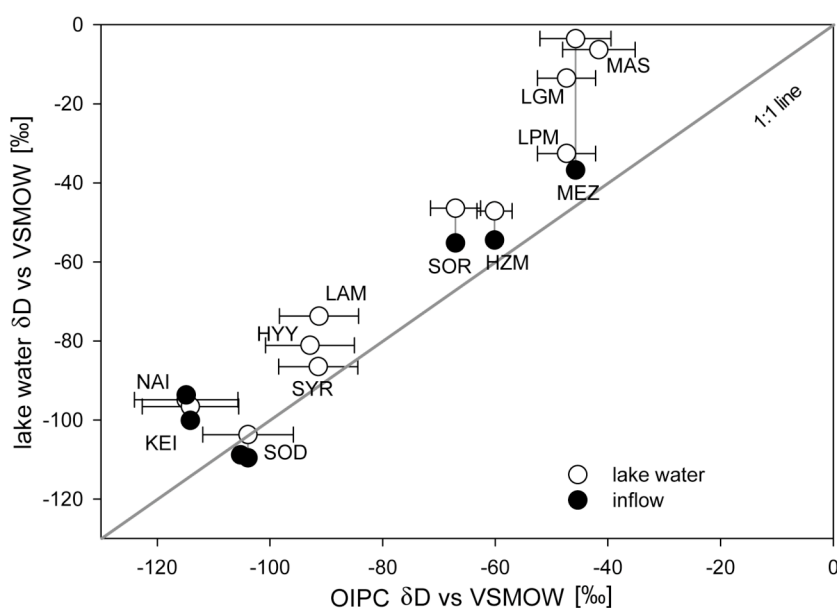


Figure 3: Comparison between lake water  $\delta D$  values (open circles: high-productivity zone, usually in 1-2 m depth; filled circles: inflows) and meteoric water  $\delta D$  values, calculated using OIPC (see text). Dotted lines show the enrichment/depletion of inflow water vs. lake water. Error bars for the  $\delta D$  of OIPC data constitute the calculated 95% confidence interval and are also applicable for the inflow data. Error bars (standard deviation) for  $\delta D$  lake water lie within the points.

### 3.2.4. $\delta D$ values of the n-alkanes

In general the  $\delta D$  values of n-alkanes from one sample can differ by more than 100‰. The main reasons for this variation are different biological sources of these n-alkanes, using different water sources for biosynthesis. Using  $\delta D$  values, four main n-alkane groups can be distinguished:

- 1) Even carbon numbered short-chain n-alkanes (n-C<sub>12</sub>, n-C<sub>14</sub>, n-C<sub>16</sub>, n-C<sub>18</sub>, n-C<sub>20</sub>) and n-C<sub>13</sub> and n-C<sub>15</sub>
- 2) Odd carbon numbered n-alkanes of medium chain length (n-C<sub>17</sub>, n-C<sub>19</sub>, n-C<sub>21</sub>, n-C<sub>23</sub>)
- 3) Even carbon numbered medium to long-chain n-alkanes (n-C<sub>22</sub>, n-C<sub>24</sub>, n-C<sub>26</sub>, n-C<sub>28</sub>, n-C<sub>30</sub>)
- 4) Odd carbon numbered long-chain n-alkanes (n-C<sub>25</sub> to n-C<sub>31</sub>)

In the following we will discuss the variations of the  $\delta D$  values of those n-alkane groups along the transect in detail.

#### **3.2.4.1. $\delta D$ values of the even carbon numbered short-chain n-alkanes (n-C<sub>12</sub>, n-C<sub>14</sub>, n-C<sub>16</sub>, n-C<sub>18</sub>, n-C<sub>20</sub>) and n-C<sub>13</sub> and n-C<sub>15</sub>**

$\delta D$  values for n-C<sub>16</sub> and n-C<sub>18</sub> could be determined on only 5 lake samples along the transect due to overall low concentration of these substances (Table 5). They usually are the heaviest measured  $\delta D$  values of the sample. Interestingly,  $\delta D$  values of the compounds are lighter in the south than in the north. The  $\delta D$  value for n-C<sub>18</sub> is significantly lighter than the other alkanes in the sediments from SOR and LGM sites, probably indicating a different H source for n-C<sub>18</sub> in these two eutrophic lakes.  $\delta D$  values from n-C<sub>16</sub> and n-C<sub>18</sub> correlate positively with latitude and negatively with mean annual temperature and meteoric water  $\delta D$  values (Table 5, Figure 4), although the number of samples is limited. The other short-chained substances seem to follow this trend, but statistical evaluation is not possible due to the limited amount of data.  $\epsilon_{\text{substance/water}}$  is increasing from the north to the south for those short-chained n-alkanes.  $\epsilon_{\text{C}_{16}/\text{w}}$  and  $\epsilon_{\text{C}_{18}/\text{w}}$  show a statistically significant inverse relationship with meteoric water  $\delta D$  and  $\delta D$  lake water values and correlate with temperature (Table 6, Figure 5). This is surprising since n-C<sub>16</sub> and n-C<sub>18</sub> are together with n-C<sub>17</sub> widely used as indicators for algal input. However, both n-alkane  $\delta D$  values significantly differ from n-C<sub>17</sub>  $\delta D$  values.

Our results suggest that  $\delta D$  values of n-C<sub>16</sub> and n-C<sub>18</sub> are independent of the meteoric water  $\delta D$  value and show an inverse relationship with mean annual temperature. The fractionation between water and n-C<sub>16</sub> and n-C<sub>18</sub> appears to be larger in areas with higher temperatures. To our knowledge such a relationship has not been reported until now.

Such large differences between the  $\delta D$  values of n-C<sub>16</sub>, n-C<sub>18</sub> and n-C<sub>17</sub> have not been observed in palaeozoic samples or oils (LI et al., 2001).

Several explanations might be considered:

1) Dependence of  $\epsilon_{\text{substance/water}}$  on growth rate.

Since  $\epsilon$  is smaller in the north (shorter vegetation period, less nutrient supply) than in the south for these possibly non-photosynthetic bacteria derived compounds, a fractionation dependent on growth rate is possible. Sessions et al. (1999) observed a wider range of  $\epsilon$  values for lipids in actively growing organisms than in plants. However, growth-rate dependency of  $\epsilon$  for fatty acids of a methane-oxidizing bacteria could not be confirmed (SESSIONS et al., 2002).

2) Different sources for these compounds in the north and in the south. Since  $\epsilon_{\text{substance/water}}$  values in the south are closer to  $\epsilon$  values of n-C<sub>17</sub>, they could share the same algal source in the southern lakes. The high  $\delta D$  values in the north could be related to methane-oxidizing bacteria, since Sessions et al. (2002) predict  $\delta D$  values of fatty acids produced by methanotrophs to be between  $-50$  to  $-170\%$ . However, it is unlikely that in the sediments of the oligotrophic Scandinavian lakes significant amounts of methane are produced. Another possible source for the isotopically light compounds in the north could be fossil hydrogen, possibly introduced by petroleum contamination. But contamination seems unlikely for the Scandinavian lakes (NAI, SOD), since population density is very low and most lakes are several kilometres away from rarely used roads. It is also unlikely for Lake Grystinge (SOR), used for drinking water storage with restricted access. Also petroleum contribution should not only affect the short-chained alkanes, but all alkanes, which is not evident. Fossil oil or shale samples lack the clear odd over even carbon number predomination expressed in the high CPI indices for the analysed samples.

It is unlikely that transport by wind over long distances accumulates significant amounts of those alkanes in the most remote areas, but not in more densely populated areas of southern Italy. The only lakes where motorboat traffic was allowed (LAM and MAS) do not contain sufficient amounts of short-chain alkanes and do not show lower than average CPI indices.

However, a possible mixing of petroleum derived alkanes with biologically derived alkanes cannot be ruled out, a way to test this hypothesis would be compound-specific radiocarbon analysis to determine the amount of fossil (“dead”) carbon.

Further research is necessary to clarify the origin of H in the short-chained n-alkanes with even carbon numbers and if different fractionations of the hydrogen isotopes occur in heterotrophs compared to autotrophic organisms.

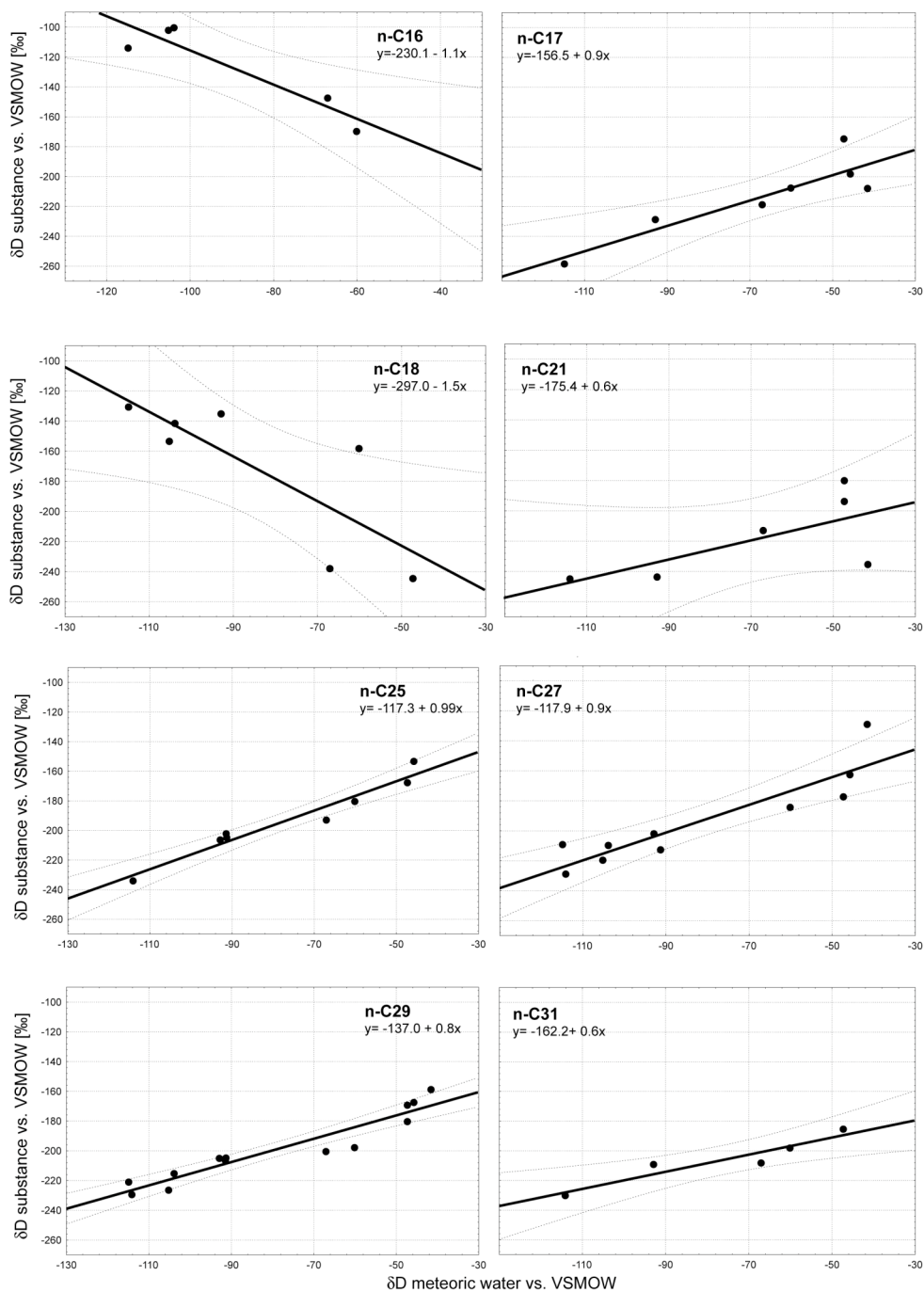


Figure 4:  $\delta D$  values of the n-C<sub>16</sub>, n-C<sub>17</sub>, n-C<sub>18</sub>, n-C<sub>21</sub>, n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub> and n-C<sub>31</sub> alkane vs. the meteoric water  $\delta D$  value (calculated using OIPC, (BOWEN and REVENAUGH, 2003)). Dotted lines show the 95% confidence interval of the regression line.

### 3.2.4.2. $\delta D$ values of the n-C<sub>17</sub>, n-C<sub>19</sub>, n-C<sub>21</sub>, n-C<sub>23</sub> alkanes

These substances, especially n-C<sub>17</sub> are of particular interest since they are widely used as a palaeoclimatic proxy.  $\delta D$  values of the n-C<sub>17</sub> alkane were determined in the samples from 8

lakes. The other samples either contained no n-C<sub>17</sub> or the substance could not properly be separated on the GC column. However, n-C<sub>17</sub> shows a clear negative correlation with latitude (Table 5, Figure 6) and a positive one with mean annual temperature.  $\delta D$  values of n-C<sub>17</sub> strongly correlate to the lake water  $\delta D$  value and to the  $\delta D$  value of meteoric water with an intercept of  $-156.5$  (Figure 4) suggesting that the meteoric water is the H source for n-C<sub>17</sub>.  $\epsilon_{C_{17}/W}$  values from the lakes range from  $-134$  to  $-174$  ‰ with the mean at  $-157$  ‰ (Table 6), about the same value as observed in laboratory experiments (SESSIONS et al., 1999).  $\epsilon_{C_{17}/W}$  is constant along the N-S transect (Table 6), covering different climates and lakes of different trophic state, supporting the hypothesis that the fractionation of hydrogen isotopes during incorporation into organic matter is only dependent on the biochemical pathway used and does not depend on environmental parameters. These results demonstrate that the n-C<sub>17</sub> alkane excellently records the lake water  $\delta D$  value and can be used to reconstruct the  $\delta D$  value of lake water (see also Figure 6).

n-C<sub>21</sub> and n-C<sub>23</sub> are considered to be derived from aquatic plants living in the water (FICKEN et al., 2000).  $\delta D$  values are often, but not always similar to n-C<sub>17</sub> with more scatter (Table 5). They are lighter in the north than in the south, following the same trend as n-C<sub>17</sub>, but, except for n-C<sub>19</sub> (although just 3 samples provided enough substance and no co-elution), do not correlate significantly with latitude, temperature or water  $\delta D$  values. This might be due to the higher standard deviations of  $\delta D$  values of n-C<sub>19</sub>, n-C<sub>21</sub> and n-C<sub>23</sub> relative to n-C<sub>17</sub>, since they often sit on a hump of unresolved compounds in the chromatogram.

Different water plants use water from different depths, characterized by different  $\delta D$  values, or they might have different evapotranspiration rates. n-C<sub>21</sub> and n-C<sub>23</sub>  $\delta D$  values seem to record the isotopic composition of lake water, but for reconstruction of the lake water  $\delta D$  value n-C<sub>17</sub> is much more recommended.

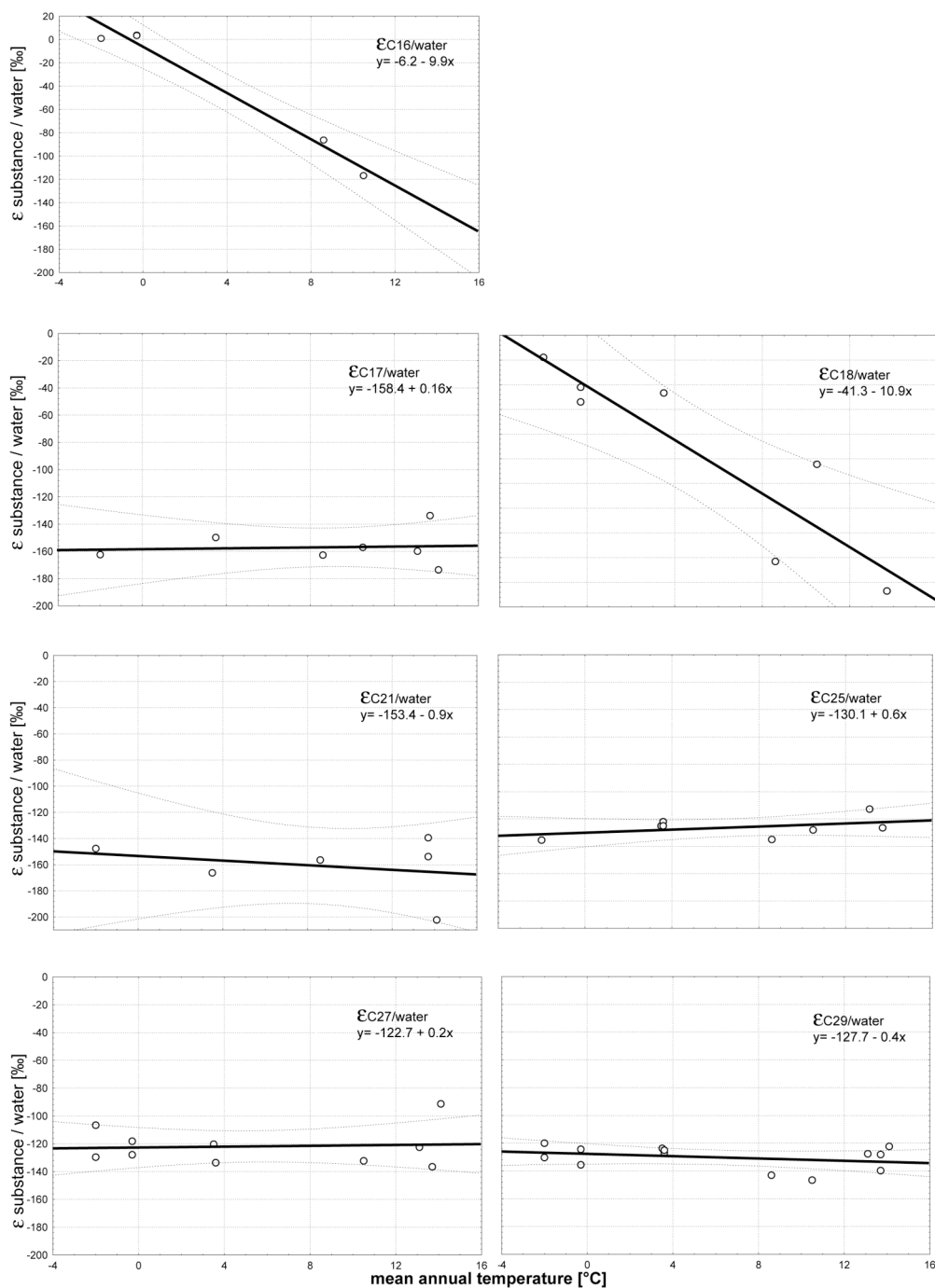


Figure 5:  $\epsilon_{\text{substance/water}}$  values calculated for the n-C<sub>16</sub>, n-C<sub>17</sub>, n-C<sub>18</sub>, n-C<sub>21</sub>, n-C<sub>25</sub>, n-C<sub>27</sub> and the n-C<sub>29</sub> alkanes plotted vs. mean annual temperature on the lake sites. Note: only the relationships for n-C<sub>16</sub> and n-C<sub>18</sub> are statistically significant (n-C<sub>16</sub>:  $p=0.002$ ,  $r=-0.99$ ; n-C<sub>18</sub>:  $p=0.006$ ,  $r=-0.90$ ).

### 3.2.4.3. $\delta D$ values of the even carbon numbered medium to long-chain n-alkanes (n-C<sub>22</sub>, n-C<sub>24</sub>, n-C<sub>26</sub>, n-C<sub>28</sub>, n-C<sub>30</sub>)

Since the substances are present in very low concentrations, only a few samples yielded enough substance to measure  $\delta D$  values. Interpretation of their origin is therefore speculative. However,



the even carbon numbered substances seem to be related in  $\delta D$  values to their following odd carbon numbered neighbours, being in general some per mil heavier.

$\delta D$  values of n-C<sub>22</sub> were only measured in 3 samples (KEI, SOD007 and SOR) and seem to be related to n-C<sub>21</sub> and n-C<sub>23</sub>, usually being several per mil heavier (Table 4).

n-C<sub>24</sub> (only measured in KEI and SOR) is about 20‰ heavier than n-C<sub>25</sub>. n-C<sub>26</sub>, n-C<sub>28</sub> and n-C<sub>30</sub> are also 10-20‰ heavier than n-C<sub>27</sub>, n-C<sub>29</sub> and n-C<sub>31</sub> in the same sample.

Overall it seems that the source of n-C<sub>22</sub> is the same source as for n-C<sub>21</sub> and n-C<sub>23</sub>, and n-C<sub>24</sub> to n-C<sub>30</sub> share the same source as their odd carbon numbered neighbours n-C<sub>25</sub> to n-C<sub>31</sub>, but involving some enrichment mechanism, resulting in 10 to 20‰ heavier  $\delta D$  values. Since this enrichment is not reported in plants (CHIKARAISHI and NARAOKA, 2003), it is likely that these alkanes are produced during microbial induced degradation or recycling processes of the odd-carbon numbered alkanes taking place in the sediment. However, it is also possible, that a different biological origin, for instance grass, which would be subject to different amounts of evapotranspiration than trees, is the reason for this slight enrichment.

#### 3.2.4.4. $\delta D$ values of the odd carbon numbered long-chain n-alkanes (n-C<sub>25</sub> to n-C<sub>31</sub>)

These four substances, commonly considered as indicators for higher terrestrial plant input, are the most abundant aliphatic compounds in all lake sediments sampled here, surpassed only by n-C<sub>17</sub> in some samples. All of these biomarkers show similar  $\delta D$  values in a given sample.

They negatively correlate with latitude and positively with mean annual temperature and  $\delta D$  values of meteoric water and lake water (Table 5, Figure 4). Substances are isotopically lighter in the north and about 70‰ heavier in the south (Figure 6).

$\epsilon_{C_{29/W}}$  ranges for all 12 lakes between -120 to -147‰ with the mean at -130‰ (Table 6). This is 27‰ heavier than the average  $\epsilon_{C_{17/W}}$ . Several studies have confirmed that leaf water in plants is enriched by 20 to 80‰ relative to soil water due to evapotranspiration processes in the leaf (ZIEGLER, 1989). Furthermore Chikaraishi & Naraoka (2003) report an  $\epsilon_{water}$  value for the long-chain n-alkanes from several C3 plants of -117‰, consistent with our data.

The long-chained odd carbon numbered n-alkanes record the meteoric water  $\delta D$  value. They are in general 30‰ heavier than n-C<sub>17</sub> (Figure 6), due to evaporation processes in the leaves of the plants. If we consider that the aquatic and terrestrial n-alkanes share the same H source, meteoric water, the difference between  $\delta D$  values of both substances could serve as a proxy for evapotranspiration of the lake ecosystem. The mean difference between the terrestrial and the aquatic  $\delta D$  values is highest for the three largest lakes MAS, LAM and NAI in terms of lake

area and catchment area with values between 40 and 70 ‰. In these lakes higher input of allochthonous biomass from distant areas with different isotopic composition of meteoric water can lead to higher differences of both  $\delta D$  values. The difference ranges around 10 to 20 ‰ for the other lakes, explainable by the enrichment of leaf water due to evaporation processes in the leaf. For the lakes LGM and LPM, which are at the same site and should experience the same amount of evapotranspiration, it is virtually identical (about 9‰). However, since we have no evapotranspiration measurements from the sites, we cannot completely explain the differences between the  $\delta D$  values of the terrestrial vs. aquatic substances and confirm that it is indeed a marker of the evapotranspiration of the lake ecosystem. n-alkanes originate from the epicuticular waxes of leaves, developed shortly after the unfolding of the leaf in spring (GULZ, 1994), when evapotranspiration is not yet very strong. However, the n-alkane composition of the wax is changing throughout the year, differing from tree species (PIASENTIER et al., 2000). If most of the n-alkanes are produced in spring, we would not see a stronger enrichment relative to the aquatic n-alkanes for sites with higher evapotranspiration in summer. This would explain the rather constant difference of 30 ‰ along the transect.

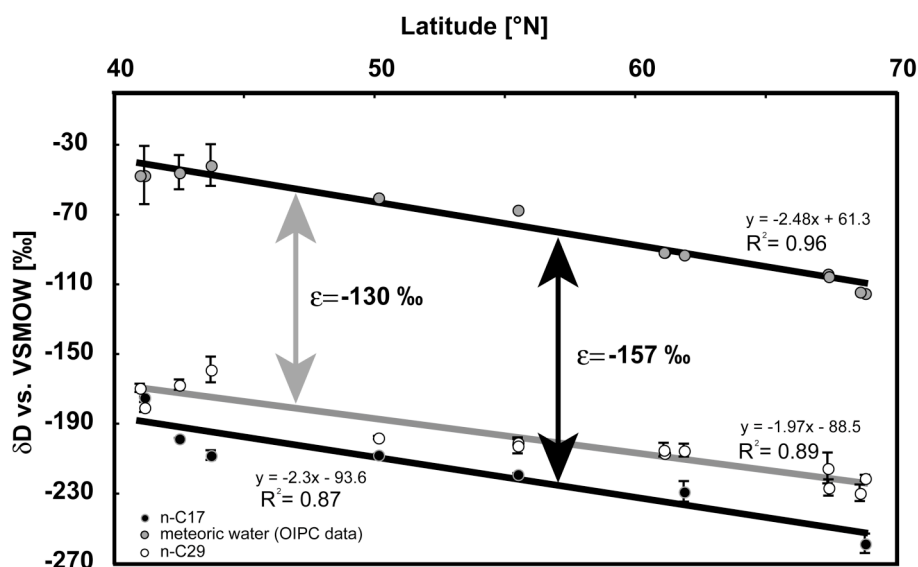


Figure 6: Variation of meteoric water  $\delta D$  values,  $\delta D$  values and the isotopic fractionation  $\epsilon$  for the aquatic n-C<sub>17</sub> and the terrestrial n-C<sub>29</sub> alkane along the N-S European transect

### 3.3. Conclusions

Hydrogen isotope ratios of recent lacustrine sedimentary n-alkanes clearly record the isotopic composition of the meteoric source water in lakes from different climates. In particular n-C<sub>17</sub> and n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub> and n-C<sub>31</sub> can be used to reconstruct the source water  $\delta D$  value. The

constant fractionation  $\epsilon_{C_{17}/W}$  of about  $-157\text{‰}$  along the transect supports the hypothesis fractionation of H during biosynthesis is independent of environmental parameters.

The enrichment of about  $30\text{‰}$  of the terrestrial long-chain n-alkanes to the aquatic n-C<sub>17</sub> is likely due to evaporation processes in plant leaves. The variation in the difference between terrestrial and aquatic n-alkanes might be influenced by input of allochthonous biomass of distant origin on the sites. Since it is not clear if the leaf wax n-alkanes are synthesized *de novo* year-round, further investigation involving evapotranspiration measurements and study of possible seasonal variations in leaf wax n-alkane  $\delta D$  values will clarify if this difference can serve as a valuable proxy for the evapotranspiration of the lake ecosystem.

The hydrogen source of the short-chain n-alkanes n-C<sub>12</sub> to n-C<sub>20</sub> (excluding n-C<sub>17</sub> and n-C<sub>19</sub>) is probably not the meteoric water pool. Non-photosynthetic bacteria synthesizing these compounds or a contribution of fossil hydrogen (petroleum contamination) might be responsible. Further research on the differences of H isotope fractionation in autotrophic vs. heterotrophic organisms is necessary. Compound-specific radiocarbon measurements on the short-chained alkanes could help clarify their origin.

Our results demonstrate that hydrogen isotope ratios of recent sedimentary n-alkanes excellently record the  $\delta D$  value of the lake water (Figure 6) and hence enable climatic reconstruction.

Moreover, the use of terrestrial and aquatic compounds might be used as a proxy for evapotranspiration of the lake environment. n-alkanes are abundant in sediments and relatively easy to purify, so the application of compound-specific hydrogen isotope measurements to samples from the geological past opens new possibilities for the reconstruction of changes in the water cycle.



Table 6:  $\epsilon_{\text{substance/water}}$  values and  $2\sigma$  standard deviation (SD) for each site and mean values along the transect. Bold values indicate constant  $\epsilon_{\text{substance/water}}$  values along the transect

sample code	$\epsilon_{\text{substance/meteoric water}}$																			
	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31
[‰ vs. VSMOW]																				
NAI	-35		-6		1	-162	-18										-107		-120	
KEI										-148	-132	-136	-113	-135	-122	-130		-130		-131
SOD003	-43		-21		4		-42					-147					-118		-124	
SOD007					3		-54		-22		-182	-182					-128		-136	
HYY						-150	-47			-166		-138		-125		-120		-124		-128
SYR												-128		-122						-127
LAM												-199		-125		-134				-125
SOR					-86	-163	-183	-187	-84	-156	-161		-118	-135	-118		-124	-143	-122	-151
HZM	-83	-85	-70	-94	-117	-157	-104					-175		-128		-132		-147		-147
MAS						-174		-167		-202							-91		-122	
MEZ						-160						-124		-113		-122		-128		
LGM				-87		-134	-207	-162		-154									-140	-145
LPM										-139				-126		-137	-129	-128		
<b>mean</b>	-54	-85	-32	-90	-39	<b>-157</b>	-94	<b>-172</b>	-53	<b>-161</b>	<b>-158</b>	<b>-153</b>	<b>-115</b>	<b>-126</b>	<b>-120</b>	<b>-122</b>	<b>-126</b>	<b>-130</b>	<b>-122</b>	<b>-141</b>
<b>SD</b>	26		34	5	58	<b>12</b>	74	<b>13</b>	44	<b>22</b>	<b>25</b>	<b>28</b>	<b>3</b>	<b>7</b>	<b>2</b>	<b>14</b>	<b>3</b>	<b>8</b>	<b>10</b>	

## 4. Compound-specific $\delta D$ values of n-alkanes from terrestrial plants along a climatic gradient – Implications for the sedimentary biomarker record

### 4.1. Introduction

The variation of the stable isotope ratios of hydrogen in meteoric water are caused by a variety of climatic parameters, such as temperature, evaporation and water vapour source (CRAIG, 1961). Since meteoric water is the only hydrogen source for most of the organisms on earth, the potential of organic matter to record this climate signal has been a focus of research for some time (EPSTEIN et al., 1976). Especially plant cellulose was found to record the source water signal (FENG and EPSTEIN, 1995). Unfortunately, cellulose is not stable over geological time scales and therefore not abundant in the sedimentary record. Bulk lipids are geologically stable biomarkers, which are also known to record the source water  $\delta D$  value (STERNBERG, 1988). However, distinct isotopic fractionations during biosynthesis of various lipids lead to variations in bulk lipid  $\delta D$  values, which are not related to isotopic variations in source water. The development of compound-specific hydrogen isotope analysis in the last years overcomes this problem and permits the measurement of  $\delta D$  values on individual organic compounds. In sediments from the geological past n-alkanes are among the most abundant lipids, since they are very stable compounds. n-Alkanes are relatively easy to extract and purify and all hydrogen atoms in n-alkanes are carbon-bound and therefore non-exchangeable for temperatures up to 150°C (SCHIMMELMANN et al., 1999). Furthermore, n-alkanes originate from different biological sources, n-alkanes with 17 and 19 carbon atoms are derived from algae, n-alkanes with 23 carbon atoms can be produced by submerged aquatic plants (FICKEN et al., 2000) and sphagnum species (BAAS et al., 2000), which also produce n-C<sub>25</sub>. The n-alkanes with 25 to 31 carbon atoms, but especially n-C<sub>27</sub> and n-C<sub>29</sub> are produced in the leaf waxes of terrestrial higher plants (EGLINTON and HAMILTON, 1967). Very high amounts of n-alkanes are present in deciduous tree leaves, compared to significantly lower concentrations in coniferous tree needles. The n-C<sub>29</sub> and especially n-C<sub>31</sub> are also major constituents of grasses (MAFFEI, 1996). Sessions *et al.* (1999) have demonstrated in laboratory experiments that the source water  $\delta D$  value is recorded in the organisms' n-alkanes. In the previous chapter 3 (SACHSE et al., 2004b), it is shown that aquatic and terrestrial n-alkanes from recent lake sediments along a N-S European climatic gradient record the meteoric water isotope composition in natural ecosystems. The observed fractionation between source water and n-alkanes was constant at -157‰ for the

aquatic substances, which is in agreement with results from laboratory studies (SESSIONS et al., 1999). Sachse *et al.* (2004b) found that the terrestrial n-alkanes are enriched between 10 and 60‰ relative to the aquatic biomarkers. This is in agreement with previous work, where hydrogen isotope fractionations between source water and n-alkanes ( $\epsilon_{w/a}$ ) of about  $-117 \pm 27\%$  for C3 plants have been reported (CHIKARAISHI and NARAOKA, 2003). However, the observed isotopic difference between higher terrestrial plants and aquatic organisms does not originate from a different biosynthetic pathway. The leaf water of plants, which is the hydrogen source for all biosynthetic products, is subject to evapotranspiration, leading to an enrichment between 20 to 80‰ (LEANNEY et al., 1985). Therefore, two isotopically distinct source waters used for the biosynthesis of terrestrial and aquatic biomarkers are the reason for the observed differences. Sachse et al. (2004) have argued that the difference between aquatic and terrestrial biomarker  $\delta D$  values in small, closed lake systems, which are fed by the meteoric water, could serve as a proxy for the evapotranspiration of the lake ecosystem. However, the nature of the terrestrial n-alkane signal in lake sediments represents a mixture of different plant species, in temperate climates most leaves will be deposited in the lake in autumn. Therefore the n-alkane hydrogen isotope composition of the dominating mostly deciduous tree vegetation around the lakes was investigated and compared with the sedimentary biomarker record investigated in the previous study (SACHSE et al., 2004b). The n-alkane composition and  $\delta D$  values of 31 plant biomass samples (*Betula*, *Fagus*, *Quercus*, *Alnus*, *Carpinus* and *Myrtus* as well as non stomata containing *Sphagnum*, *Cladonia* and Moss) from 14 sites along a climatic gradient from Northern Finland to Southern Italy has been analysed (Table 1). 11 of the sites were previously investigated for the n-alkane  $\delta D$  values in adjacent lake sediments (see chapter 3) and are compared to the leaf wax n-alkane  $\delta D$  values. Variability among different species and different n-alkanes will be explored. Conclusions are drawn regarding the use of the terrestrial n-alkane  $\delta D$  signal as a palaeoclimate parameter.

## 4.2. Results and Discussion

### 4.2.1. n-alkane concentrations of biomass

In general the leaves from deciduous trees contain the highest amounts of extractable n-alkanes (as a mean 1066 mg/kg dry weight) but with a high variability between 6540 and 100 mg/kg. No systematic variation of their concentration with species or site was observed. *Sphagnum* species contain about 91 mg/kg dry weight of n-alkanes (with a variation between 61 to 129 mg/kg). Mosses and *Cladonia*, only sampled in Scandinavia contain very small amounts of n-alkanes (a mean of 34 mg/kg dry weight with a variation between 8 and 69 mg/kg). Some

coniferous needle samples from the Scandinavian sites were also extracted, but contained even less extractable n-alkanes than Mosses and Cladonia. The low concentration did not permit the measurement of isotope values, therefore they are excluded from the discussion. These observations suggest that deciduous tree leaves are the major contributors to the sedimentary organic matter of terrestrial origin, with only minor influence by *Sphagnum* species and a negligible input by Mosses and *Cladonia* species as well as coniferous tree needles. Also, deciduous trees produce far more biomass than the other plants.

However, since sampling inhomogeneity and the extraction procedure are known to influence the measured total concentration of hydrocarbons, we will rely in the following discussion on the relative amounts of n-alkanes.

Deciduous tree leaves contain n-alkanes with 25 to 31 carbon atoms and a strong odd over even carbon number predominance (expressed as the CPI) is observed (Table 7), which is in agreement with previous observations (EGLINTON and HAMILTON, 1967; GULZ, 1994; PIASENTIER et al., 2000). All *Betula* species in Scandinavia contain also relatively high amounts of n-C<sub>23</sub>, a substance previously attributed only to water plants (FICKEN et al., 2000) or sphagnum species (BAAS et al., 2000). However, the dominant compound in *Betula* species is n-C<sub>27</sub> in the north Scandinavian samples. In 2 species from southern Scandinavia (LAM) and Germany (HZM) n-C<sub>31</sub> becomes dominant, n-C<sub>23</sub> is almost absent from these two samples. The average chain length (ACL) of *Betula* species is subsequently increasing from N to S from 25.5 (FIN002) to 28.3 (HZM). The ACL index of all analysed species is also increasing from N to S. This suggests that trees in areas with a longer vegetation period and more potential incoming radiation protect their leaves with longer chain n-alkanes from water loss.

The n-C<sub>27</sub> alkane is also the dominant compound in *Fagus*, where it is virtually the only present n-alkane. This is in agreement with previous studies which found high concentrations of n-C<sub>27</sub> in beech leaves and soils under beech stands (GLEIXNER et al., 2005; GULZ, 1994).

In all *Quercus* species, only present in Italy, n-C<sub>29</sub> is the major n-alkane, with lower contributions by the n-C<sub>25</sub>, n-C<sub>27</sub> and n-C<sub>31</sub> alkanes. The only sampled *Alnus* species (MAS) contained almost exclusively n-C<sub>29</sub>, with minor contributions from C<sub>27</sub> and C<sub>31</sub>. One *Carpinus* species (MEZ) contains n-C<sub>29</sub> and n-C<sub>31</sub> as the major n-alkanes.



Table 7: n-Alkane distributions of the sampled plant biomass: maximum chain length ( $C_{max}$ ), carbon preference index (CPI and  $CPI_{23-33}$ ) and average chain length (ACL)

sample code	sampled biomass	$C_{max}$	CPI	CPI 23-33	ACL
FIN002	<i>Betula pubescens</i>	27	9.9	13.2	25.5
NAI	<i>Betula pendula</i>	27	62.7	89.8	25.6
	Moss	27	1.3	4.2	22.3
	<i>Sphagnum</i>	27	5.4	14	24.9
KEI	<i>Betula pubescens</i>	27	62.0	92.5	25.8
	<i>Cladonia</i>	29	0.9	7.1	22.6
SOD003	<i>Betula pubescens</i>	27	34.1	39.5	26.1
	<i>Cladonia</i>	27	3.1	3.8	25.6
	<i>Fagus sylvatica</i>	27	13.8	14.7	26.8
SOD007	<i>Betula pendula</i>	27	11.5	12	25.8
	<i>Cladonia</i>	27	5.0	5.2	25.7
	<i>Sphagnum</i>	23	7.1	8.4	25.7
SOD004	<i>Betula pendula</i>	27	12.8	13.7	26.9
	Moss	27	1.8	2.8	24.5
HYY	<i>Betula pendula</i>	27	16.0	17.2	25.8
	Moss	27	7.2	8.6	26.5
	<i>Sphagnum</i>	27	10.2	11.5	25.8
SYR	<i>Myrte</i>	31	25.3	29.8	30.3
	<i>Betula pendula</i>	27	30.9	58.5	26.1
	Moss	31	2.3	5.1	26.4
LAM	<i>Betula pendula</i>	31	30.2	32.5	28.1
	<i>Cladonia</i>	27	3.5	4.3	26.4
HZM	<i>Betula pendula</i>	31	15.0	15.5	28.3
	<i>Fagus sylvatica</i>	27	59.8	59.8	27
MAS	<i>Quercus variabilis</i>	31	2.6	2.8	29.6
	<i>Alnus incana</i>	29	6.2	7.1	28.3
	<i>Quercus cerris</i>	29	14.4	14.4	28.9
ITA001	<i>Quercus robur</i>	29	15.6	15.6	28.5
MEZ	<i>Quercus petraea</i>	29	7.5	8.2	28.3
	<i>Carpinus betulus</i>	31	-	-	30.3
LGM	<i>Fagus sylvatica</i>	27	19.9	26.7	26.9
LPM					

One *Myrtus* plant sampled at SYR contained high amounts of n- $C_{29}$  and especially n- $C_{31}$ . Also, it was the only sampled plant that contained significant amounts of n- $C_{33}$ .

*Sphagnum* species, only sampled at NAI, SOD007 and HYY, also contain n-alkanes with 23 to 31 carbon atoms, with n- $C_{27}$  and n- $C_{23}$  as major compounds. The carbon preference index (CPI) for sphagnum is lower than for deciduous tree leaves, whereas the average chain length (ACL) is in the same range as observed for broadleaf tree leaves (Table 7).

Mosses and *Cladonia* species, sampled in Scandinavia (KEI, NAI, SOD001, SOD007, SOD004, HYY, LAM and SYR) contained not only the long-chain n-alkanes, but also smaller amounts of short-chain alkanes, down to n- $C_{14}$  in some cases. In the shorter chain n-alkanes, no odd over even carbon number predominance is visible. This leads to much lower CPI values and slightly lower ACL values of these plants compared to *Sphagnum* and deciduous tree leaves (Table 7).

#### 4.2.2. $\delta D$ values of n-alkanes

The measured  $\delta D$  values on the n-alkanes n-C<sub>23</sub> to n-C<sub>33</sub> vary between -231 (n-C<sub>29</sub> from *Myrtus* at SYR) and -141‰ (n-C<sub>27</sub> from *Quercus* at ITA002 and MEZ) (Table 8). Only for the n-C<sub>27</sub> alkane of one moss at NAI site a very heavy  $\delta D$  value of -108‰ was observed.

Variability of the  $\delta D$  values of the different n-alkanes of one plant sample can be as large as 60‰ (*Cladonia* at KEI) but averages around 30‰. This variability is larger for *Sphagnum*, *Cladonia* and Mosses than for deciduous leaves. In deciduous tree leaves the  $\delta D$  value of n-C<sub>23</sub>, if present, is often the heaviest measured  $\delta D$  value.

$\delta D$  values of the n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub> and n-C<sub>31</sub> alkanes are lighter in the north and heavier in the south, as expected from the meteoric water  $\delta D$  gradient. Also, the mean  $\delta D$  value (n-C<sub>23</sub> to n-C<sub>33</sub>) and the concentration weighted mean  $\delta D$  values are in general heavier in the south and lighter in the north (Table 8).

n-alkane  $\delta D$  values from *Sphagnum* species at the measured sites are lower or similar to deciduous tree leaf  $\delta D$  values at the same site.

n-alkanes from Mosses, which were only sampled in Scandinavia, have higher  $\delta D$  values than *Sphagnum* or deciduous leaves, except for SOD007, where  $\delta D$  values are similar to *Sphagnum*.

$\delta D$  values from *Cladonia* are similar to  $\delta D$  values from deciduous trees leaves at the same site.

$\delta D$  values of the n-alkanes of different broadleaf trees from the same site have similar  $\delta D$  values. This demonstrates, that the meteorological conditions at a site lead to similar deuterium enrichment in the leaf water. One notable exception is the *Myrtus* plant sampled at SYR, which is characterized by very low n-alkane  $\delta D$  values of -231, -226 and -225‰ for the n-C<sub>29</sub>, n-C<sub>31</sub> and n-C<sub>33</sub> alkanes respectively, about 50‰ lighter than observed for *Betula* at the same site. Since *Myrtus* leaves have a very thick wax layer, the leaf is better protected from evaporative water loss, avoiding stronger deuterium enrichment in the leaf water.

When comparing the meteoric source water isotopic composition with the n-alkane  $\delta D$  values from the deciduous leaves (Table 9), the best correlation is found for n-C<sub>27</sub>, the n-alkane, which was found in nearly every plant. Other significant correlations were found for n-C<sub>25</sub>, n-C<sub>31</sub> and the mean and concentration weighted mean  $\delta D$  values of all n-alkanes from n-C<sub>23</sub> to n-C<sub>31</sub> (Figure 7). n-C<sub>23</sub> and surprisingly n-C<sub>29</sub>, the most abundant n-alkane in the lake sediments analysed for the previous study (SACHSE et al., 2004b) do not show a significant relationship with the source water  $\delta D$  value. However, n-C<sub>23</sub> was only found in significant amounts in the Scandinavian plant samples, whereas n-C<sub>29</sub> was most abundant in the Southern European samples. Therefore these 2 alkanes do not cover the whole climatic gradient, which could be the reason for the poor relationship with the source water  $\delta D$  value. The other n-alkanes correlate

with mean annual  $\delta D$  values, August and September  $\delta D$  values as well as with lake or stream water  $\delta D$  values (except for n-C<sub>25</sub>) (Table 9, Figure 7).

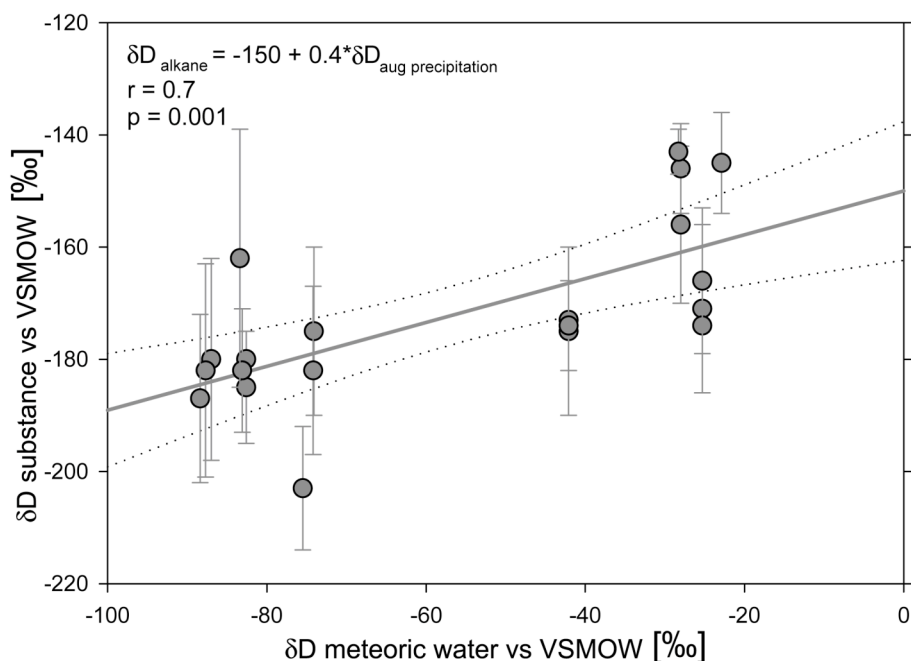


Figure 7: Correlation of august precipitation  $\delta D$  values and weighted mean n-alkane  $\delta D$  values from deciduous leaves throughout the climatic gradient. The dotted line shows the 95% confidence interval. The error bars are representative of the variability of the concentration weighted mean  $\delta D$  value of the leaf wax n-alkanes within one sample.

None of the correlations is a 1:1 relationship. All equations have a slope lower than one, suggesting that the apparent isotopic fractionation ( $\epsilon$ ) is lower for lower  $\delta D$  source water values. However, since the isotopic composition of the source water for plants, the meteoric water, is modified in the leaf water due to evapotranspiration, and this enrichment was in fact higher in Scandinavia during Summer 2002, the deviation from a slope of one is a measure of the influence of leaf water enrichment in plants. The highest correlation coefficients were found for the August meteoric water value. Since our sampling took place in August and September, this suggests, that the deciduous tree leaves record the meteoric water from the previous weeks. These data indicate, that n-alkanes are synthesised year-round in the wax layer of leaves. However, the correlation coefficients are only slightly higher than for mean annual or September values, therefore this interpretation remains speculative. Research should focus on possible seasonal variations of n-alkane  $\delta D$  values in the leaf waxes of deciduous trees.

Table 8:  $\delta D$  values and standard deviation (SD,  $2\sigma$ ) of the n-alkanes n-C<sub>23</sub> to n-C<sub>33</sub> and the concentration weighted mean  $\delta D$  values of the sampled biomass

sample code	sampled biomass	n-C <sub>23</sub>	SD	n-C <sub>24</sub>	SD	n-C <sub>25</sub>	SD	n-C <sub>26</sub>	SD	n-C <sub>27</sub>	SD	n-C <sub>28</sub>	SD	n-C <sub>29</sub>	SD	n-C <sub>30</sub>	SD	n-C <sub>31</sub>	SD	n-C <sub>32</sub>	SD	n-C <sub>33</sub>	SD	mean	SD	weighted mean		
FIN002	<i>Betula pubescens</i>	-168	1	-165	7	-198	1	-170	7	-189	0														-185	15	-187	
NAI	<i>Betula pendula</i>	-156	7			-187	3			-191	5														-178	19	-182	
	Moss									-108	4														-108	-	-108	
	<i>Sphagnum</i>	-162	2			-197	2			-213	1														-191	26	-197	
KEI	<i>Betula pubescens</i>	-157	6			-170	6			-193	2														-173	18	-180	
	<i>Cladonia</i>	-143	6							-167	20			-207	8			-199	5						-179	30	-189	
SOD003	<i>Betula pubescens</i>	-171	2			-188	2			-188	0														-183	10	-185	
	<i>Cladonia</i>									-173	1														-173	-	-173	
	<i>Fagus sylvatica</i>									-180	2														-180	-	-180	
SOD007	<i>Betula pendula</i>	-135	15			-162	6			-170	6			-185	10			-193	2						-169	23	-162	
	<i>Cladonia</i>	-189	1	-201	2	-214	1	-195	0	-204	0	-171	3	-190	1			-179	1						-195	14	-200	
	<i>Sphagnum</i>	-206	5			-194	3			-191	12			-217	4			-201	13						-202	10	-201	
SOD004	<i>Betula pendula</i>	-174	2			-188	1	-176	3	-189	0			-167	0			-166	2						-177	11	-182	
	Moss	-145	3			-169	10			-176	2														-163	16	-164	
HYY	<i>Betula pendula</i>	-192	1			-210	0	-193	3	-205	0			-189	4			-186	6						-196	11	-203	
	Moss					-175	4			-183	7														-179	5	-181	
	<i>Sphagnum</i>	-178	1			-217	5			-200	4														-198	19	-200	
SYR	<i>Myrte</i>													-231	3	-199	7	-226	2	-210	12	-225	1		-227	4	-228	
	<i>Betula pendula</i>	-155	4			-183	2			-189	2							-186	4						-170	15	-182	
	Moss									-182	6			-198	12			-186	4						-189	9	-190	
LAM	<i>Betula pendula</i>					-189	4			-183	2			-160	7			-162	2						-174	15	-175	
	<i>Cladonia</i>									-179	8														-179	-	-179	
HZM	<i>Betula pendula</i>					-186	3			-175	1			-169	1			-168	1						-174	8	-174	
	<i>Fagus sylvatica</i>									-173	1														-173	-	-173	
MAS	<i>Quercus variabilis</i>													-181	10			-161	3						-171	15	-171	
	<i>Alnus incana</i>													-174	8										-174	-	-174	
	<i>Quercus cerris</i>									-159	3			-174	5			-148	3						-161	13	-166	
ITA001	<i>Quercus robur</i>					-149	4			-141	4			-157	3			-136	6						-146	9	-145	
MEZ	<i>Quercus petraea</i>									-141	6			-161	3											-151	14	-156
	<i>Carpinus betulus</i>									-153	9							-142	9						-148	8	-146	
LGM	<i>Fagus sylvatica</i>					-149	1			-142	1			-149	3										-147	4	-143	
LPM																												

Table 9: Correlation of the n-alkane  $\delta D$  values with meteoric water (r is the Pearson correlation coefficient, p – value  $>0.05$  indicates a significant relationship, a and b are the coefficients for the linear regression equation  $y = ax+b$ ), bold values indicate significant correlations

		n-C <sub>23</sub>	n-C <sub>25</sub>	n-C <sub>27</sub>	n-C <sub>29</sub>	n-C <sub>31</sub>	mean C <sub>23-31</sub>	concentration weighted mean C <sub>23-31</sub>
mean annual $\delta D$ (OIPC)	r	-0.265	<b>0.631</b>	<b>0.825</b>	0.174	<b>0.773</b>	<b>0.674</b>	<b>0.67</b>
	p	0.526	<b>0.028</b>	<b>0</b>	0.609	<b>0.009</b>	<b>0.001</b>	<b>0.001</b>
	a		0.4	<b>0.6</b>		<b>0.5</b>	<b>0.3</b>	<b>0.3</b>
	b		-146.3	<b>-129.3</b>		<b>-128.7</b>	<b>-147.2</b>	<b>-145.8</b>
lake water $\delta D$	r	-0.428	0.557	<b>0.815</b>	0.098	<b>0.784</b>	<b>0.681</b>	<b>0.66</b>
	p	0.29	0.075	<b>0</b>	0.789	<b>0.012</b>	<b>0.001</b>	<b>0.002</b>
	a			<b>0.4</b>		<b>0.3</b>	<b>0.2</b>	<b>0.2</b>
	b			<b>-149.9</b>		<b>-150</b>	<b>-159.3</b>	<b>-159.9</b>
august $\delta D$ (OIPC)	r	-0.294	<b>0.677</b>	<b>0.843</b>	0.199	<b>0.772</b>	<b>0.692</b>	<b>0.688</b>
	p	0.479	<b>0.016</b>	<b>0</b>	0.557	<b>0.009</b>	<b>0.001</b>	<b>0.001</b>
	a		<b>0.5</b>	<b>0.6</b>		<b>0.5</b>	<b>0.3</b>	<b>0.4</b>
	b		<b>-149.3</b>	<b>-136.1</b>		<b>-136.5</b>	<b>-150.8</b>	<b>-150</b>
september $\delta D$ (OIPC)	r	-0.3	<b>0.59</b>	<b>0.801</b>	0.185	<b>0.768</b>	<b>0.659</b>	<b>0.654</b>
	p	0.471	<b>0.043</b>	<b>0</b>	0.586	<b>0.009</b>	<b>0.002</b>	<b>0.002</b>
	a		<b>0.4</b>	<b>0.6</b>		<b>0.5</b>	<b>0.3</b>	<b>0.3</b>
	b		<b>-151.9</b>	<b>-134.9</b>		<b>-131.4</b>	<b>-149.7</b>	<b>-148.8</b>

#### 4.2.2. Hydrogen isotope fractionation between source water and biomass n-alkanes

The mean hydrogen isotope fractionation between source water (august precipitation) and the weighted mean n-alkane  $\delta D$  values ( $\epsilon_{w/a}$ ) of all plants is  $-118\text{‰}$ , which is slightly higher than found in the sedimentary record. This might be due to higher evaporative enrichment of the leaf water in summer, whereas the sediments will receive most of the leaves in autumn. However, our sampling might also not be representative for the total terrestrial input into the lake sediments.  $\epsilon_{w/a}$  varies between  $-86\text{‰}$  and  $-166\text{‰}$ , excluding the exceptionally high value of  $-22\text{‰}$  for the Moss at the NAI site (Figure 8). Accounting for the net biochemical fractionation of hydrogen during biosynthesis of  $-157\text{‰}$  (SACHSE et al., 2004b; SESSIONS et al., 1999) this results in an enrichment relative to plant source water of up to  $76\text{‰}$ . This agrees well with the range observed in leaf water (ZIEGLER, 1989), which is the source water for biosynthesis. The mean  $\epsilon_{w/a}$  for all deciduous trees is  $-121\text{‰}$ , excluding the light *Myrtus*  $\delta D$  values. For *Myrtus* at SYR an  $\epsilon_{w/a}$  value of  $-166\text{‰}$  is observed, suggesting that no or only a very small enrichment of leaf water has taken place. The thick wax layer might be able to restrict evaporative enrichment of leaf water to a minimum. In general, deciduous tree leaves have very similar  $\epsilon_{w/a}$  values at the same sites (Figure 8), but can vary substantially even between neighbouring sites, as evident for the three finish sites close to the town of Sodankylä (SOD003, SOD004 and SOD007). We conclude, that  $\epsilon_{w/a}$  in broadleaf tree leaves is a measure of the meteorological conditions at the site. Overall, the plants without stomata, Moss and *Cladonia*, have smaller mean  $\epsilon_{w/a}$  values of -

87 and  $-113\text{‰}$  respectively, and therefore show a stronger isotopic enrichment of leaf water compared to broadleaf trees, which contain stomata. Stomata therefore regulate water loss of higher plants and prevent strong loss of water by evapotranspiration. A small  $\epsilon_{w/a}$  value of only  $-22\text{‰}$  is observed for the moss at the KEI site. This indicates that an extremely strong evaporative enrichment has taken place in the plants tissue, which is reasonable, since mosses can survive dry periods and regenerate, when water is again available.

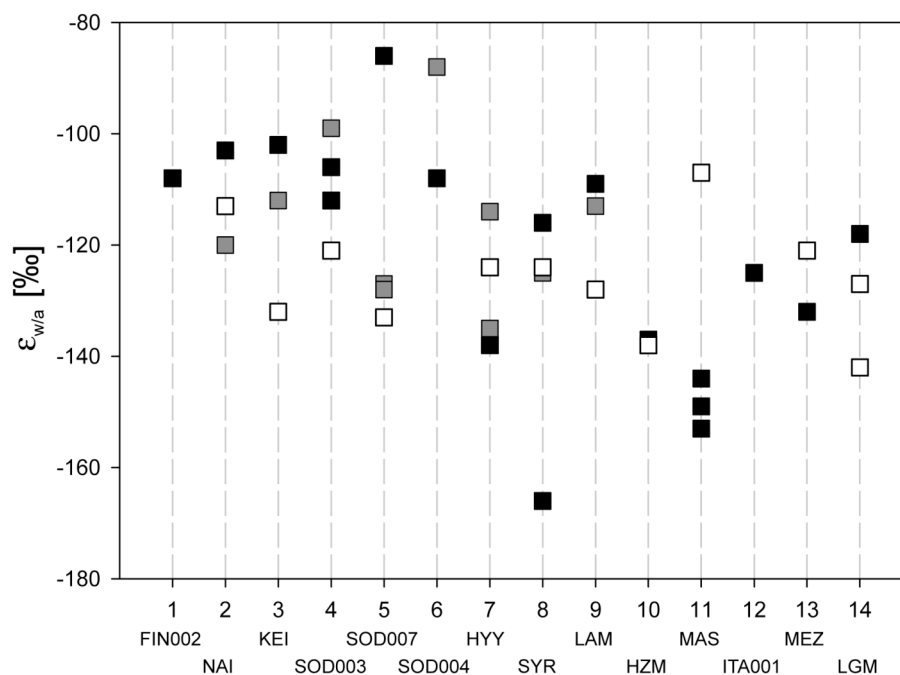


Figure 8: Comparison of  $\epsilon_{w/a}$  values for sedimentary n-alkanes (open squares) and deciduous tree leaf n-alkanes (black squares) and non-stomata containing plants (grey squares) for the analysed sites.

*Sphagnum* species, which also have no stomata, have a mean  $\epsilon_{w/a}$  of  $-128\text{‰}$ , which is lower than  $\epsilon_{w/a}$  values for deciduous tree leaves at the same site. This is reasonable, since *Sphagnum* grows in a wet environment, evaporative enrichment should not be as strong as for deciduous leaves.

$\epsilon_{w/a}$  is higher in northern Scandinavia, lower in central Europe and higher in Southern Italy (Figure 8). Since summer 2002 was exceptionally hot and dry in Scandinavia, but cool and wet in Central Europe, the observed pattern agrees with meteorological observations. Moreover, the average chain length of all analysed biomass samples shows a linear relationship with  $\epsilon_{w/a}$  (Figure 9). This relationship is equally strong when only considering the stomata containing broadleaf species (Figure 9). With increasing chain length the  $\epsilon_{w/a}$  value increases, therefore the evaporative enrichment of deuterium in the leaf water decreases. This indicates that longer chain compounds provide a better protection from evaporative loss of water for plants. It has been

shown that the hydrocarbon composition in the leaves of *Rosmarinus officinalis* changes with changes in moisture and temperature on a seasonal scale (MAFFEI et al., 1993). Therefore, plants might increase the production of longer chain n-alkanes in the leaf waxes as a response to dryer conditions and to prevent evaporative water loss.

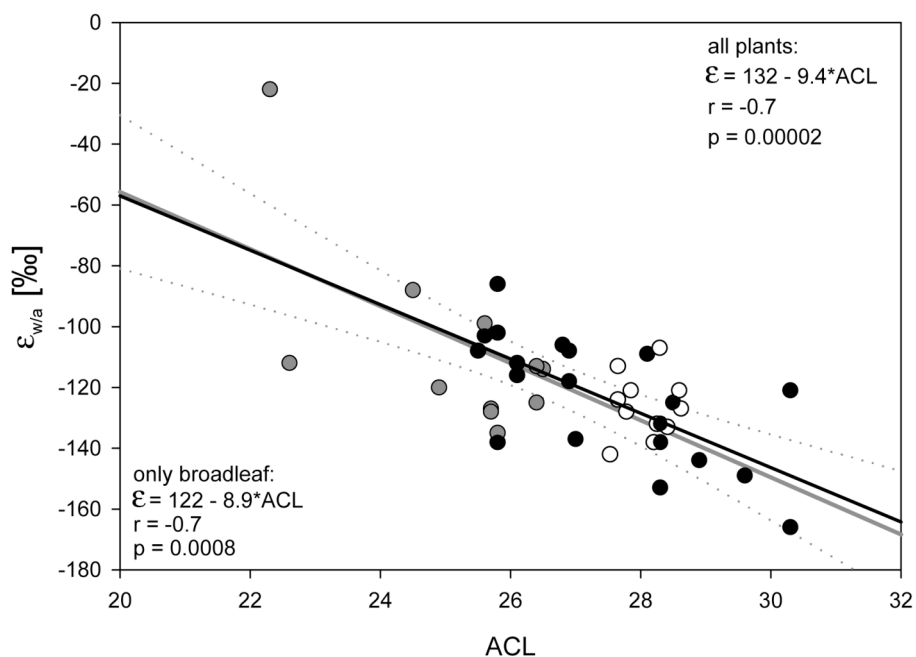


Figure 9: Average chain length (ACL) versus  $\epsilon_{w/a}$  of non-stomata biomass (grey circles), deciduous tree leaf (black circles) and sediment (open circles) samples. The grey line is the linear fit for all biomass samples, the black line is for deciduous tree leaves only. The dotted line represents the 95% confidence interval (only shown for all plants).

However, the observed linear relationship between ACL and  $\epsilon_{w/a}$  is not observed in the associated lake sediments. The ACL values (n-C<sub>25</sub> to n-C<sub>31</sub>) of the sedimentary n-alkanes vary only slightly along the transect (27.7 to 28.6). The sedimentary record of a lake integrates over several species and receives most of its input in autumn, although not exclusively, since strong precipitation events also deliver leaves into the lake. The sedimentary values fall within the mean values of the broadleaf tree leaves (Figure 9), which are the major contributors, and hence represent spatial and temporal average values.

#### 4.2.3. Comparison with sedimentary n-alkane distributions and $\delta D$ values

The distribution of the terrestrial long-chain n-alkanes in the sediments shows a much smaller variability than for the plant biomass. The ACL indices of all sediments are similar with an average value of 28, whereas the ACL indices of broadleaf tree samples vary between 25 and

Table 10: Comparison of the fractionation factors between plant n-alkanes and source water to associated sediment values

sample code	sampled biomass	biomass weighted mean $\delta D$ C <sub>23-33</sub>	fractionation $\epsilon_{w/b}$ biomass	fractionation $\epsilon_{w/s}$ sediment
FIN002	<i>Betula pubescens</i>	-187	-108	-
NAI	<i>Betula pendula</i>	-182	-103	-113
	Moss	-108	-22	
	<i>Sphagnum</i>	-197	-120	
KEI	<i>Betula pubescens</i>	-180	-102	-132
	<i>Cladonia</i>	-189	-112	
SOD003	<i>Betula pubescens</i>	-185	-112	-121
	<i>Cladonia</i>	-173	-99	
	<i>Fagus sylvatica</i>	-180	-106	
SOD007	<i>Betula pendula</i>	-162	-86	-133
	<i>Cladonia</i>	-200	-127	
	<i>Sphagnum</i>	-201	-128	
SOD004	<i>Betula pendula</i>	-182	-108	-
	Moss	-164	-88	
HYY	<i>Betula pendula</i>	-203	-138	-124
	Moss	-181	-114	
	<i>Sphagnum</i>	-200	-135	
SYR	<i>Myrtus</i>	-228	-166	-124
	<i>Betula pendula</i>	-182	-116	
	Moss	-190	-125	
LAM	<i>Betula pendula</i>	-175	-109	-128
	<i>Cladonia</i>	-179	-113	
HZM	<i>Betula pendula</i>	-174	-138	-138
	<i>Fagus sylvatica</i>	-173	-137	
MAS	<i>Quercus variabilis</i>	-171	-149	-107
	<i>Alnus incana</i>	-174	-153	
	<i>Quercus cerris</i>	-166	-144	
ITA001	<i>Quercus robur</i>	-145	-125	-
MEZ	<i>Quercus petraea</i>	-156	-132	-121
	<i>Carpinus betulus</i>	-146	-121	
LGM	<i>Fagus sylvatica</i>	-143	-118	-142
LPM				-127

30 (Figure 9). At the investigated sites it is not possible to determine the dominant broadleaf tree species from the distribution of n-alkanes in the sediments. The observed n-alkane distributions, for instance for *Betula*, show, that even the same species can have variable n-alkane distributions. Also, seasonal variations in n-alkane distributions in leaves have been observed (GULZ, 1994). The vegetation at site HZM consist of almost exclusively of *Fagus* species, which only contain the n-C<sub>27</sub> n-alkane. However, the sedimentary record of lake HZM shows a



uniform distribution of the n-C<sub>25</sub> to n-C<sub>31</sub> n-alkanes. Therefore, the use of n-alkane distributions in lake sediments as a chemotaxonomic indicator is not recommended.

The mean  $\delta D$  values of the sedimentary n-C<sub>25</sub> to n-C<sub>31</sub> alkanes (n-C<sub>23</sub> from the sediments is omitted, since its lower  $\delta D$  values suggest they originate from aquatic plants rather than terrestrial plants) taken from the previous study (SACHSE et al., 2004b) are heavier than the weighted mean plant biomass  $\delta D$  values at nearly all sites (Figure 10, Table 10).

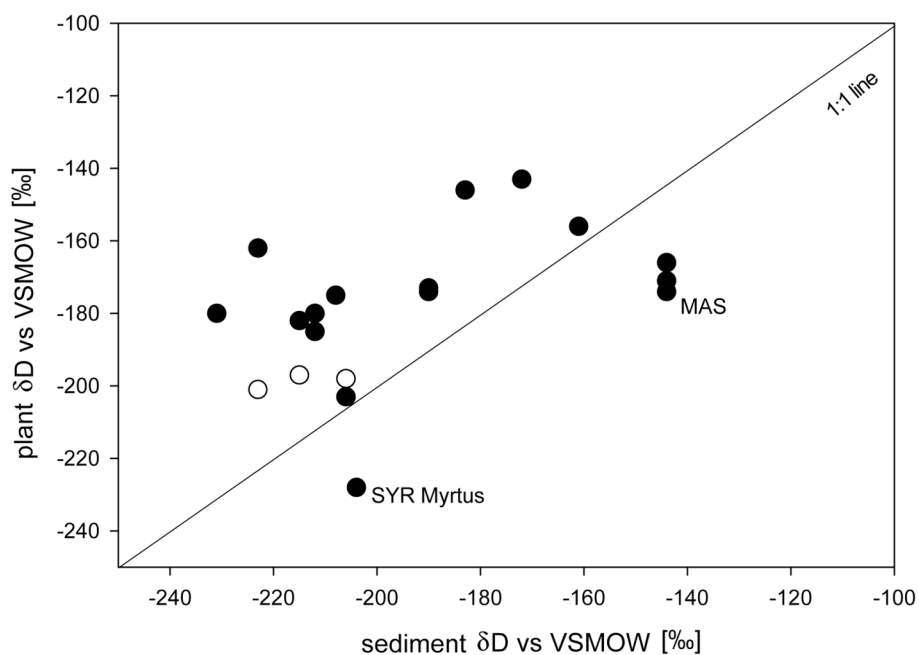


Figure 10: Comparison of the concentration weighted mean broadleaf tree n-alkane  $\delta D$  values (filled circles), *Sphagnum*  $\delta D$  values (open circles) with mean sedimentary n-alkane  $\delta D$  values.

The n-alkanes from *Myrtus* at SYR have 20‰ lighter  $\delta D$  values than the sediment, possibly due to their thick protective wax layer as discussed above. The leaf wax n-alkanes from MAS have 40‰ lighter  $\delta D$  values. Since all 3 sampled species from MAS have virtually the same  $\delta D$  and  $\epsilon_{w/a}$  values and the big size of the lake and its catchment area compared to the other lakes, these values might not be representative of the lake catchment. All other plant biomass  $\delta D$  values are heavier, some values equal, than the associated sediment n-alkane  $\delta D$  values (Figure 10). Since our sampling took place in late summer, when evapotranspiration and therefore leaf water isotopic enrichment should be high, this observation is reasonable. In autumn, when most leaves will fall into the lakes and get incorporated into the sedimentary record, a lighter  $\delta D$  value would be expected, because of lower evaporation of leaf water. Consequently, the plant n-alkane  $\delta D$  values seem to record seasonal variations.

Sediment as well as plant  $\epsilon_{w/a}$  values show the same pattern: higher values in Northern Scandinavia, lower values in Central Europe and higher values in Italy (Figure 8), suggesting that the trend of leaf water enrichment is preserved in the sedimentary record. These findings indicate that changing  $\delta D$  values of deciduous tree derived n-alkanes in the sedimentary record can be used as a proxy for changes in leaf water enrichment and therefore environmental factors such as relative humidity and precipitation.

### 4.3. Conclusions

The comparison of n-alkane  $\delta D$  values and concentrations from different plants along a climatic gradient and associated sedimentary n-alkane  $\delta D$  values indicates that:

- the relative abundance of specific n-alkanes within one species is not constant over the transect, longer chain n-alkanes are present in *Betula* species from Southern Scandinavia and Central Europe compared to the Northern Scandinavia. This makes the use of n-alkane distributions in sediments as an indicator for specific species nearly impossible. The n-C<sub>23</sub> alkane, previously only attributed to submerged aquatic plants and *Sphagnum* was also found in significant amounts in *Betula* leaves. Therefore care should be exercised when using sedimentary n-alkanes to separate aquatic and terrestrial organic matter input, since n-C<sub>23</sub> can be derived from both sources. These findings explain the high variability of n-C<sub>23</sub>  $\delta D$  values observed in lake sediments in the previous study (SACHSE et al., 2004b).
- $\delta D$  values of different deciduous tree leave n-alkanes are similar within a site. They are variable even within species, when comparing different, neighbouring sites. However, different water use strategies are visible between deciduous trees and *Sphagnum* species, which are characterized by lighter  $\delta D$  values (and therefore less evaporative enrichment of water in the tissue) at the same site, since they grow in wet environments. Mosses and *Cladonia*, which contain no stomata to regulate water loss, have similar or heavier  $\delta D$  values than broadleaf trees at the same site. These results suggest that plant anatomy (stomata vs. non-stomata containing plants) and site conditions on a small scale (relative humidity, soil moisture, precipitation) and not biosynthetic differences among species are the major drivers for variations in  $\epsilon_{w/a}$ .

- $\delta D$  values of the deciduous tree leaf n-alkanes correlate highest to the august precipitation  $\delta D$  values. This indicates that seasonal variations in n-alkane  $\delta D$  values do exist. Also, sedimentary n-alkane  $\delta D$  values are in general similar or lower than the sampled plant material from the summer. Further research on the timescale and magnitude of seasonal variations in leaf wax n-alkanes is necessary to fully understand the origin of the terrestrial  $\delta D$  signal in the sedimentary record.
- the average chain length (ACL) of the dominating vegetation is increasing from N to S, suggesting that plants subject to higher degrees of evaporation protect their leaves with longer chain compounds. Evidently, a significant negative relationship is observed between the ACL value of the biomass and the isotopic fractionation between source water and n-alkanes ( $\epsilon_{w/a}$ ) suggesting that longer chain compounds indeed provide a better protection from evaporative water loss through the leaves.

These results prove, that leaf wax n-alkane  $\delta D$  values from terrestrial plants, especially of broadleaf trees, record the  $\delta D$  value of the precipitation, modified by site specific meteorological conditions (evapotranspiration, relative humidity and soil moisture). The comparison of biomass and sedimentary n-alkane distributions and  $\delta D$  values shows that the sedimentary record integrates spatially as well as temporary. Therefore it is suggested that variations in the terrestrial biomarker  $\delta D$  values in lake sediments can be used as an indicator of changing climatic conditions, if the water source is not changing.

## 5. Seasonal variations in deciduous tree leaf wax n-alkane $\delta\text{D}$ and $\delta^{13}\text{C}$ values: Implications for their use as a palaeoclimate proxy

### 5.1. Introduction

The stable isotope ratios of hydrogen ( $\delta\text{D}$  values) and oxygen ( $\delta^{18}\text{O}$  values) in meteoric water depend on a variety of climatic parameters, such as temperature, humidity and evaporation (CRAIG, 1961; GAT, 1996). Since meteoric water is the major oxygen and hydrogen source for nearly every organism on Earth, the potential of organic matter to record the meteoric water isotope signal has been a focus of research. Cellulose was found to record the  $\delta\text{D}$  and  $\delta^{18}\text{O}$  value of the meteoric water and has been applied in palaeoclimate reconstruction using tree rings (FENG and EPSTEIN, 1995; YAPP and EPSTEIN, 1982). With growing interest in this application, the associated fractionation processes have been studied in detail. It is well known that the water in the leaves of plants becomes enriched in the heavy isotopes  $^{18}\text{O}$  and D during evapotranspiration processes (LEANNEY et al., 1985). Models have been developed to describe the isotopic enrichments of leaf water compared to plant source water (CRAIG and GORDON, 1965; FLANAGAN and EHLERINGER, 1991; RODEN and EHLERINGER, 1999). These models have shown, that the isotopic enrichment in leaf water is mainly controlled by changes in relative humidity, but also transpiration rate (BARBOUR and FARQUHAR, 2000). A number of studies have successfully reconstructed changes in the hydrological cycle using tree-ring cellulose  $\delta\text{D}$  and/or  $\delta^{18}\text{O}$  values (MAYR et al., 2003; MCCARROLL and LOADER, 2004; PENDALL et al., 1999).

Recently, the compound-specific hydrogen isotope composition of lipid biomarkers in sediments has been demonstrated to record the source water  $\delta\text{D}$  value with high fidelity (HUANG et al., 2004; SACHSE et al., 2004b; SAUER et al., 2001; SESSIONS et al., 1999). The biosynthetic fractionation of the hydrogen isotopes during biosynthesis was found to be independent of environmental parameters; for n-alkanes an isotopic fractionation between source water and n-alkanes of  $-157\text{‰}$  was observed (SACHSE et al., 2004b; SESSIONS et al., 1999). Lipids like n-alkanes are much more stable and more abundant in the sedimentary record than cellulose, making them ideal candidates for reconstruction of changes in the water cycle over geological timescales. Furthermore, n-alkanes contain only carbon-bound hydrogen, which is non-exchangeable (SCHIMMELMANN et al., 1999), and are relatively easy to extract and purify. These compounds hold information on their biological sources, since n-alkanes of different chain-lengths are produced by different organisms. Algae, for example, mainly produce n-alkanes

with 17 and/or 19 carbon atoms (n-C<sub>17</sub>, n-C<sub>19</sub>), submerged aquatic plants are dominated by n-C<sub>23</sub> (FICKEN et al., 2000) and the long-chain n-alkanes with odd carbon numbers n-C<sub>25</sub> to n-C<sub>31</sub> are found in the leaf waxes of higher terrestrial plants (EGLINTON and HAMILTON, 1967). Large amounts of these alkanes are found in the leaves of broadleaf trees, whereas coniferous tree needles contain much smaller amounts. The different biological sources of n-alkanes permit a differentiation of aquatic and terrestrial organic matter found in lake sediments. Sachse et al. (2004b) examined recent sediments of small ground-water fed lakes along a climatic gradient from northern Finland to Southern Italy and found that the terrestrial derived n-alkanes are enriched in D relative to the n-alkanes of aquatic origin by 10 to 60‰. Sachse *et al.* (2004b) suggested that if the plants use the same meteoric water source as the aquatic organisms, the isotopic difference between the terrestrial and aquatic n-alkanes in lake sediments would be a proxy of leaf water enrichment and consequently the amount of evapotranspiration and relative humidity. However, it has not been demonstrated up to now

- a) if n-alkanes show seasonal variations in  $\delta D$  values
- b) if leaf wax n-alkane  $\delta D$  values record the source water  $\delta D$  signal as a cumulative annual signal or
- c) if leaf wax n-alkanes  $\delta D$  values record variations in the source water over a certain timeframe

In this study those issues will be addressed in order to decipher the origin of the  $\delta D$  signal from terrestrial biomarkers in the sedimentary record.

Leaf wax n-alkane concentrations and  $\delta D$  and  $\delta^{13}C$  values obtained from 2 sites, a Maple and a Beech stand in Germany, are compared over the course of the 2004 growing season.

Additionally, the relationship between  $\delta D$  values from the Beech site and meteorological data, monitored throughout the year at the site is explored. The measured  $\delta D$  values from the Beech leaf n-alkanes are compared to modelled leaf water isotope values.

## **5.2. Materials and Methods**

### **5.2.1. Study site and sample collection**

Both sampling sites are shown on Figure 1. The Maple site is located in south-western Germany, close to the village of Thann, about 45 km NE of Regensburg. The forest area (2 ha) consists of Maple (*Acer pseudoplatanus*), planted under an umbrella of Pine and Spruce (*Pinus sylvestris* and *Picea abies*). Shaded Maple leaves were sampled every one to two weeks along a path into the forest.

The Beech sampling site is located within the “Hainich National Park”, near the city of Eisenach in Central Germany (51°04'46"N, 10°27'08"E, 440 m a.s.l.) in a suboceanic/subcontinental climate with an annual mean air temperature of 8°C and 750-800 mm average annual precipitation. The area has been protected as a National Park since 1997, since it is one of the largest virgin Beech forests in Europe. The forest is dominated by Beech (*Fagus sylvatica* with 65%), Ash (*Fraxinus excelsior*, 25%) and Maple (*Acer pseudoplatanus* and *plantonoides*, 7%). Other deciduous and, to a smaller extent coniferous trees are also present. The lack of management has resulted in a wide distribution of tree age classes with a maximum of up to 250 years. A more detailed site description is given in Knohl *et al.* (2003).

Sampling of leaves took place approximately every two weeks during the 2004 growing season (April to early November) along a ca. 50 m transect from a track to the meteorological measurement tower site.

For both sites approximately 20 shaded leaves from different trees at a height of about 2 m were pooled for analysis, to obtain a representative, average value..

### **5.2.2. Meteorological measurements at the Hainich site**

Meteorological and ecosystem data were measured at an eddy covariance flux tower, whose footprint covers the sampled area at the Hainich site. Air pressure (PTB101B, Vaisala, Helsinki, Finland), air temperature (at 2 m) and air humidity (HMP35D, Vaisala, Helsinki, Finland), photosynthetically active radiation (LI-190SA, LiCor Inc., Lincoln, NE, U.S.A.) and water vapour concentration (LiCor 6262-3, LiCor Inc., Lincoln, NE, U.S.A.) were monitored continuously. Precipitation was collected at the tower site (RainGauge, Young, Traverse City, MI, U.S.A.). In addition, soil moisture was measured at 8 cm depth (Theta probes, ML-2x, Delta T, Cambridge, U.K.). A detailed description of the available measurement equipment at the Hainich site is given in Knohl *et al.* (2003).

### **5.2.3. Modelling leaf water isotopic enrichment**

The classical model describing the enrichment of  $^{18}O$  and D of a water body due to evaporation was developed by Craig & Gordon (1965). The model was later expanded to include leaf boundary layers (FARQUHAR *et al.*, 1989; FLANAGAN and EHLERINGER, 1991). To calculate the leaf water isotopic composition the model described in (RODEN *et al.*, 2000; RODEN and EHLERINGER, 1999) will be used. It should be noted that this model predicts the isotope composition of leaf water at the site of evaporation, e.g. the chloroplast, where the photosynthesis and carbohydrate metabolism take place. In contrast, bulk leaf water in plants is

isotopically more heterogeneous (YAKIR et al., 1989), due to specific anatomical, morphological and physiological features of leaves. Since the isotope composition of the hydrogen source for biosynthesis will be modelled, there is no need to account for these processes.

The model is available as an electronic spreadsheet for download under

[ftp://ecophys.biology.utah.edu/Tree\\_Ring/](ftp://ecophys.biology.utah.edu/Tree_Ring/):

$$R_{wl} = \alpha^* \left[ \alpha_k R_{wx} \left( \frac{e_i - e_s}{e_i} \right) + \alpha_{kb} R_{wx} \left( \frac{e_s - e_a}{e_i} \right) + R_a \left( \frac{e_a}{e_i} \right) \right] \quad \text{Equation 2}$$

The subscripts *wl*, *wx* and *a* refer to leaf water, xylem water (which is equivalent to the source water for the plant) and bulk air, respectively. *e* is the vapour pressure (subscripts *i*, *s* and *a* refer to intercellular air spaces, leaf surface and bulk air, respectively).

The fractionation factors involved are well established: the equilibrium fractionation between liquid and water vapour ( $\alpha^*$ ) is slightly temperature dependent (MAJOUBE, 1971), the kinetic fractionation associated with diffusion in air ( $\alpha_k$ ) has been determined by Merlivat (1978). The kinetic fractionation during diffusion through the boundary layer ( $\alpha_{kb}$ ) is calculated raising  $\alpha_k$  to the 2/3 power (FLANAGAN et al., 1991).

The major environmental factors controlling the hydrogen isotope composition of leaf water ( $R_{wl}$  or  $\delta D_{wl}$ ) are the relative humidity (which is used in the calculation of  $e_a$ ) and the isotopic composition of the source water ( $R_{wx}$ ) and the atmospheric water vapour ( $R_a$ ).  $e_i$  is a function of leaf temperature. The vapour pressure at the leaf surface,  $e_s$  is the only parameter where leaf physiology is considered, and it is estimated using stomatal conductance and transpiration rate. However, only differences in an order of magnitude in the estimation of stomatal conductance and transpiration rate are likely to affect the modelled leaf water isotopic composition by a few ‰ (RODEN et al., 2000).

As model input a value of  $0.1 \text{ mol m}^{-2} \text{ s}^{-1}$  for stomatal conductance is used, which is reasonable for Beech leaves (HERBST, 1995) and a value of  $1 \text{ mol m}^{-2} \text{ s}^{-1}$  for the boundary layer conductance, which is used estimating leaf transpiration and finally  $e_s$ .

Since the temperature at the leaf surface, where the modelled processes take place is rarely equal to air temperature,  $\alpha^*$  is calculated using a 2° higher leaf temperature, which is true for high radiation loads, when most photosynthetic activity is expected. However, high transpiration rates and low radiation can result in a cooler leaf than air temperature. It should be noted, that the sensitivity of the model to changes in leaf temperature is rather small, a 1°C decrease in temperature results in about 1‰ decrease in leaf water  $\delta D$  values, which is within the standard deviation of our measurement.  $\alpha_k$  is assumed to have a value of 1.025 (MERLIVAT, 1978).

For  $\delta_i$ , the source water isotopic composition, the monthly  $\delta D$  values of meteoric water, calculated using the Online Isotopes In Precipitation Calculator (OIPC, accessible at <http://www.waterisotopes.org>), which uses the IAEA-GNIP meteoric water database (IAEA, 2001) and interpolation algorithms described in Bowen & Revenaugh (2003) are applied. The calculated isotopic composition of meteoric water was found to accurately predict the actual measured isotopic composition of lake and stream waters throughout Europe (SACHSE et al., 2004b).

The isotopic composition of the atmospheric water ( $\delta D_a$ ) exerts a major influence on  $\delta_{iw}$ . Since there are no direct measurements of  $\delta D_a$ , these values have been estimated using empirical, temperature dependent equations obtained by Jacob & Sonntag (1991). The authors investigated an 8-year record of precipitation and water vapour at Heidelberg in Southern Germany, where the general climate is comparable to our study sites. Using the daily mean temperature measured at the tower one obtains a mean  $\delta D_a$  value of  $-132.3\text{‰}$  ( $n=228$ ,  $SD=13.3$ ) for the period between the 1.4. 2004 and the 15.11.2004. The mean  $\delta D$  value of meteoric water obtained using the OIPC for this period is  $-56.0\text{‰}$  (using the equations by Jacob & Sonntag (1991) for estimation of  $\delta D$  of the precipitation, results in a rather similar mean value of  $-55.1\text{‰}$ ), therefore the mean isotopic difference between water vapour and precipitation is  $-76.2\text{‰}$ . Under equilibrium conditions at  $20^\circ\text{C}$  a mean isotope fractionation of  $-85.1\text{‰}$  would be expected, which is similar to the empirically estimated values, when considering the mean standard deviation of the n-alkane  $\delta D$  measurement of  $4\text{‰}$ . Therefore a steady state between water vapour and precipitation is assumed, which is true only for the wetter periods. However, using the above mentioned estimations results in reasonable values. Therefore, the mean isotopic difference between atmospheric water vapour and precipitation at our site is assumed to be  $-76.2\text{‰}$  and  $\delta D_a$  values are calculated accordingly.

### 5.3. Results

#### 5.3.1. n-alkane concentrations

##### 1. Thann site (Maple)

The total concentration of n-alkanes from Maple leaves ranges between 25 and 100 mg per kg dry weight. The total concentration of n-alkanes is highest in spring and fairly constant over summer, before decreasing in August. However, we will rely here more on the relative amounts of n-alkanes, since sampling heterogeneity and the extraction procedure might be responsible for small concentration changes. Maple leaves contain the n-alkanes n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub> and n-C<sub>31</sub>. n-C<sub>25</sub> makes up 6% of the annual mean and is only a minor constituent. The major n-alkane



is n-C<sub>29</sub>, with 38%, whereas n-C<sub>27</sub> and n-C<sub>31</sub> both compromise about 28%. The amount of n-C<sub>25</sub> is rather constant over the growing season, whereas the relative amount of n-C<sub>27</sub> and n-C<sub>31</sub> is decreasing from 38% in May to 22% and from 37 to 17% in November, respectively. n-C<sub>29</sub> is constantly increasing from 25 to 57% over the course of the sampling period (Figure 11).

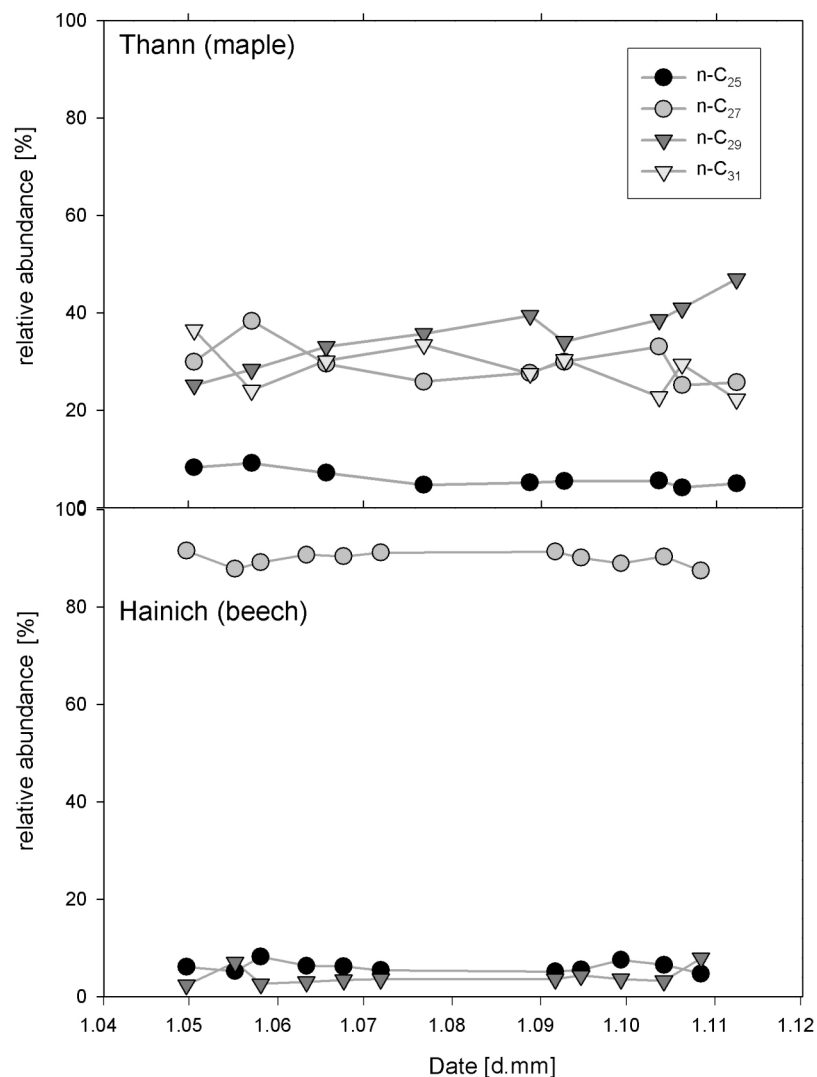


Figure 11: Seasonal variations in the relative amounts of n-alkanes from Maple (top) and Beech (bottom) leaves

#### Hainich site (Beech)

The total concentration of n-alkanes from Beech leaves ranges between 150 to 260 mg per kg sample dry weight, and is slightly higher than for Maple leaves. The highest total concentrations were also observed in spring, with decreasing amounts over the summer period. The analysed Beech leaves contained only 6% of n-C<sub>25</sub> and 4% of n-C<sub>29</sub> as annual means. The major constituent was n-C<sub>27</sub>, which made up 90% of all n-alkanes in Beech leaves. No change in the relative amount of n-alkanes over the growing season was apparent (Figure 11).

### 5.3.2. n-alkane $\delta D$ and $\delta^{13}C$ values

#### 1. Thann site (Maple)

Isotopic ratios were measured on the n-alkanes n-C<sub>27</sub>, n-C<sub>29</sub> and n-C<sub>31</sub> extracted from the Maple leaves. The  $\delta^{13}C$  values of n-C<sub>29</sub> decreased throughout the growing season from -37.1 to -38.4‰, whereas the  $\delta^{13}C$  values of n-C<sub>27</sub> and n-C<sub>31</sub> were about 2‰ heavier and increased throughout the year by about 1‰ (Table 11, Figure 12).

Table 11:  $\delta^{13}C$  and  $\delta D$  values of the n-C<sub>27</sub> n-C<sub>29</sub> and n-C<sub>31</sub> alkanes extracted from Maple leaves during the 2004 growing season

Thann (maple) sampling date	n-C <sub>27</sub>		$\delta D$		n-C <sub>29</sub>		$\delta D$		n-C <sub>31</sub>		$\delta D$	
	$\delta^{13}C$	stdv	$\delta D$	stdv	$\delta^{13}C$	stdv	$\delta D$	stdv	$\delta^{13}C$	stdv	$\delta D$	stdv
03.05.2004	-37.2	0.1	-154	1	-37.1	0.1	-169	6	-36.5	0.1	-178	1
23.05.2004	-38.4	0.3	-158	3	-38.2	0.1	-159	19	-37.4	0.6	-169	4
18.06.2004	-37.1	0.1	-169	0	-38.7	0.3	-181	4	-37.3	0.5	-178	5
22.07.2004	-36.8	0.2	-158	3	-38.3	0.3	-183	1	-36.4	0.0	-179	3
28.08.2004	-37.1	0.1	-142	8	-38.3	0.1	-175	7	-36.5	0.3	-164	7
09.09.2004	-37.5	0.3	-129	10	-38.2	0.2	-169	6	-37.1	0.2	-157	9
12.10.2004	-37.2	0.1	-172	2	-39.2	0.2	-187	5	-36.8	0.0	-177	2
20.10.2004	-37.0	0.8	-137	1	-39.0	0.4	-176	2	-37.1	0.3	-159	7
08.11.2004	-36.6	0.2	-178	1	-38.4	0.2	-192	3	-35.2	0.2	-182	3

$\delta D$  values of all 3 n-alkanes were highly variable. n-C<sub>29</sub> was characterized by the lowest  $\delta D$  values, n-C<sub>31</sub> was about 5-10 ‰ heavier and n-C<sub>27</sub> was again 10-20‰ heavier. All three n-alkanes showed a similar variability over the year. Higher values were observed in early May. A subsequent rise in  $\delta D$  values was apparent until late August, the values decreased again in late September, before showing another increase in early October. To mid-October,  $\delta D$  values of all three n-alkanes showed a decrease.

Table 12:  $\delta^{13}C$  and  $\delta D$  values of the n-C<sub>27</sub> alkane from Beech leaves

Hainich (beech) sampling date	n-C <sub>27</sub> $\delta^{13}C$	stdv	$\delta D$	stdv
30.04.2004	-35.5	0.1	-156	1
17.05.2004	-35.8	0.1	-159	2
26.05.2004	-34.8	0.1	-168	3
11.06.2004	-36.9	0.1	-175	3
24.06.2004	-36.0	0.1	-158	3
07.07.2004	-35.4	0.3	-168	1
06.09.2004	-33.6	0.1	-160	4
15.09.2004	-34.3	0.1	-160	7
29.09.2004	-35.2	1.1	-140	4
14.10.2004	-34.8	0.2	-159	3
27.10.2004	-34.5	0.6	-156	2

## 2. Hainich site (Beech)

$\delta D$  and  $\delta^{13}C$  values were determined only on the n-C<sub>27</sub> alkane, the concentration of the other compounds was too low for the measurement of isotope ratios (Table 12, Figure 12).

$\delta^{13}C$  values of the n-C<sub>27</sub> alkane rose to a value of -34.8‰ in late May, before they dropped to -36.9‰ in June. A high value of -33.6‰ was observed again in early September, before values stabilized at around -35‰.

$\delta D$  values of the n-C<sub>27</sub> alkane decreased from a relatively high value of -155‰ in late April to -180‰ in early June. An increase in  $\delta D$  values was observed in late June. Another strong increase with the highest measured value in the 2004 growing season, -140‰ was observed in early October. Afterwards values decreased to -160‰. It is evident, that mean  $\delta D$  values in September and October were slightly higher than from April to July.

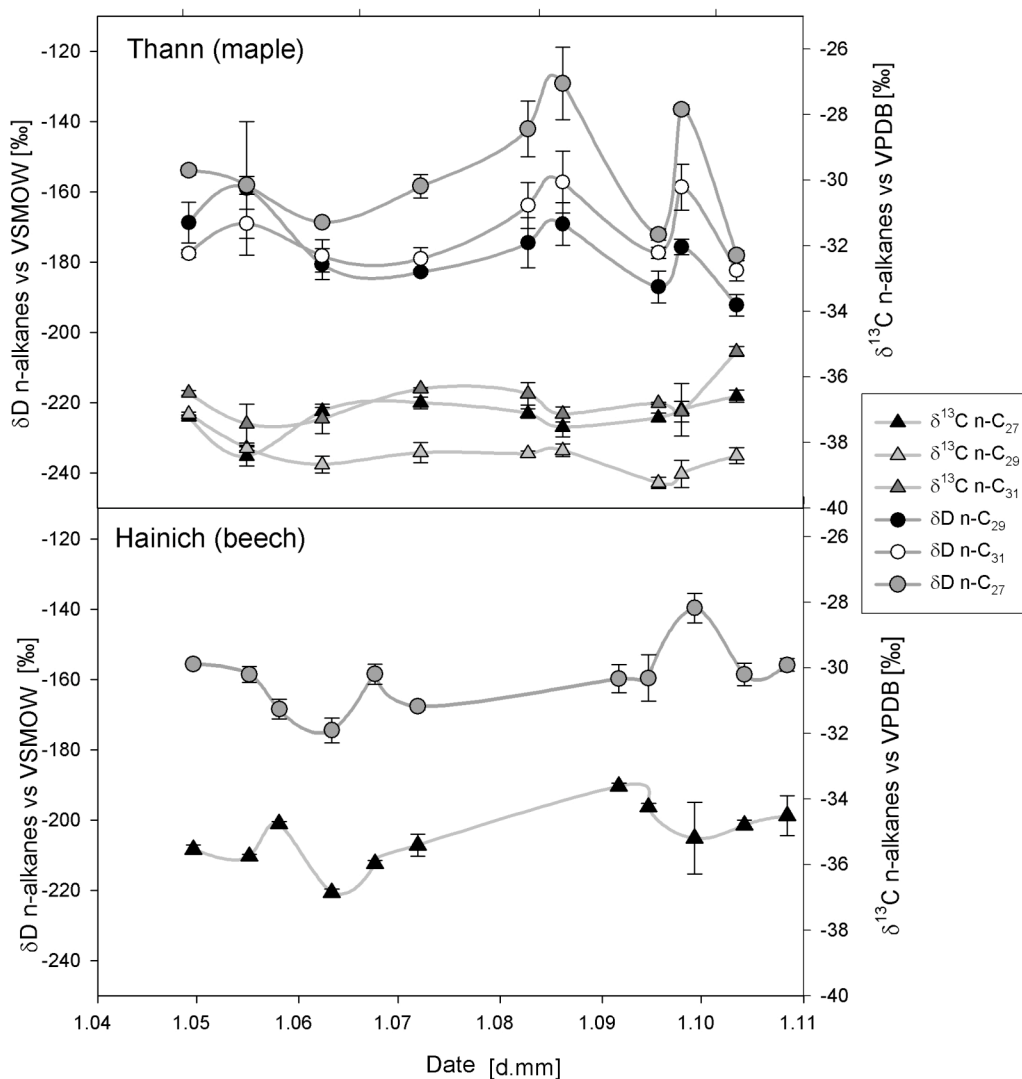


Figure 12: Seasonal variations in the  $\delta D$  and  $\delta^{13}C$  values of n-alkanes from Maple (top) and Beech (bottom) leaves

### 5.3.3. Modelling leaf water $\delta\text{D}$ values at the Hainich site

The modelled leaf water  $\delta\text{D}$  values for the Hainich site vary between +10‰ and -45‰ (daily values) or 0 to -30‰ as 3-day mean values over the growing season, with a mean value of -17‰ (Figure 13). This results in an average enrichment over mean annual source water  $\delta\text{D}$  value (meteoric water  $\delta\text{D} = -56‰$ ) of 39‰. The modelled  $\delta\text{D}$  leaf water values correlate with relative humidity ( $r^2=0.92$ ,  $n=260$ ), demonstrating the strong dependence of leaf water  $\delta\text{D}$  values on relative humidity. The highest  $\delta\text{D}$  values were modelled for late April, late June (up to +8‰) and early September (up to +12‰), coinciding with longer periods of low relative humidity and few rainfall events (Figure 15). Towards the end of the growing season modelled  $\delta\text{D}_{\text{wl}}$  values decrease to -40‰ in late October.

## 5.4. Discussion

### 5.4.1. Seasonal variations in n-alkane concentration

Maple leaves contained mostly the n-C<sub>27</sub>, n-C<sub>29</sub> and n-C<sub>31</sub> alkanes (Figure 11).

The observed seasonal variation, an increase in the relative amount of n-C<sub>29</sub> over the growing season and a decrease in the relative amount of n-C<sub>27</sub> and n-C<sub>31</sub> suggests that n-alkanes are synthesised *de novo* over the course of the year in Maple leaves.

No change in the relative amount of n-alkanes in Beech leaves was observed, with n-C<sub>27</sub> making up about 90% of all n-alkanes. This supports previous observations, where Beech leaves and soils under Beech stands were found to contain high amounts of n-C<sub>27</sub> (GLEIXNER et al., 2005; PIASENTIER et al., 2000). However, Piasentier et al. (2000) reported a progressive increase in the longer-chain n-alkanes during successive leaf stages from buds to mature leaves for Ash, Hazel and Beech. This phenomenon is observed in the Maple leaves with the increasing relative amount of n-C<sub>29</sub>, but not for Beech leaves. Piasentier et al. (2000) also report higher amounts of n-C<sub>25</sub> in young Beech leaves than found in this study. This suggests that intra-species variability and/or environmental factors play a role in n-alkane synthesis in the leaf wax layer as observed for *Rosmarinus officinalis* (MAFFEI et al., 1993).

### 5.4.2. Seasonal variations in n-alkane $\delta\text{D}$ and $\delta^{13}\text{C}$ values for Maple leaves

$\delta\text{D}$  and  $\delta^{13}\text{C}$  values of leaf wax n-alkanes of two deciduous trees are highly variable throughout the year (Figure 12). Those results again suggest, that n-alkanes in the leaf wax layer of higher plants are synthesised *de novo* year-round. The observed maximum variations of the n-alkane  $\delta\text{D}$  values of about 40‰ were within the range of expected leaf water enrichment (LEANNEY et

al., 1985). However, the question remains, if leaf wax n-alkanes preserve a cumulative leaf water isotope signal, or if complete or partial turnover of the leaf wax n-alkanes results in an integrated value over a certain timeframe. According to Gulz (1994) the leaf wax is completely developed shortly after unfolding of the leaf in spring but several studies have shown that the chemical composition of the wax layer is constantly changing (GULZ, 1994; PIASENTIER et al., 2000). This would imply that the deuterium content of the n-alkanes should vary accordingly. The decreasing total n-alkane concentration over the growing season suggests that more n-alkanes are produced in spring than in summer. However, the changing relative amounts of n-alkanes for the Maple leaves and the high variability of the n-alkane  $\delta D$  values, especially in summer, for Maple and Beech leaves suggest *de novo* production all over the year. It is known that abrasion by wind removes significant amounts of n-alkanes from the leaf wax layer (SCHEFUSS et al., 2003), which would imply the need for a year-round production of n-alkanes, to ensure the protection from evaporative water loss from the leaves.

The  $\delta D$  values of n-alkanes from the Maple leaves from Thann show relatively high values at the beginning of the 2004 growing season in late April and early May, before they decrease to lower values in June. Although this might be related to an enriched source water, it is more likely that isotopically enriched reserves, mainly stored as starch from the previous year, are used up during the for the biosynthesis during the early growth season. It is known that tree growth in early spring mainly depends on reserves from the previous year (DAMESIN and LELARGE, 2003), which has also been observed in tree-ring cellulose from early-wood (HELLE and SCHLESER, 2004). Thus, the  $\delta D$  signal preserved in the leaf wax n-alkanes in spring might reflect dry conditions from last autumn and not the current spring conditions.

The relatively high difference between  $\delta D$  values of the different n-alkanes (as much as 50‰ for the August event between n-C<sub>29</sub> and n-C<sub>27</sub>, Figure 12) suggests a pronounced heterogeneity in the hydrogen source. Either the more enriched substances (n-C<sub>27</sub> and n-C<sub>31</sub>) are preferably synthesised during these two events (although no significant change in the relative amount of these n-alkanes is observed at that time), or they are synthesised from a more enriched hydrogen pool than n-C<sub>29</sub>.

The  $\delta^{13}C$  values of the n-alkanes from Maple leaves vary only slightly by 2‰ over the growing season (Figure 12, Table 11). Interpretation of  $\delta^{13}C$  values from biomass values is difficult, since several factors can influence these values. The discrimination of  $^{13}C$  in biomass during carbon fixation can be expressed as:

$$\Delta = a + (b - a) \left( \frac{c_i}{c_a} \right) \quad \text{Equation 3}$$

(FARQUHAR et al., 1982)

The net discrimination consists of 2 processes: the discrimination during diffusion of  $\text{CO}_2$  through the stomata ( $a$ ), which is estimated to be  $-4.4\text{‰}$  and the net discrimination due to carboxylation ( $b$ ) which is about  $-27\text{‰}$ . However, the biosynthesis of specific compound classes, which are synthesised from the products of the Calvin Cycle, involves additional discriminations. Lipids, for example, are generally more depleted in  $^{13}\text{C}$  than bulk biomass (ABELSON and HOERING, 1961; COLLISTER et al., 1994).

$c_i$  and  $c_a$  are the intercellular and ambient  $\text{CO}_2$  concentrations. This ratio is controlled by stomatal conductance and photosynthetic rate. Variation of both parameters is dependant on physiological factors (eg. different species have different responses of stomatal conductance to environmental parameters) and environmental factors. Separation of the relative influences of these different factors is not straightforward. However, Yakir & Israeli (1995), have suggested that with the combined use of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of leaf tissue it is possible to separate these two factors. Scheidegger *et al.* (2000) have presented a conceptual model to separate the relative influences of stomatal conductance and photosynthetic rate based on  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values of organic matter. Since  $\delta\text{D}$  values of leaf water, the hydrogen source for the biomass, are subject to the same processes as  $\delta^{18}\text{O}$  values, their responses to the discussed processes should be rather similar. The  $\delta^{13}\text{C}$  values obtained from the Maple leaves do not show any positive or negative correlation with  $\delta\text{D}$  values, their variation over the growing season is very small (within  $2\text{‰}$ ). Little effect on  $\delta^{13}\text{C}$  values during a change in  $\delta^{18}\text{O}$  (eg.  $\delta\text{D}$ ) of biomass would be due to simultaneous changes in photosynthetic rate and stomatal conductance (SCHEIDEGGER et al., 2000). Following this interpretation a high  $\delta\text{D}$  values with no apparent changes in  $\delta^{13}\text{C}$ , as observed in early September and October for the Maple leaves, would be caused by reduced photosynthetic rate and reduced stomatal conductance during dryer periods. Low  $\delta\text{D}$  values without changes in  $\delta^{13}\text{C}$ , as observed in early October, are caused by the opposite effect during wetter periods.

#### **5.4.3. Seasonal variations in n-alkane $\delta\text{D}$ and $\delta^{13}\text{C}$ values for Beech leaves - Comparison of modelled leaf water enrichment with n-alkane $\delta\text{D}$ values**

The  $\delta\text{D}$  values of n- $\text{C}_{27}$ , the only major n-alkane found in the Beech leaves from the Hainich site, also start with relatively high values in late April (Figure 12). This again might not be due

to leaf water enrichment, but to the use of stored reserves from the previous year. Two enrichment events are observed in n-C<sub>27</sub>  $\delta D$  values, the first in June and a second, more pronounced event in late September. If the leaf water  $\delta D$  value is calculated from the  $\delta D$  value of the n-C<sub>27</sub> alkane, using a mean biosynthetic fractionation during hydrogen incorporation into n-alkanes of -157‰ (SACHSE et al., 2004b; SESSIONS et al., 1999), values vary between +10‰ and -15‰, well within the range of the modelled  $\delta D$  values (Figure 13), suggesting that the n-C<sub>27</sub>  $\delta D$  value is determined by  $\delta D$  of the leaf water. Since it is not expected that the n-alkane  $\delta D$  value on the sampling day records the current  $\delta D$  leaf water signal, a possible time lag has been investigated. 3-day mean values of modelled  $\delta D$  for subsequent 3, 9, 12, 15, 18, 21, 24, 27 and 30 day time lags were calculated for the two observed enrichment events and compared to the measured n-C<sub>27</sub>  $\delta D$  values (the high late April value is left out, since it might reflect the use of storage products rather than leaf water as a hydrogen source). Significant correlations are obtained for a time lag of 27 (r=0.99, n=5) and 30 days (r=0.93, n=5) for event one and of 18 days (r=0.95 n=5) for event 2 (Figure 14). For event one relative humidity shows also a negative relationship with n-alkane  $\delta D$  values (r=-0.75 and r=-0.73 for 27 and 30 days respectively). However, it is not statistically significant. For event two relative humidity gave a significant negative relationship to  $\delta D$  of n-C<sub>27</sub> for an 18-day time lag (r=-0.90, n=5). The resulting equations for the linear regressions are shown in Table 13.

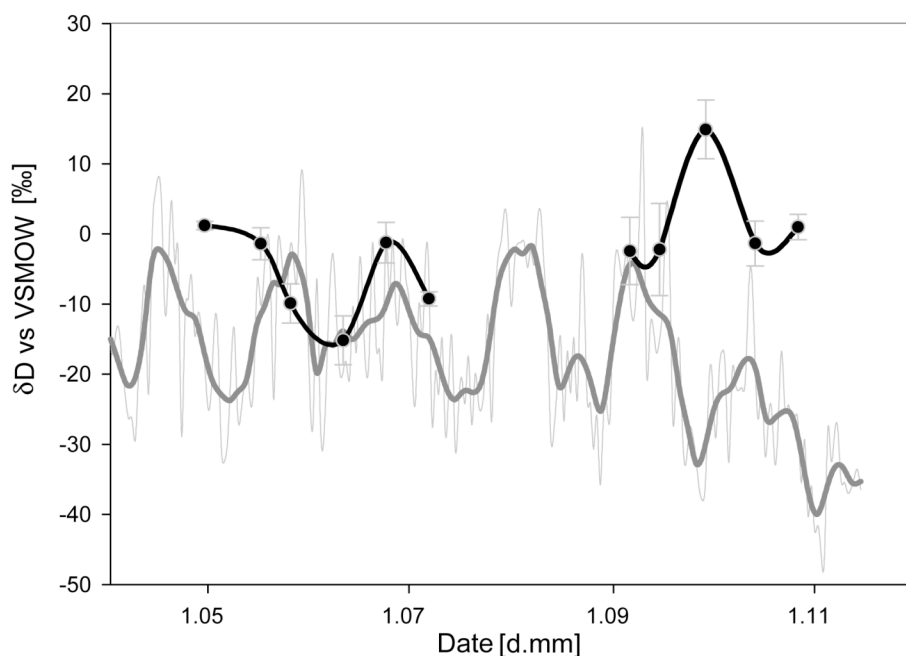


Figure 13: Modelled leaf water  $\delta D$  values and estimated  $\delta D$  values from the measurement of the n-C<sub>27</sub> alkane for the Hainich (Beech) site

The relationship for the 27-day time lag, with the highest Pearson coefficient (r=0.99) between modelled leaf water  $\delta D$  and measured n-C<sub>27</sub>  $\delta D$  is nearly a 1:1 relationship (slope is 1.04) with

an offset of  $-161.3\text{‰}$  between leaf water and n-alkane. This offset is strikingly similar to the expected biosynthetic fractionation between source water and lipid of about  $-157\text{‰}$ , demonstrating that the n-alkane  $\delta D$  value tracks the leaf water isotopic composition. For event 2 and an 18-day time lag a steeper slope (1.24) with an offset of about  $-167.9\text{‰}$  between leaf water and alkane is observed. Figure 13 shows that event 2 is characterised by the highest enrichment in the n- $C_{27}$   $\delta D$  values observed during our study. Roughly 18 days before a strong enrichment in leaf water  $\delta D$  was also found in our model runs, however, the predicted enrichment is about  $15\text{‰}$  lower than the observed enrichment in the n- $C_{27}$  alkane. When taking into account the soil moisture at 8 cm depth at the study site and the amount of precipitation over the 2004 growing season (Figure 15) it becomes apparent, that August, September and October of 2004 were much drier than May, June and July. The late summer/autumn soil moisture content was reduced to  $2/3$  of the spring/early summer value. According to a simple Rayleigh fractionation model for evaporation of water, a loss of  $1/3$  of the water results in an enrichment of  $35\text{‰}$  at  $20^\circ C$ .

Table 13: Significant correlations for 3-day mean values of modelled  $\delta D_{wl}$  and measured relative humidity (rH) against  $\delta D$  value of the n- $C_{27}$  alkane for specific time lags. The Pearson correlation coefficient (r) and the resulting linear regression equation are shown. (Note: for  $n=5$   $r>0.88$  or  $r<-0.88$  is statistically significant)

$\delta D$ n- $C_{27}$ vs	$\delta D_{wl}$		rH	
	r	equation	r	equation
<b>Event 1</b>				
27 days timelag	0.99	$\delta D_{wl} = 161.3 + 1.04 * \delta D_{C27}$	-0.75	$rH = -97.5 - 1.02 * \delta D_{C27}$
30 days timelag	0.93	$\delta D_{wl} = 174.3 + 1.11 * \delta D_{C27}$	-0.73	$rH = -140.9 - 1.28 * \delta D_{C27}$
<b>Event 2</b>				
18 days timelag	0.95	$\delta D_{wl} = 167.9 + 1.24 * \delta D_{C27}$	-0.90	$rH = -155.0 - 1.53 * \delta D_{C27}$

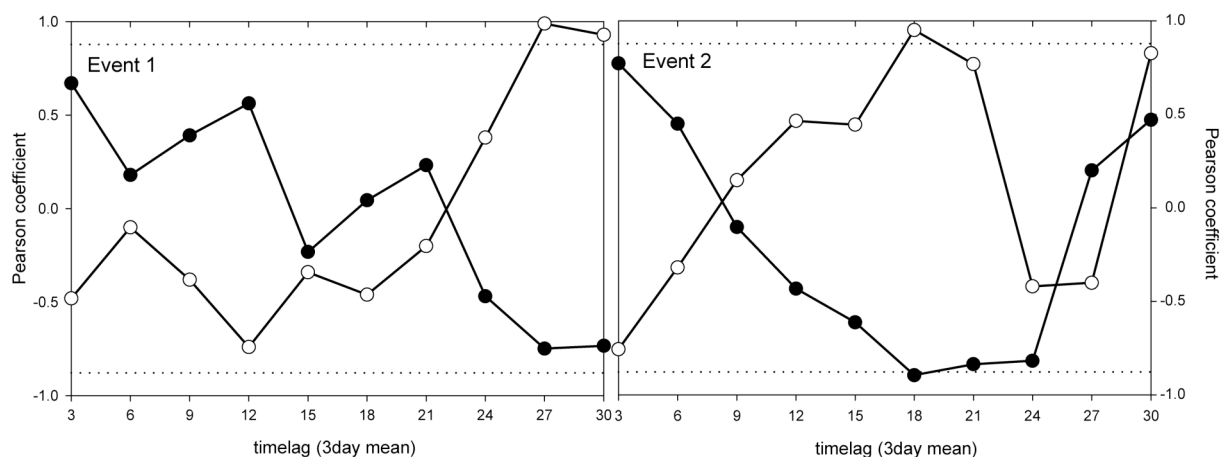


Figure 14: Plot of the Pearson correlation coefficients of modelled leaf water  $\delta D$  versus n- $C_{27}$  alkane  $\delta D$  (open circles) and relative humidity (filled circles) for the specific time lags (3 day means). Above or below the dotted lines correlations are significant ( $\alpha=0.05$ ).



However, a Rayleigh fractionation assumes a humidity of 0%, whereas the actual humidity will be higher and the resulting enrichment lower. This effect explains the higher than expected  $\delta\text{D}$  leaf water values, determined from the n-C<sub>27</sub> alkane  $\delta\text{D}$  value and their magnitude of ca. 20‰. This suggests that deciduous tree leaf wax n-alkane  $\delta\text{D}$  values track changes in the leaf water  $\delta\text{D}$  value by a 2 to 4 weeks time lag. It is not expected that n-alkanes will record every single excursion in leaf water isotope composition, but integrate over longer time periods. Longer dry or wet seasons (in the order of weeks) seem to be recorded in n-alkane  $\delta\text{D}$  values. Our data suggest that a more or less complete metabolic turnover of the hydrogen pool in n-alkanes occurs in roughly 4 weeks under these climatic conditions. Consequently, the n-alkanes would not be synthesised from recent assimilates, but would be conversion products formed from earlier formed substances, which carry an older source water signature. This mechanism would explain the rather long time lag of the leaf water hydrogen isotope signal, as observed in our study. Jetter & Schäffer (2001) have shown that after a complete removal of the wax layer of *Prunus laurocerasus* leaves a rapid reestablishment occurs. After 5 days a complete new wax layer had developed, with acetates as major constituents. Acetate concentration is decreasing at the same time as alcohols increase, which become dominant after 20 days, before concentrations of alcohols decrease. n-Alkanes show a slow but steady increase, becoming the dominant compound in the leaf wax ca. 30 days after the disruption. These findings suggest, that a conversion of acetates to alcohols by acetate hydrolysis takes place in the leaf tissue (JETTER and SCHAFFER, 2001). Although *P. laurocerasus* is different from Beech in several aspects, the data support our findings that n-alkanes seem to have slower turnover rates and therefore excursions in leaf water  $\delta\text{D}$  appear in the n-alkanes only after 2-4 weeks.

The  $\delta^{13}\text{C}$  values of the Beech leaf wax n-alkanes show a 3‰ variation over the growing season, which is slightly higher than observed for Maple leaves. Also, variations occur on shorter timescales than in the Maple leaves. In late May  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values of the n-C<sub>27</sub> alkane both fall before rising again in late June. Such a pattern could be the result when stomatal conductance exerts the major control, whereas the photosynthetic rate is not changing. Low  $\delta^{13}\text{C}$  values indicate a lower stomatal conductance and higher  $\delta^{13}\text{C}$  values are caused by higher stomatal conductance. Such a pattern would be expected when water is not limited (SCHEIDEGGER et al., 2000). The relatively high soil moisture content in June (Figure 15) at the Beech site supports this interpretation. The highest single precipitation event during the 2004 growing season occurred on May 7<sup>th</sup> with nearly 50 mm of rainfall on one day.

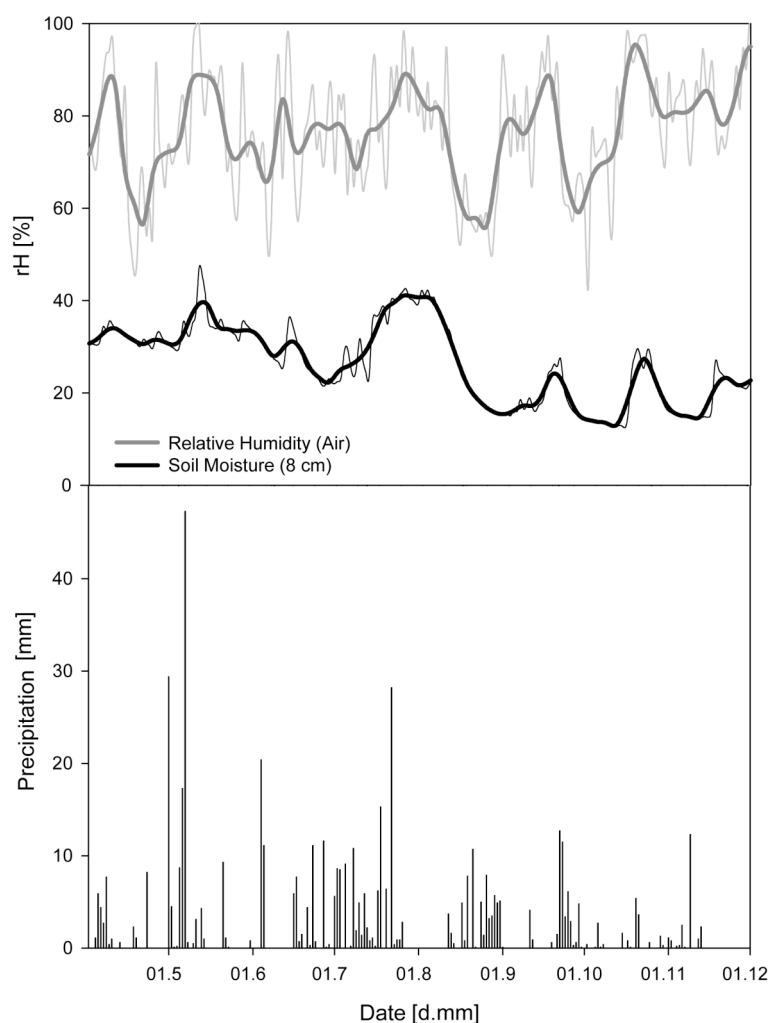


Figure 15: Relative humidity (rH), soil moisture at 8cm and precipitation amount for the 2004 growing season at the Hainich (Beech) site. Strong lines indicate 3-day mean values for rH and soil moisture.

This led to a significant increase in soil moisture in the following days (Figure 15). Due to sufficient water availability stomata will open, leading to a higher stomatal conductance. Higher stomatal conductance results in higher  $\delta^{13}C$  values of biomass, when photosynthetic activity is constant (SCHEIDEGGER et al., 2000). Interestingly, a peak in the n-C<sub>27</sub>  $\delta^{13}C$  value is observed 3 weeks after the precipitation event on 26<sup>th</sup> of May. Evidence for a rather constant photosynthetic activity is provided by the constant ratio of vapour pressure deficit (VPD) to photosynthetic activity (PAR) in May and July (Figure 16). During September and October lower  $\delta^{13}C$  values are observed at the same time as high  $\delta D$  values are measured. This isotope pattern suggests that stomatal conductance stays rather constant, but that the photosynthetic rate is lower (SCHEIDEGGER et al., 2000). To reduce water loss, stomata stay closed during the observed dryer period in autumn, so photosynthetic activity determines changes in biomass  $\delta^{13}C$  values. The VPD/PAR ratio shows much higher fluctuations from July to October (Figure 16), with lower values in late August caused by low VPD and higher values in early September, indicating less favourable conditions for photosynthesis.

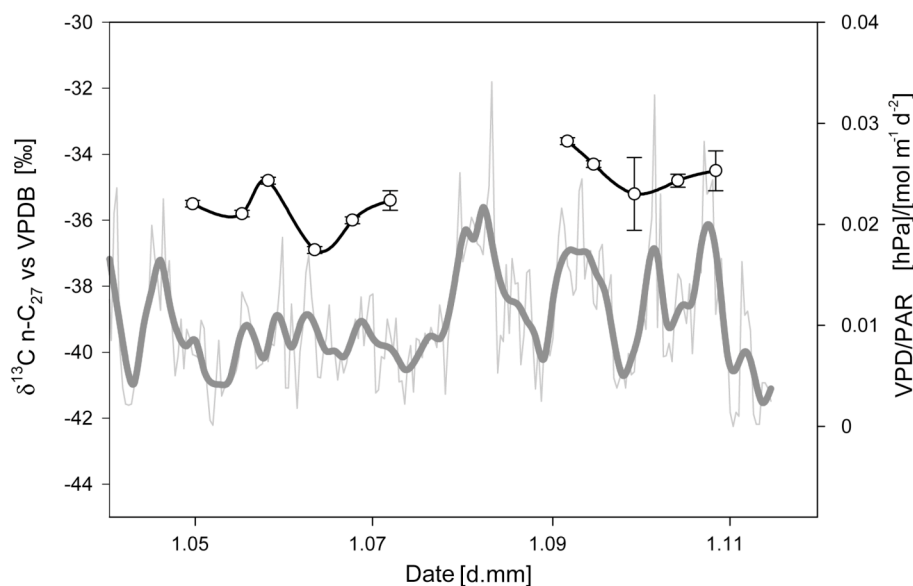


Figure 16:  $\delta^{13}C$  values of the n-C<sub>27</sub> alkane and the VPD/PAR ratio (vapour pressure deficit normalized to photosynthetically active radiation) over the 2004 growing season at the Hainich Beech site. The grey line indicates 3-day mean values for the VPD/PAR ratio.

Two weeks after the high VPD/PAR values in early September low  $\delta^{13}C$  values are observed in the n-C<sub>27</sub> alkane, which is in agreement with the interpretation of lower photosynthetic rate as the driver for the observed lower  $\delta^{13}C$  values.

The observed variation in the n-alkane  $\delta^{13}C$  values can be explained in conjunction with their  $\delta D$  values. The observed 2-4 week time-lag for  $\delta D$  in n-alkanes is also evident in  $\delta^{13}C$  values, although less pronounced and can be explained by variations in soil moisture and changing photosynthetic activity.

However, since at least two independent variables are determining the  $\delta^{13}C$  value (stomatal conductance and photosynthetic activity which are controlled by the interaction of a variety of environmental parameters) interpretation in terms of environmental change is much more difficult. By using both, hydrogen and carbon isotope ratios, an interpretation in terms of changing  $\delta^{13}C$  values due to changes in environmental parameters is possible.

The response of both isotope ratios on a molecular level to changing environmental conditions can also help to understand the flow of carbon and hydrogen through the different metabolic products of plants. Further studies could include different metabolic products (sugars, acetates, aldehydes etc.) and follow the flow of carbon and hydrogen to estimate turnover times of these products.

#### 5.4.4. Implications for the use of n-alkane $\delta D$ values as a palaeoclimate proxy

For Maple leaves, a change in the relative amounts of the n-alkanes is observed, which make the use of certain n-alkane distributions as biomarkers for specific trees nearly impossible.

However, Beech leaves were found to produce only n-C<sub>27</sub>.

Our results demonstrate that  $\delta D$  values from leaf wax n-alkanes are variable throughout the growing season. Comparison with modelled leaf water  $\delta D$  values suggests that the n-alkane  $\delta D$  values from deciduous tree leaves track the leaf water isotopic composition, integrated over a period of roughly a week by a 2 to 4 week time lag. This suggests, that n-alkanes are not produced by recent assimilates (such as sugars) but are conversion products of other compounds, possibly acetates and alcohols, which are the first compounds appearing in the leaf wax layer. The isotopic signal of the leaf water will therefore be transferred to the n-alkanes after a certain time lag. Since relative humidity is the main factor influencing the leaf water  $\delta D$  value, fossil leaf wax n-alkanes from sediments could be used to reconstruct changes in relative humidity. However, as evident in the lower autumn n-alkane  $\delta D$  values and the low soil moisture content, the soil water availability can significantly alter the modelled leaf water signal. Future models describing leaf water enrichment should therefore include a soil water model. Since most leaves from deciduous trees, incorporated into the sediments, are, at least in the temperate regions of the Earth, autumn leaves, the recorded humidity signal will be the autumn signal. However, when investigating long-term changes of relative humidity over time periods of several thousands of years, this should be of minor importance. In small lakes, where the meteoric water is the source water for the lake and the surrounding vegetation, the difference between aquatic biomarker  $\delta D$  values (for instance the algal derived n-C<sub>17</sub> alkane) and leaf wax n-alkanes could serve as a proxy for changes in leaf water enrichment and therefore relative humidity.

However, it is not clear yet, if different tree species or even different plants (grasses etc.) show different behaviour. Our data from the Maple leaves show, that significant differences in the different n-alkane  $\delta D$  values exist.

Clearly, further studies applying this approach to other tree species, grasses etc. under different climatic conditions are necessary to calibrate this new proxy to relative humidity. However, when investigating one site and by the use of multiple proxies giving additional information on the vegetation (such as pollen), reasonable estimates of changes in relative humidity should be possible.

## 6. Concluding Remarks

In this thesis the major open questions regarding the use of compound-specific hydrogen isotope ratios of sedimentary n-alkanes as a palaeoclimate proxy were resolved. Initial studies, including laboratory experiments (HUANG et al., 2002; SAUER et al., 2001; SESSIONS et al., 1999) have shown, that the  $\delta D$  values of organic compounds, such as sterols and fatty acids seem to reliably record the source water  $\delta D$  value. However, no study before has investigated, if sedimentary n-alkanes, maybe the most abundant biomarker throughout the geological time with no exchangeable hydrogen, record the source water  $\delta D$  signal in a natural system. n-Alkanes also permit a differentiation of aquatic and terrestrial organic matter input into sediments, since different n-alkanes are produced by aquatic and terrestrial organisms. Also, n-alkanes are relatively easy to extract and purify, compared to other biomarker compounds.  $\delta D$  values of these compounds should therefore hold information on the hydrogen source of terrestrial and aquatic organisms. Algae and photosynthetic bacteria living in the water column, which produce the short-chain n-alkanes like n-C<sub>17</sub>, use the lake water as their hydrogen source, whereas precipitation delivers hydrogen to terrestrial plants, whose leaf waxes contain high concentrations of the long-chained hydrocarbons n-C<sub>25</sub> to n-C<sub>33</sub>. The combined use of aquatic and terrestrial biomarker  $\delta D$  values in closed lake systems should therefore provide additional information on the evapotranspiration of the ecosystem around the lake (Figure 17).

In the third chapter of this thesis it was shown that n-alkanes from recent lake sediments along a climatic gradient from Northern Finland to Southern Italy record the source water  $\delta D$  value with high fidelity. The mean hydrogen isotope fractionation during n-alkane biosynthesis for the aquatic n-alkanes in natural ecosystems is constant at -157‰, regardless of lake trophic state or climate, which matches with laboratory experiments (SESSIONS et al., 1999). This result presents a solid base for the application of aquatic n-alkane  $\delta D$  values to reconstruct variations in the hydrogen isotope ratio of the source water, and therefore climate. Furthermore, the n-alkanes of terrestrial origin were enriched in deuterium relative to the aquatic n-alkanes by between 10 and 60‰. This enrichment is not due to a different isotopic fractionation ( $\epsilon$ ) during n-alkane biosynthesis, but due to evapotranspiration processes in the plants leaves. So, the apparent isotope fractionation between meteoric water and n-alkanes for terrestrial plants is therefore smaller, than compared to aquatic organisms (Figure 17). However, the question remained, if the terrestrial n-alkane  $\delta D$  signal in these lake sediments is a mean annual signal or if n-alkanes in the leaf waxes are synthesised *de novo* year round. Therefore plant biomass, mostly leaves from the dominating deciduous trees at the lake shore was analysed. The results presented in

chapter 4 show, that the  $\delta D$  values of the plant biomass n-alkanes are in general more enriched than the sedimentary n-alkanes of terrestrial origin. These data suggest that n-alkanes from the leaf waxes record seasonal variations, since the sampling took place in summer, when evapotranspiration is expected to enrich the leaf water in deuterium, compared to the autumn, when most leaves will be deposited into the lake sediments. The results also reveal that  $\delta D$  values of the leaf wax n-alkanes are similar for different deciduous tree species at the same site, but variable even between the same species at neighbouring sites. This suggests, that changes in terrestrial plant n-alkane  $\delta D$  values record variability in local climate, such as changes in relative humidity. Furthermore, it was observed that with increasing average chain length of the n-alkanes from the leaves of deciduous trees, the hydrogen isotopic fractionation between source water ( $\epsilon$ ) and n-alkanes increases. This increase of  $\epsilon$  is due to decreasing enrichment of leaf water, due to evapotranspiration. Therefore, broadleaf trees with longer chain n-alkanes are better protected from evaporative water loss. n-Alkanes from plants without stomata, such as mosses, are in general more enriched than n-alkanes from deciduous tree leaves at the same site, indicating that stomata play an important role in regulation the water loss of plants.

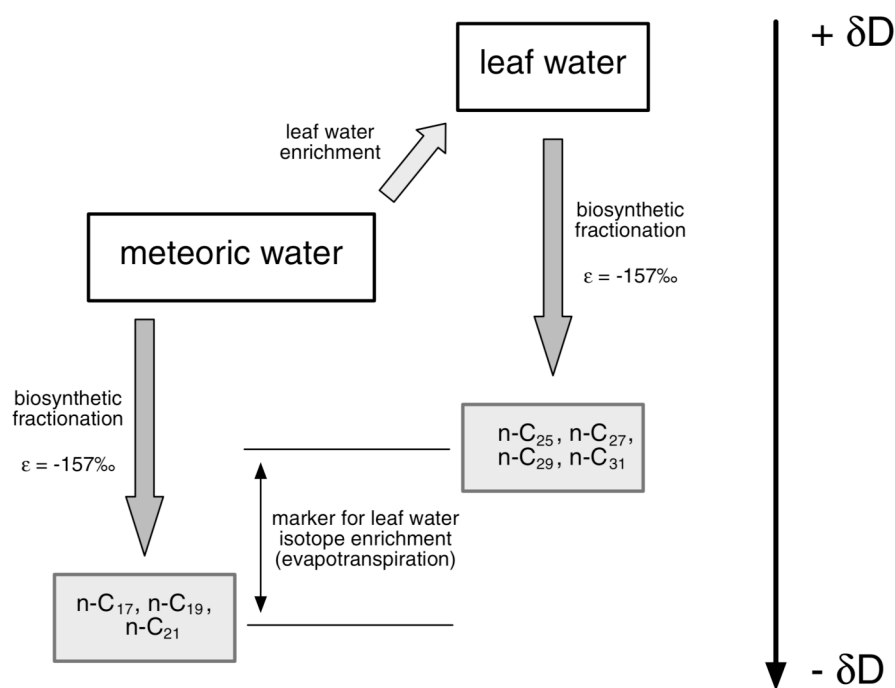


Figure 17: Isotopic relationships between meteoric water and aquatic (n-C<sub>17</sub>, n-C<sub>19</sub>, n-C<sub>21</sub>) and terrestrial n-alkanes (n-C<sub>25</sub>, n-C<sub>27</sub>, n-C<sub>29</sub>, n-C<sub>31</sub>) in lake sediments

To assess the magnitude and timing of possible seasonal variations in leaf wax n-alkane  $\delta D$  values, leaf samples from a Maple and Beech stand in Central Germany were sampled and analysed over the 2004 growing season. Both deciduous tree leaves show significant variations

in n-alkane  $\delta D$  and, to a smaller extent,  $\delta^{13}C$  values over the growing season. It is well established, that for instance  $\delta D$  values of cellulose from tree rings can record seasonal variations in leaf water (BARBOUR et al., 2002). These findings have resulted in a number of attempts to model the leaf water enrichment as a function of source water and environmental parameters (GAN et al., 2003; RODEN et al., 2000; RODEN and EHLERINGER, 1999).

Observations and models have shown, that the source water and water vapour isotope composition and relative humidity are the main drivers for changes in leaf water isotope enrichment. For the Beech stand, the Hainich forest, meteorological data are monitored throughout the year. Therefore, the deuterium enrichment of leaf water was modelled over the 2004 growing season. Comparison of the modelled leaf water  $\delta D$  values with the leaf wax n-alkane  $\delta D$  values revealed a 2-4 week time lag between isotope excursions in modelled leaf water and n-alkane  $\delta D$  values. Studies on the development of the wax layer of leaves have shown, that after an artificial wax removal a complex biosynthesis takes place to re-establish the protective layer. First aldehydes are formed and become dominant in the wax, after 10 days they are converted into alcohols and only after 30 days n-alkanes become the dominant compound in the wax. These results support our isotope data and imply that n-alkanes are not synthesised by recent assimilates, but are conversion products of earlier formed compounds, such as alcohols.

These results highlight the amount of information preserved in n-alkane  $\delta D$  values and open new perspectives in reconstructing changes in the water cycle. In a recent study Radke et al. (2005) could show, that high temperatures during sediment burial alter the n-alkane  $\delta D$  value in Permian and Jurassic sediments. However, since they observed a linear relationship between sediment maturity and n-alkane  $\delta D$  values, a correction of  $\delta D$  values from mature sediments seems possible, if the burial history of the sediments is known. This would extend the use of compound-specific hydrogen isotope ratios to reconstruct palaeoclimate and palaeohydrology to periods in Earth history where only few proxy data exist.

It is expected, that  $\delta D$  values of n-alkanes and other biomarker compounds will be increasingly employed to elucidate the influence of past climate change on the water cycle and vice versa. Based on the results from this thesis several perspectives for future applications in this emerging field become possible:

*(1) The connection of global climate change and the global water cycle in the recent geological past*

The dominant mode of global climate variability on multi-year timescales, the El Niño Southern Oscillation (ENSO) phenomenon affects the whole climate system, since global precipitation patterns are altered during ENSO events. Worrying is the prediction of an increase in frequency and/or intensity of ENSO events in a warmer climate (IPCC, 2001). However, there is no agreement among palaeoclimatologists on the magnitude of change in frequency or intensity of ENSO during the last ice age, a time when the climate state was significantly different from today. There also is evidence that the monsoonal cycles in Asia have changed several times abruptly since the last deglaciation, but the timing and intensity of some of these episodes remain elusive (MORRILL *et al.*, 2003). The exact timings, frequencies and intensities and therefore causes and feedbacks of these major climate phenomena, by their nature closely connected to the water cycle, are not well understood. Compound-specific  $\delta D$  values of sedimentary biomarkers from lake sediments in the affected areas can offer new information on the variability of these climatic phenomena and elucidate the causes of these changes and therefore permit better predictions of the consequences of current human interference with the climate system.

*(2) Application of compound specific  $\delta D$  values for plant-water studies*

The growing interest in understanding plant-water relationships in ecological studies demands for a detailed comprehension of the pathways and storage capacities of water in different plants. By investigating the flow of hydrogen isotopes through the different metabolic products in plants, this new analytical approach can deliver substantial information on the hydrogen and therefore water flow in plants. As the results presented in Chapter 5 show, n-alkanes in leaf waxes are conversion products of earlier formed substances. Future studies could follow the hydrogen flow into different metabolic compartments, to better understand the order in which metabolites are formed and to explain, at which stages in lipid biosynthesis the major isotope fractionation steps take place. Also, these studies could clarify, if hydrogen isotopes in different metabolic compartments record the meteorological conditions over different timeframes. This would provide a tool to reconstruct the prevalent meteorological conditions experienced by the plant.

*(3) Determining changes in ecosystem hydrology*

In a recent study Krull *et al.* (2005) could show, that the combined use of compound-specific  $\delta D$  and  $\delta^{13}C$  analysis of n-alkanes from soil organic matter can be used to



successfully differentiate between water sources of different vegetation types (grasses versus trees) in natural ecosystems. These results show the potential of n-alkane  $\delta D$  values to trace different water use strategies of plants. In the context of the increasing shortage of water in the arid regions of the Earth, understanding of these processes is particularly important in ecosystems altered by humans, such as deforestation or agriculture.

## 7. Summary

The aim of this thesis was, to explore if the hydrogen isotope ratio of biomarker compounds in lake sediments can be used as a novel tool to reconstruct changes in the water cycle over geological timescales. Therefore surface sediments from small lakes, as well as plant biomass from the lake catchments along a climatic gradient from Northern Finland to Southern Italy were analysed to test, if modern climate variability is recorded in their n-alkane  $\delta D$  values. To clarify the origin of the dominating terrestrial n-alkane input into the lakes and to account for possible seasonal variations in leaf wax n-alkane  $\delta D$  values and their causes, leaves of Beech and Maple trees from two sites in Germany were sampled over one growing season.

The results presented here demonstrate various climatic informations are preserved in biomarker  $\delta D$  values. In particular, this new proxy allows the reconstruction of changes in the isotopic composition of lake water, precipitation and relative humidity, all of which are induced by climatic changes.

In particular, the following results, related to the questions asked at the beginning (see 1.2.), represent a solid base for the application of n-alkane  $\delta D$  values for palaeoclimate reconstruction:

- 1) n-alkane  $\delta D$  values from surface sediments of small ground-water fed lakes along a climatic gradient have been shown to reliably record the source water  $\delta D$  values. N-Alkanes of aquatic origin (n-C<sub>17</sub>) clearly record the source water  $\delta D$  signal and therefore permit the reconstruction of changes in lake water  $\delta D$  values and hence variations in climate.
- 2) The isotopic fractionation between source water and n-alkanes in a natural ecosystem of aquatic organisms was found to be constant at -157‰ along the climatic gradient, which matches results from laboratory studies (SESSIONS et al., 1999). Since the investigated lakes are characterized by different trophic states and cover different climates, we conclude that environmental parameters exert only minor influence on this biochemical fractionation. This is particularly important, when applying n-alkane  $\delta D$  values to palaeoclimate questions. However, the observed constant isotopic fractionation facilitates the use of n-alkane  $\delta D$  values to reconstruct the source water  $\delta D$  value.
- 3) n-Alkanes of terrestrial origin found in the investigated lake sediments are enriched in D by 10-60‰, due to the evaporative enrichment of D in leaf water. Higher  $\delta D$  values

were observed for n-Alkanes extracted from leaves and plant biomass of the dominating vegetation around these lakes. This suggests that the higher evaporation during the summer (when leaves were sampled) lead to a D enrichment of leaf water and n-alkanes. With increasing average chain length of the n-alkanes from deciduous leaves a larger isotopic fractionation between source water and n-alkanes was observed, suggesting that longer chain n-alkanes in the wax layer of leaves provide a better protection from evaporative water loss and therefore decrease the enrichment of leaf water in plants.

4) If both, terrestrial and aquatic organisms use the same water source, the meteoric water, the observed isotopic difference between both biomarkers in lake sediments can be used to reconstruct leaf water enrichment, which in turn is determined by evapotranspiration. In small, ground water fed lakes, the difference between aquatic and terrestrial n-alkane  $\delta D$  values, can therefore be used as a proxy for the amount of evapotranspiration of the ecosystem at the lake shore.

5) n-alkanes from Maple and Beech leaves show significant seasonal variation in  $\delta D$  as well as  $\delta^{13}C$  values. Comparison with modelled leaf water  $\delta D$  values from the Hainich site reveals a 2-4 week time lag between isotope excursions in modelled leaf water and leaf wax n-alkane  $\delta D$  values. These observations provide evidence that n-alkanes are not synthesised by recent assimilates, such as sugars, but are conversion products from earlier formed substances such as alcohols.

Since air relative humidity is the main driver for changes in leaf water  $\delta D$  values, we conclude that leaf wax n-alkanes reliably track the leaf water  $\delta D$  value and therefore relative humidity. Since most leaves, at least in the temperate climates of the Earth, will be deposited into the lake sedimentary archives in autumn, the preserved  $\delta D$  signal will be the autumn signal.

These results show that compound-specific hydrogen isotope ratios from sedimentary archives record the current climatic conditions of the studied lake ecosystems and hence can be used to reconstruct changes in the water cycle.

## 8. Zusammenfassung

Das Ziel der vorliegenden Dissertation war, zu überprüfen, ob sich substanzspezifische Wasserstoffisotopenverhältnisse ( $\delta D$ ) einzelner Biomarker Substanzen aus Seesedimenten als neuer Ansatz zur Rekonstruktion von Änderungen des Wasserkreislaufes in der geologischen Vergangenheit nutzen lassen. Dazu wurden rezente Sedimente aus Seen entlang eines Klimagradienten von Nordfinnland bis Süditalien sowie Pflanzenbiomasse aus deren Einzugsgebieten analysiert, um zu überprüfen, ob die gegenwärtige Klimavariabilität in den  $\delta D$  Werten der n-Alkane aufgezeichnet wird. Um die Einflussfaktoren auf den  $\delta D$  Wert der n-Alkane terrestrischen Ursprungs in den Sedimenten zu bestimmen, wurden zusätzlich Blätter von Buche und Ahorn an zwei Standorten in Deutschland über eine Wachstumsperiode beprobt und analysiert.

Die vorliegenden Ergebnisse zeigen die Bandbreite der klimatischen Informationen, die in den  $\delta D$  Werten von n-Alkanen aufgezeichnet werden können und ermöglichen die Anwendung dieses neuen Paläoklimaindikatoren zur Rekonstruktion der Isotopenzusammensetzung des Seewassers als auch des Niederschlages sowie der relativen Luftfeuchte. Die Rekonstruktion von Änderungen in diesen Parametern erlaubt Rückschlüsse auf klimatische Variationen. Die folgenden Ergebnisse dieser Dissertation bilden eine umfassende Grundlage für die Anwendung substanzspezifischer  $\delta D$  Werte von n-Alkanen als neuer Paläoklimaindikator:

- 1)  $\delta D$  Werte von n-Alkanen aus rezenten Oberflächensedimenten grundwassergespeicherter Seen entlang eines klimatischen Gradienten zeichnen die Wasserstoffisotopenzusammensetzung der Wasserstoffquelle von Organismen auf. n-Alkane die von Algen produziert werden ( $n-C_{17}$ ) können somit genutzt werden, um die Veränderungen in der Isotopenzusammensetzung des Seewassers, und somit des Klimas, zu rekonstruieren.
- 2) Es konnte gezeigt werden, dass die Wasserstoffisotopenfraktionierung ( $\epsilon$ ) zwischen Wasserstoffquelle (meteorisches Wasser) und n-Alkanen in natürlichen Ökosystemen konstant  $-157\text{‰}$  über den gesamten Klimagradienten beträgt. Dieses Ergebnis bestätigt Laboruntersuchungen. Da sich die untersuchten Seen in verschiedenen Klimazonen befinden und durch unterschiedliche Trophiestufen gekennzeichnet sind, können Umweltfaktoren, wie z.B. Temperatur als Steuerungsfaktoren für die Isotopenfraktionierung während der n-Alkan Biosynthese ausgeschlossen werden.

Diese konstante Fraktionierung ermöglicht die Rekonstruktion der Isotopenzusammensetzung der Wasserstoffquelle anhand der  $\delta D$  Werte von n-Alkanen aus Sedimenten.

- 3) n-Alkane terrestrischen Ursprungs, die in den Blattwachsen, vor allem von Laubbäumen synthetisiert werden, sind in den Seesedimenten um 10-60‰ angereichert in Deuterium, hervorgerufen durch die Evapotranspiration in Blättern, die das schwere Isotop im Blattwasser anreichert. n-Alkane aus Pflanzenbiomasse vom Ufer der untersuchten Seen zeigten nochmals schwerere  $\delta D$  Werte als die sedimentären n-Alkane. Grund dafür ist eine höhere Evapotranspiration im zur Zeit der Probennahme im Sommer, relativ zum Herbst, wenn die meisten Blätter in die Seen eingetragen werden. Außerdem wurde bei längerer durchschnittlicher Kettenlänge der n-Alkane eine größere Isotopenfraktionierung relativ zum meteorischen Wasser beobachtet, und damit eine geringere Anreicherung von Deuterium im Blattwasser der Pflanzen. Diese Ergebnisse zeigen, dass längerkettige n-Alkane einen effektiveren Schutz vor Evapotranspiration bieten.
- 4) Wenn terrestrische und aquatische Organismen dieselbe Wasserstoffquelle, das meteorische Wasser, haben, wie beispielsweise an den untersuchten grundwassergespeisten Seen, kann der isotopische Abstand zwischen n-Alkanen terrestrischer und n-Alkanen aquatischer Herkunft zur Rekonstruktion der Deuterium Anreicherung im Blattwasser und somit als Maß für die Evapotranspiration des Ökosystems am Seeufer genutzt werden.
- 5) Es konnte gezeigt werden, dass  $\delta D$  als auch  $\delta^{13}C$  Werte von n-Alkanen aus Buchen- und Ahornblättern starke Schwankungen über eine Wachstumsperiode aufweisen. Der Vergleich mit modellierten Blattwasserisotopenverhältnissen zeigt, dass das  $\delta D$  Signal des Blattwassers erst nach 2-4 Wochen in den  $\delta D$  Werten der n-Alkane erscheint. Diese Ergebnisse zeigen, dass n-Alkane in Blattwachsen nicht direkt aus den Assimilaten der Photosynthese gebildet werden, sondern aus anderen, zuerst gebildeten Metaboliten, wie z.B. Alkoholen.

Da die relative Luftfeuchtigkeit der Hauptsteuerungsfaktor für Variationen im  $\delta D$  Wert des Blattwassers ist, kann geschlossen werden, dass n-Alkane der Blattwache das Isotopensignal des Blattwassers und somit die relative Luftfeuchtigkeit aufzeichnen. Da

Blätter zum größten Teil im Herbst in Seen eingetragen werden, ist das im Sediment enthaltene Isotopensignal das der relativen Luftfeuchtigkeit des Herbstes.

Die vorliegenden Ergebnisse bilden die Basis für die Anwendung dieser neuen Methode zur Klimarekonstruktion und zeigen, wie vielfältig  $\delta D$  Werte von n-Alkanen aus Sedimenten zur Rekonstruktion von Veränderungen im Wasserkreislauf genutzt werden können.

## Acknowledgements

Many people have provided help and support during my work for this PhD thesis. Especially I want to acknowledge the help from my advisors at Friedrich-Schiller University Jena and Max-Planck Institute for Biogeochemistry:

**Dr. Gerd Gleixner** (MPI-BGC Jena) for offering me to work in this emerging field of organic biogeochemistry and his strong support during the work for my thesis.

I would like to thank my university supervisor **Prof. Reinhard Gaupp** (Friedrich Schiller University Jena) for his continued support.

I would like to thank my friends and colleagues at MPI-BGC for providing an excellent research atmosphere and support throughout my thesis' work:

**Ansgar Kahmen** and **Volker Hahn** for their friendship and fruitful discussions on drafts of the manuscripts.

**Steffen Rühlow** and **Jens Radke** for the help while sampling and for teaching me how to run a GC-IRMS system. The IsoLab at MPI-BGC, especially **Willi Brand**, **Roland Werner** and **Heike Geilmann** for the measurement of water samples and standards. The workshop at MPI-BGC, especially **Bernd Schlöffel** and **Frank Voigt** are acknowledged for construction of our sampling raft.

Without help from the following people the 2002 lake sampling would not have been possible: I am grateful to **Gerhard Daut** (University of Jena), **Jörg Negendank** and **Cathrin Brüchmann** (GFZ Potsdam) for providing sampling equipment. **Dan Hammarlund** (University of Lund), **Tuomas Laurilla** (Finnish Meteorological Institute, Sodankylä), **Lauri Arvola** (Lammi Biological Station), **Timo Vesala** (Helsinki University), **Niels-Otto Jensen** (Risø National Laboratory), **Günther Schleser** and **Andreas Lücke** (FZ Jülich) and **Günther Seufert** (JRC, San Rossore) are acknowledged for providing help with selection of the lakes, support on-site and data on the lakes.

I thank **Ines Mügler** and **Andrej Thiele** for their help with sample extraction and measurement and **Alexander Telz** for leaf sampling at the Hainich.

I would like to thank the library, the computer department and the administration of MPI-BGC for their support.

I would like to thank **Alex Sessions** (CalTech, Pasadena, USA) and **Julian Sachs** (MIT, Cambridge, USA) who gave very helpful comments on chapter 3; **Evelyn Krull** (CSIRO, Glen Osmond, Australia), **Graham Farquhar** (RSBC, Canberra, Australia) and **Jim Ehleringer** (University of Utah, Salt Lake City, USA) for their comments on Chapter 5.

I also acknowledge the **Deutsche Forschungsgemeinschaft** for funding my PhD position under project G1262/8.

I would like to thank the **Max Planck Society** for providing the excellent scientific infrastructure at MPI-BGC.

I am deeply grateful to my parents **Sabine** and **Konrad Sachse** for their love, support and encouragement throughout my entire career. I would like to thank my girlfriend, **Luzie Polatzek**, for her enduring support during work for this thesis.



## 9. References

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### EDUCATION:

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- 1995-1997** Studies in Geology at Institute for Earth Sciences, Friedrich-Schiller University Jena, Germany
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- 3/2002** Diploma in Geology, grade: 1.1
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- 11/1993-1/1995** civil service at the hospital of the Friedrich Schiller University Jena
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- 1998-2001** elected member of the student council at Institute for Geosciences of the University of Jena
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.....

## **Selbständigkeitserklärung**

Ich erkläre, dass ich die vorliegende Arbeit selbständig und unter Verwendung der angegebenen Hilfsmittel, persönlicher Mitteilungen und Quellen angefertigt habe.

.....

Ort, Datum

.....

Dirk Sachse