Carbon isotope composition of middle Eocene leaves from the Messel Pit, Germany

Michaela Grein, Anita Roth-Nebelsick & Volker Wilde

Abstract

The $^{13}C/^{12}C$ ratios ($\delta^{13}C$) of leaves from the middle Eocene of the Messel Pit (Middle Messel Formation) were measured to determine the ratios of leaf-internal to ambient carbon dioxide concentration (c_i/c_a) for the respective time. For extant plants this parameter provides information about their ecophysiological state. Fossil leaves belonging to three species were analyzed: *Laurophyllum lanigeroides* (Lauraceae), *Daphnogene crebrigranosa* (Lauraceae) and *Rhodomyrtophyllum sinuatum* (Myrtaceae). In order to determine the range of $\delta^{13}C$ across a single leaf (intra-leaf variability) samples from the basal, central and apical region were separately prepared and analyzed. The results are compared to $\delta^{13}C$ and c_i/c_a ratios in extant evergreen Lauraceae (*Laurus nobilis*, *Cinnamomum camphora*, *Persea americana*) and Myrtaceae (*Myrtus communis*, *Psidium littorale/cattleianum*).

The $\delta^{13}C$ of the fossil cuticles varies from –30 ‰ to –27 ‰ in the Lauraceae and from –29 ‰ to –26 ‰ in the Myrtaceae, which are typical ranges for extant C_3-plants. Results of intra-leaf analyses indicate that $\delta^{13}C$ varies slightly across the leaves but intra-leaf variability is statistically not significant. Mean Eocene c_i/c_a-ratios as calculated from the measured $\delta^{13}C$ values varied from 0.78 to 0.87. The c_i/c_a-ratios of extant Lauraceae are substantially lower (average 0.69) than for the fossil material. In Myrtaceae, c_i/c_a-ratios are almost the same for extant and fossil material (extant Myrtaceae average at about 0.8).

Keywords: middle Eocene, Messel, leaves, carbon isotopes, c_i/c_a-ratio.

Zusammenfassung

Das $^{13}C/^{12}C$-Verhältnis ($\delta^{13}C$) von fossilen Blättern aus dem Mitteleozän der Grube Messel (Mittlere Messel Formation) wurde gemessen und daraus das Verhältnis von blattinternem zu atmosphärischem Kohlendioxid (c_i/c_a) für die betreffende Zeit bestimmt. Bei heutigen Pflanzen erlaubt diese Größe eine Einschätzung des ökophysiologischen Zustands. Es wurden die Blätter von drei fossilen Arten analysiert: *Laurophyllum lanigeroides* (Lauraceae), *Daphnogene crebrigranosa* (Lauraceae) und *Rhodomyrtophyllum sinuatum* (Myrtaceae). Um Schwankungen des $\delta^{13}C$ innerhalb eines Blattes (blattinterne Variabilität) bestimmen zu können, wurden getrennte Proben aus dem basalen, dem mittleren und dem apikalen Bereich genommen und analysiert. Die Ergebnisse wurden mit dem $\delta^{13}C$ und c_i/c_a rezenter immergrüner Lauraceae (*Laurus nobilis*, *Cinnamomum camphora*, *Persea americana*) und Myrtaceae (*Myrtus communis*, *Psidium littorale/cattleianum*) verglichen.

Das $\delta^{13}C$ der fossilen Kutikulen variiert von –30 ‰ bis –27 ‰ innerhalb der Lauraceae und von –29 ‰ bis –26 ‰ innerhalb der Myrtaceae, was im Bereich moderner C_3-Pflanzen liegt. Die Ergebnisse der blattinternen Analysen zeigen, dass der $\delta^{13}C$-Wert innerhalb eines Blattes variiert, diese blattinterne Variabilität statistisch jedoch nicht signifikant ist. Das c_i/c_a-Verhältnis für das Mitteleozän, das mit den gemessenen $\delta^{13}C$-Werten berechnet wurde, schwankt zwischen durchschnittlich 0,78 und 0,87. Die c_i/c_a-Verhältnisse für rezente Lauraceae sind deutlich geringer (durchschnittlich 0,69) als die für die beiden fossilen Arten errechneten Werte. Bei den Myrtaceae unterscheiden sich die mitteleozänen und rezenten c_i/c_a-Verhältnisse kaum, rezente Myrtaceae liegen hier durchschnittlich bei etwa 0,8.

Contents

1. Introduction

Although "greenhouse conditions" prevailed during the Eocene (e. g. Zachos et al. 1994; Greenwood & Wing 1995; Wilf et al. 1998; Gingerich 2006; Jahren 2007; Hollis et al. 2009) substantial faunal and floral changes occurred which were caused by the almost continuous global cooling that followed the Early Eocene Climatic Optimum (e. g. Zachos et al. 2001; Francis & Poole 2002; Dupont-Nivet et al. 2007; various contributions in Prothero et al. 2003). Most information about Eocene climate dynamics is still derived from the marine realm (e. g. Zachos et al. 2001). In order to understand the entire Eocene climate system and its changes, terrestrial data are equally important. Fossil leaves represent an easily accessible and plentiful terrestrial archive that allows an insight into the climate history of the Earth.

Many studies dealing with the response of plants to changing climates and varying CO_2 concentrations include measurements of the carbon isotopic composition ($^{13}C/^{12}C$) of leaf tissues (e. g. Lloyd & Farquhar 1994; Kürschner 1996; Lockheart et al. 1998; Heaton 1999; Nguyen Tu et al. 2004; Schweizer et al. 2007; Aucour et al. 2008). The carbon isotope discrimination of C_3-plants in favor of the light ^{12}C isotopes shifts the carbon isotope ratio towards more negative values between approximately –36 ‰ to –22 ‰. The carbon isotope discrimination, however, differs among species and among individuals in a population. Moreover, it is related to the c_i/c_a-ratio (ratio between intra-leaf and atmospheric CO_2) which is in turn influenced by a number of environmental parameters such as leaf temperature, humidity and irradiation (e. g. Farquhar et al. 1982a, 1982b, 1989; Evans et al. 1986; Leavitt & Newberry 1992; Ehleringer & Cerling 1995). Particularly, the c_i/c_a-ratio indicates the degree of stomatal conductance (= gas permeability of the epidermis). In the present study, we analyzed the carbon isotope composition of fossil leaf material from the middle Eocene Messel Formation. Since a high stomatal conductance usually requires adequate availability of water, the carbon isotope signal of leaves provides information on the ecophysiological state of the plants and therefore on environmental conditions which prevailed at the Messel Lake.

Acknowledgements

We thank the staff of the Department Messelforschung, Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt am Main, for excellent technical assistance and support and the Botanical Garden of the University of Tübingen for providing fresh leaf material. Furthermore, the technical support of Bernd Steinhilber, Institute for Geosciences, Tübingen, is gratefully acknowledged. We thank James Nebelsick, Institute for Geosciences, Tübingen, Germany, for critically reading the English manuscript. The authors are very grateful to Thomas Tütken for his constructive review.

This study was financially supported by the Federal State of Baden-Württemberg by a grant to M. G. within the program "Landesgraduiertenförderung".

2. Material and Methods

2.1. Fossil and extant leaf material

For the present study, fossil leaf material from the early middle Eocene of the Messel Pit (Middle Messel Formation) near Darmstadt (Hesse, Germany), a UNESCO World Nature Heritage Site, has been analyzed. In the Messel Pit the typical laminated oil shale-filling of an Eocene maar lake (Felder & Harms 2004) was commercially mined for more than hundred years. The oil shale became famous for numerous fossils showing an excellent preservation (e. g. Gruber & Micklich 2007; Collinson et al. 2010). The eruption giving rise to the maar lake at Messel has been dated at about 47.8 Ma (Mertz & Renne 2005).

$\delta^{13}C$ measurements were carried out for six leaves of *Laurophyllum lanigeroides* (Lauraceae), four leaves of *Daphnogene crebrigranosa* (Lauraceae) and seven leaves of *Rhodomyrtophyllum sinuatum* (Myrtaceae). For detailed descriptions and discussions of the species compare Wilde (1989). All investigated fossil leaves are stored in the paleobotanical collection of the Senckenberg Forschungsinstitut und Naturmuseum (Frankfurt am Main, Germany).

Furthermore, recent leaves from potted plants of the same families as the fossil specimens were analyzed: *Laurus nobilis* (Lauraceae, five leaves), *Cinnamomum camphora* (Lauraceae, three leaves), *Persea americana* (Lauraceae, two leaves), *Myrtus communis* (Myrtaceae, five leaves) and *Psidium littorale/cattleianum* (Myrtaceae, three leaves). The recent plant material was collected in the Botanical Garden of Tübingen (Baden-Württemberg, Germany).

2.2. Preparation of leaf material

Here, we used remnant tissues of completely to almost completely preserved fossil leaves. Whenever possible, three samples per specimen were analyzed in order to illustrate intra-leaf variability of $\delta^{13}C$ except for *Daphnogene crebrigranosa* where only one sample per leaf was available. Measurements were carried out on four leaves of *Daphnogene crebrigranosa* (four measurements), six leaves of *Laurophyllum lanigeroides* (20 measurements) and seven leaves of *Rhodomyrtophyllum sinuatum* (16 measurements). Samples from the basal, central and apical (or tip) region were cut out of the leaf blade and thoroughly rinsed with water (Fig. 1). In order to remove the adhering sediment all fossil material was treated with hydrofluoric acid (HF) and subsequently rinsed thoroughly with water. To avoid accidental measurements on oil shale fragments, samples were afterwards macerated in Schulze's Solution, a mixture of crystalline potassium chlorate ($KClO_3$) and nitric acid (HNO_3), until their colour turned from black to light brown or yellow. After maceration samples were neutralized in ammonia solution (NH_3+H_2O) followed by washing in water again. This is the standard preparation

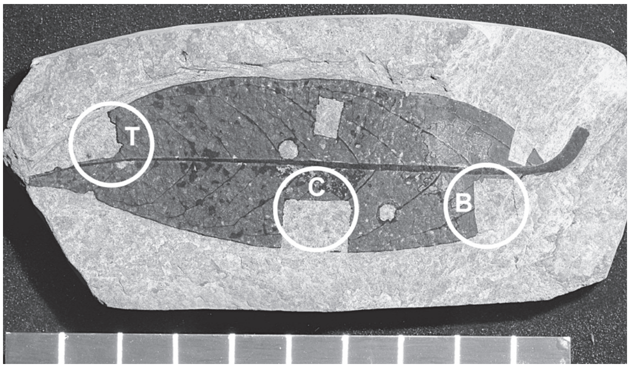

Fig. 1. Leaf of *Laurophyllum lanigeroides* showing the sampling sites for isotope analysis. The locations tip (T), center (C) and base (B) are marked (photo: M. Mueller. SFN – Frankfurt/Main). – Length of scale: 10 cm.

procedure for fossil cuticles applied at the palaeobotanical laboratory of the Senckenberg Forschungsinstitut und Naturmuseum (Frankfurt/Main). In this study, maceration with Schulze´s Solution allowed for a precise separation of cuticle fragments from oil shale fragments under a binocular.

Extant plant material was just rinsed with water without any further chemical preparation. For isotope measurements, samples of extant and fossil material were finally dried at room-temperature for at least seven days followed by drying in an oven for at least three days at about 30–60 °C. Additionally, a methodical test was carried out on extant bulk leaf material of Lauraceae and Myrtaceae to determine whether the $\delta^{13}C$ signal is affected by the chemical treatment.

2.3. Carbon isotope composition and c_i/c_a-ratio

The C-isotopic composition ($\delta^{13}C$) was measured at the Department for Geochemistry, Institute for Geosciences, University of Tübingen (Germany) with a Carlo Erba NC 2500 Elemental Analyzer connected to a Thermo Finnigan Delta Plus XL mass spectrometer. Samples were normalized to the international graphite standard USGS 24 with a certified $\delta^{13}C$ value of –16 ‰ VPDB. The reproducibility of $\delta^{13}C$ values was ± 0.1 ‰.

The ratio of leaf-internal to atmospheric carbon dioxide concentration (c_i/c_a) was calculated according to the equation of Farquhar et al. (1989) which is suitable for C_3 plants. The carbon isotope discrimination (Δ) is calculated as

$$(1) \quad \Delta = \frac{\delta^{13}C_{atm} - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}}$$

with

$$(2) \quad \Delta = a + (b - a) \times \frac{c_i}{c_a}$$

$\delta^{13}C_{atm}$ is the carbon isotope composition of the atmosphere, $\delta^{13}C_{plant}$ is the carbon isotope composition of the plant tissue, a is the fractionation caused by diffusion of CO_2 through stomata (a = 4.4 ‰) and b is the fractionation due to carboxylation by Rubisco (b = 27.0 ‰). Middle Eocene atmospheric $\delta^{13}C_{atm}$ was derived from the carbon isotope composition of marine carbonates $\delta^{13}C_{CaCO3}$ which is approximately +0.8 ‰ at 47 Ma (mean curve) provided by Zachos et al. (2001).

Based on the assumption that the $\delta^{13}C_{atmCO2}$ is regulated on a long-term basis by equilibrium with $\delta^{13}C_{CaCO3}$ of marine carbonates with $\delta^{13}C_{CO2}$ values being about 7 ‰ more negative (Mora et al. 1996; Beerling et al. 1998; Beerling 2000; Beerling & Royer 2002) this results in $\delta^{13}C_{atm}$ = –6.2 ‰ for the middle Eocene atmosphere. Modern atmospheric carbon isotope composition is about $\delta^{13}C_{atm}$ = –8.2 ‰ (e. g. Nguyen Tu et al. 2004; Keeling et al. 2010). These differences in Eocene and modern $\delta^{13}C_{atm}$ directly influence the calculated carbon isotope discrimination and, thus, the c_i/c_a-ratio derived from $\delta^{13}C$ (see also Tab. 1).

Statistical analysis was performed by using SPSS 16.01.

3. Results

Measurements of $\delta^{13}C$ on the fossil leaf material indicate some differences in the carbon isotope composition of the middle Eocene Lauraceae (n = 10) and Myrtaceae

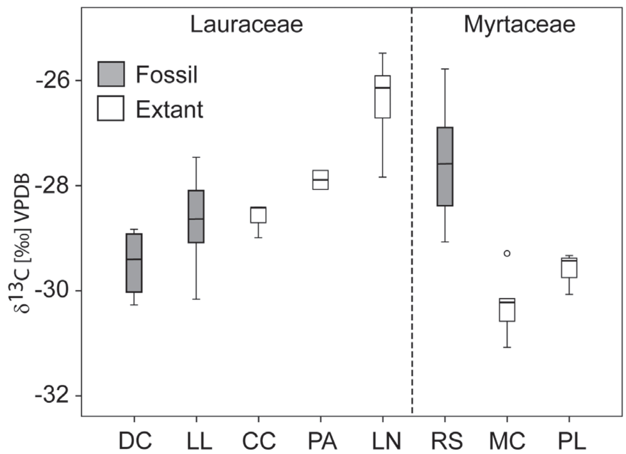

Fig. 2. Carbon isotope composition ($\delta^{13}C$) of fossil and extant leaves. Lauraceae: DC: *Daphnogene crebrigranosa*; LL: *Laurophyllum lanigeroides*; CC: *Cinnamomum camphora*; PA: *Persea americana*; LN: *Laurus nobilis*. Myrtaceae: RS: *Rhodomyrtophyllum sinuatum*; MC: *Myrtus communis*; PL: *Psidium littorale*. With the exception of DC, the data represent the pooled results from all three leaf positions. The results are presented as box–whisker plots. The vertical lines connect the highest and lowest value, and the boxes span the 50 % interquartile. The median is indicated by the horizontal line within the boxes. Single outliers are drawn as circles.

Tab. 1. Carbon isotope composition ($\delta^{13}C$) and derived c_i/c_a-ratios for extant and middle Eocene Lauraceae (L) and Myrtaceae (M). Number of leaves (n), calculated carbon isotope discrimination (Δ) and $\delta^{13}C$-derived c_i/c_a-ratio presented as average values with standard deviation. An excel-sheet containing all single leaf $\delta^{13}C$ measurements of both fossil and extant leaves can be obtained from the authors on request.

species (family)	n	age	$\delta^{13}C$ ‰	Δ ‰	c_i/c_a
Laurus nobilis (L)	5	extant	–26.4 ± 0.9	18.7 ± 1.0	0.63 ± 0.04
Cinnamomum camphora (L)	3	extant	–28.6 ± 0.3	21.0 ± 0.3	0.73 ± 0.02
Persea americana (L)	2	extant	–27.9 ± 0.3	20.3 ± 0.3	0.70 ± 0.01
Laurophyllum lanigeroides (L)	6	Eocene	–28.6 ± 0.8	23.1 ± 0.8	0.83 ± 0.04
Daphnogene crebrigranosa (L)	4	Eocene	–29.5 ± 0.7	24.0 ± 0.7	0.87 ± 0.03
Myrtus communis (M)	5	extant	–30.3 ± 0.7	22.8 ± 0.7	0.81 ± 0.03
Psidium littorale/cattleianum (M)	3	extant	–29.6 ± 0.4	22.1 ± 0.4	0.78 ± 0.02
Rhodomyrtophyllum sinuatum (M)	7	Eocene	–27.6 ± 0.9	22.0 ± 1.0	0.78 ± 0.04
Messel (combined results)	17	Eocene	–28.4 ± 1.1	22.9 ± 1.2	0.82 ± 0.05

(n = 7) from Messel. The $\delta^{13}C$ varied from about –30 ‰ to –27 ‰ (24 single measurements) in the two species of Lauraceae and from –29 ‰ to –26 ‰ in the single species of Myrtaceae (16 single measurements) (Fig. 2). Mean c_i/c_a-ratios derived from $\delta^{13}C$ measurements of the fossils varied from 0.78 in *Rhodomyrtophyllum sinuatum* to 0.87 in *Daphnogene crebrigranosa* (Tab. 1); values for *Laurophyllum lanigeroides* are intermediate with c_i/c_a= 0.83.

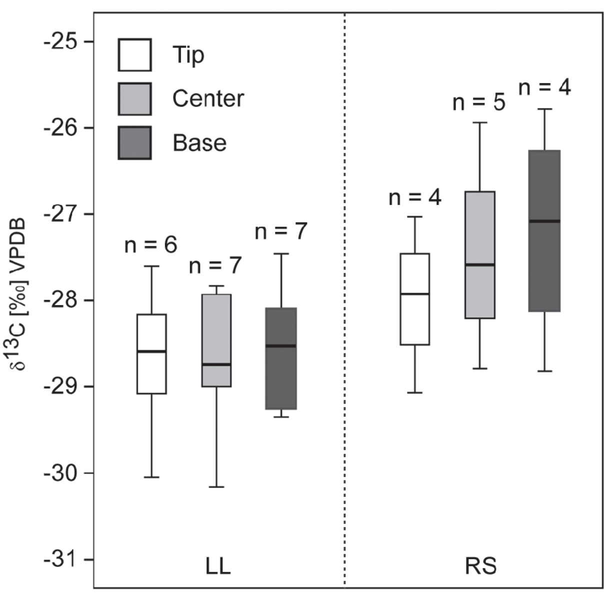

Fig. 3. Carbon isotope composition ($\delta^{13}C$) of fossil leaf material of *Laurophyllum lanigeroides* (LL) and *Rhodomyrtophyllum sinuatum* (RS) from the three different positions on the leaf (n = number of measurements). The results are represented as box–whisker plots. The vertical lines connect the highest and lowest value, and the boxes span the 50 % interquartile. The median is indicated by the horizontal line within the boxes.

The mean c_i/c_a-ratios for extant Lauraceae are clearly lower (between 0.63 and 0.73) than the ratios for the fossil species (0.83 to 0.87). For fossil and extant Myrtaceae, c_i/c_a-ratios hardly differ with values ranging between 0.78 and 0.81. The average value for all of the analyzed fossil leaves from the middle Eocene of Messel is $\delta^{13}C$ = –28.4 ± 1.1 ‰ (n = 17) corresponding to an average c_i/c_a-ratio of 0.82 ± 0.05.

Results of intra-leaf analyses (Fig. 3) indicate a slight variation in $\delta^{13}C$ within the three sample regions between 2.2 ‰ (mean) and 2.5 ‰ (maximum) for *L. lanigeroides* and between 2.6 ‰ (mean) and 3.0 ‰ (maximum) for *R. sinuatum*. However, application of one-way ANOVA (ANalysis Of VAriance) shows that the variation is statistically not significant. Intra-leaf differences show a Significance Level of 90.8 % (F-value = 0.97) for *L. lanigeroides* and 60.7 % (F-value = 0.53) for *R. sinuatum*.

As to the extant families, ranges for intra-leaf variability within apex, center and base vary between 1.2 ‰ (mean) and 2.9 ‰ (maximum) within the Lauraceae and between 1.4 ‰ (mean) and 3.2 ‰ (maximum) within the Myrtaceae.

Interleaf differences are significant for both *L. lanigeroides* (Significance Level < 0.1 %, F-value = 27.16) and *R. sinuatum* (Significance Level = 0.1 %, F-value = 15.39). Interspecific differences between *L. lanigeroides* and *R. sinuatum* are also significant (Significance Level = 0.06 %).

4. Discussion

$\delta^{13}C$ values of modern C_3 plants range typically from –36 to –22 ‰ (e. g. Farquhar et al. 1989; O'Leary 1995; Cerling et al. 2004; Kingston & Harrison 2007; and citations therein) with local and temporal (diurnal/seasonal) variations, depending on the species. During the past centuries, the $\delta^{13}C$ values of modern plants were influenced by changes in the carbon isotope composition of the atmospheric CO_2 due to human activity. The concentration of atmospheric CO_2, however, appears to have only minor influence on $\delta^{13}C$ (NGUYEN TU et al. 2004). The $\delta^{13}C$ values of the Messel leaves fit into the range typical for modern C_3-species. Our average value for $\delta^{13}C = -28.4 \pm 1.1$ ‰ (n = 17) is somewhat more negative than the values of SCHWEIZER et al. (2007), who analyzed the carbon isotopic composition of a larger number (n = 63) of unspecified "terrestrial leaves" from the Messel Pit. The $\delta^{13}C$ values of their "terrestrial leaves" ranged between approximately –30 ‰ and –25 ‰ with an average of –26.8 ‰ ± 0.8 ‰.

Following SCHWEIZER et al. (2007) this could have been caused by the fact that fossil leaves are particularly sensitive to contamination and diagenetic alteration because of their large surface area-to-volume ratio. However, the results of SCHWEIZER et al. (2007) indicate that the Messel oil shale is depleted in ^{13}C ($\delta^{13}C_{shale} = -28.54 \pm 2.7$ ‰) relative to "terrestrial leaves" and leaves of water lilies. The compositional differences between the fossil leaves and the oil shale matrix in their study therefore obviously confirms that isotopic exchange between the fossils and their matrix was not extensive enough to produce distinct isotopic alteration (SCHWEIZER et al. 2007). The fact that our $\delta^{13}C$ values for the fossil leaves are close to the oil shale values provided by SCHWEIZER et al. (2007) therefore appears to be either accidental or it could be interpreted in such a way that diagenetic changes, if experienced by the fossil leaf material, were likely very small and possibly neutralized by the pretreatment. It is also possible that the differences in the mean values for leaves in both studies may have been caused by the fact that our study is based on material from three distinct species whereas SCHWEIZER et al. (2007) used a larger random sample of unspecified "terrestrial leaves". The interspecific differences found in the present study corroborate the assumption that the fossil material carries the original ^{13}C signal.

The c_i/c_a-ratio and, thus, carbon isotope discrimination for modern plants depends on stomatal conductance and the photosynthetic rate which are in turn influenced by several environmental factors such as light, water supply, nutrients, atmospheric CO_2 concentration and temperature (e. g. FARQUHAR et al. 1989; TIESZEN 1991; EHLERINGER & CERLING 1995; O'LEARY 1995). The c_i/c_a-ratio is frequently maintained at a constant (or almost constant) level typically around 0.7 (e. g. EHLERINGER & CERLING 1995; LARCHER 2003). A high c_i/c_a-ratio (>0.7) indicates non-conservative water use, that is, the absence of strict water saving strategies resulting in a trend for high stomatal conductance, and a high assimilation rate (e. g. LLOYD & FARQUHAR 1994; FRANKS & FARQUHAR 1999). The c_i/c_a-ratio varies substantially within various terrestrial habitats. For example, differences exist between Australian evergreen trees, Mediterranean evergreen sclerophyllous shrubs, xerophytic trees, dry tropical trees and rain forest trees (e. g. BEYSCHLAG et al. 1987; LLOYD et al. 1992; SHERIFF 1992; LLOYD & FARQUHAR 1994; ISHIDA et al. 1996). In several tropical rainforest species, the c_i/c_a-ratios estimated from carbon isotope discrimination are relatively high indicating a relatively small stomatal limitation to assimilation rate and non-conservative water use (e. g. LLOYD & FARQUHAR 1994; ISHIDA et al. 1996; GONZALEZ-RODRIGUEZ et al. 2001).

The c_i/c_a-ratio also varies among plants living within the same habitat. EHLERINGER et al. (1987) studied 128 plant species (C_3, C_4 and CAM) from a subtropical monsoon forest in southern China. Among Myrtaceae $\delta^{13}C$ values ranged between about –30 to –27 ‰ (two trees and two shrubs from open and intermediate habitats) and among Lauraceae $\delta^{13}C$ values ranged from approximately –35 to –29 ‰ (five trees and one shrub from intermediate and closed habitats). The study shows that among C_3 species, $\delta^{13}C$ tended to become more negative with decreasing light availability from open to closed habitats. EHLERINGER et al. (1986, 1987) also demonstrated that in some species the c_i/c_a-ratio changed because of decreasing intercellular CO_2 concentration with increasing light.

The relatively high average values for the c_i/c_a-ratio of the three taxa from Messel, amounting to a mean value of about 0.82, indicates a tropical climate with high water availability. Among the fossil species, the Lauraceae were obviously more lavish regarding to efficiency in water use than the Myrtaceae. If the c_i/c_a-ratios of the fossil Lauraceae are compared to the c_i/c_a-ratios which were determined for the plants growing at the Botanical Garden in Tübingen University, then the fossil values are much higher. However, high c_i/c_a-ratios were also recorded for Lauraceae species growing in their natural environment, such as *Laurus azorica* (Lauraceae) with a c_i/c_a-ratio varying from 0.67 to 0.95 (GONZALEZ-RODRIGUEZ et al. 2001) and two subtropical Lauraceae, *Cryptocarya chinensis* and *Lindera chunii*, varying between approximately 0.8 and 0.9 (DE LILLIS & SUN 1990). It may therefore be supposed that the low values of the material from Botanical Garden are due to the potted condition of the plants. However, the two Myrtaceae species from the Botanical Garden which are also potted plants showed quite high c_i/c_a-ratios. Additionally, all individuals were well-watered.

On the basis of the present data set, it is therefore difficult to determine systematic differences in c_i/c_a-ratios between fossil and extant Lauraceae and Myrtaceae. The fact, however, that all fossil material from Messel shows high c_i/c_a-ratios which are typical of tropical and subtropical plants growing at sites with high humidity indicates that the climate was warm and humid. The $\delta^{13}C$ data obtained within this study therefore corroborate results of other studies in which warm and humid conditions were suggested for the early middle Eocene at Messel (SCHAARSCHMIDT 1988; WILDE 1989 and citations therein; WILDE 2005; WILDE & MICKLICH 2007; GREIN et al. in press).

5. Conclusions

$\delta^{13}C$ values of three species of fossil dicotyledonous angiosperm leaves from the early middle Eocene oil shale of Lake Messel range between –30 to –26 ‰ which fits into the range typical for modern C_3 plants. Intra-leaf analyses indicate slight $\delta^{13}C$ variations across individual leaves which are, however, statistically not significant and much lower than the variability within the leaves of a distinct species.

High c_i/c_a-ratios of 0.78 to 0.87 have been inferred from $\delta^{13}C$ values of fossil Myrtaceae and Lauraceae leaves of the middle Eocene oil shale of the Messel Pit (Germany). They indicate a relatively small stomatal limitation to assimilation rate and non-conservative water use of these plants. The $\delta^{13}C$ data are therefore in accordance with the warm and humid climate that has previously been suggested for the early middle Eocene at Messel.

6. References

AUCOUR, A.-M., GOMEZ, B., SHEPPARD, S. M. F. & THÉVENARD, F. (2008): $\delta^{13}C$ and stomatal number variability in the Cretaceous conifer *Frenelopsis*. – Palaeogeography, Palaeoclimatology, Palaeoecology, **257**: 462–473.

BEERLING, D. J. (2000): Increased terrestrial carbon storage across the Palaeocene–Eocene boundary. – Palaeogeography, Palaeoclimatology, Palaeoecology, **161**: 395–405.

BEERLING, D. J. & ROYER, D. L. (2002): Fossil plants as indicators of the Phanerozoic global carbon cycle. – Annual Review of Earth and Planetary Sciences, **30**: 527–556.

BEERLING, D. J., MCELWAIN, J. C. & OSBORNE, C. P. (1998): Stomatal responses of the 'living fossil' *Ginkgo biloba* L. to changes in atmospheric CO_2 concentrations. – Journal of Experimental Botany, **49**: 1603–1607.

BEYSCHLAG, W., LANGE, O. L. & TENHUNEN, J. D. (1987): Diurnal patterns of leaf internal CO_2 partial pressure of the sclerophyll shrub *Arbutus unedo* growing in Portugal. – In: TENHUNEN J. D., CATARINO F. M., LANGE O. L. & OECHEL W. C. (eds.): Plant Response to Stress. Functional Analysis in Mediterranean Ecosystems. NATO ASI Series, Series G, Ecological Sciences, **15**: 355–368; Berlin (Springer-Verlag).

CERLING, T. E., HART, J. A. & HART, T. B. (2004): Stable isotope ecology in the Ituri Forest. – Oecologia, **138**: 5–12.

COLLINSON, M. E., MANCHESTER, S. R., WILDE, V. & HAYES, P. (2010): Fruit and seed floras from exceptionally preserved biotas in the European Paleogene. – Bulletin of Geosciences, **85**: 155–162.

DE LILLIS, M. & SUN, G. C. (1990): Stomatal response patterns and photosynthesis of dominant trees in a tropical monsoon forest. – Acta Oecologica, **11**: 545–555.

DUPONT-NIVET, G., KRIJGSMAN, W., LANGEREIS, C. G., ABELS, H. A., DAI, S. & FANG, X. (2007): Tibetan plateau aridification linked to global cooling at the Eocene–Oligocene transition. – Nature, **445**: 635–638.

EHLERINGER, J. R. & CERLING, T. E. (1995): Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 concentrations in plants. – Tree Physiology, **15**: 105–111.

EHLERINGER, J. R., FIELD, C. B., LIN, Z. F. & KUO, C. Y. (1986): Leaf carbon isotope ratio and mineral composition in subtropical plants along an irradiance cline. – Oecologia, **70**: 520–526.

EHLERINGER, J. R., LIN, Z. F., FIELD, C. B., SUN, G. C. & KUO, C. Y. (1987): Leaf carbon isotope ratios of plants from a subtropical monsoon forest. – Oecologia, **72**: 109–114.

EVANS, J. R., SHARKEY, T. D., BERRY, J. A. & FARQUHAR, G. D. (1986): Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. – Australian Journal of Plant Physiology, **13**: 281–292.

FARQUHAR, G. D., BALL, M. C., VON CAEMMERER, S. & ROKSANDIC, Z. (1982a): Effect of salinity and humidity on $\delta^{13}C$ value of halophytes. Evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. – Oecologia, **52**: 121–124.

FARQUHAR, G. D., O'LEARY, M. H. & BERRY, J. A. (1982b): On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. – Australian Journal of Plant Physiology, **9**: 121–137.

FARQUHAR, G. D., EHLERINGER, J. R. & HUBICK, K. T. (1989): Carbon isotope discrimination and photosynthesis. – Annual Review of Plant Physiology and Plant Molecular Biology, **40**: 503–537.

FELDER, M. & HARMS, F.-J. (2004): Lithologie und genetische Interpretation der vulkano-sedimentären Ablagerungen aus der Grube Messel anhand der Forschungsbohrung Messel 2001 und weiterer Bohrungen. – Courier Forschungsinstitut Senckenberg, **252**: 151–203.

FRANCIS, J. E. & POOLE, I. (2002): Cretaceous and early Tertiary climates of Antarctica: Evidence from fossil wood. – Palaeogeography, Palaeoclimatology, Palaeoecology, **182**: 47–64.

FRANKS, P. J. & FARQUHAR, G. D. (1999): A relationship between humidity response, growth form and photosynthetic operating point in C_3 plants. – Plant, Cell and Environment, **22**: 1337–1349.

GINGERICH, P. D. (2006): Environment and evolution through the Paleocene-Eocene thermal maximum. – Trends in Ecology and Evolution, **21**: 246–253.

GONZALEZ-RODRIGUEZ, A. M., MORALES, D. & JIMENEZ, M. S. (2001): Gas exchange characteristics of a Canarian laurel forest tree species (*Laurus azorica*) in relation to environmental conditions and leaf canopy position. – Tree Physiology, **21**: 1039–1045.

GREENWOOD, D. R. & WING, S. L. (1995): Eocene continental climates and latitudinal temperature gradients. – Geology, **23**: 1044–1048.

GREIN, M., UTESCHER, T., WILDE, V. & ROTH-NEBELSICK, A. (in press): Reconstruction of the middle Eocene climate of Messel using palaeobotanical data. – Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen.

GRUBER, G. & MICKLICH, N. (2007): Messel – Schätze der Urzeit (Begleitbuch zur Ausstellung "Messel on Tour"). 160 pp.; Hessisches Landesmuseum Darmstadt (Theiss).

HEATON, T. H. E. (1999): Spatial, species, and temporal variations in the $^{13}C/^{12}C$ ratios of C_3 plants: Implications for palaeodiet studies. – Journal of Archaeological Science, **26**: 637–649.

HOLLIS, C. J., HANDLEY, L., CROUCH, E. M., MORGANS, H. E. G., BAKER, J. A., CREECH, J., COLLINS, K. S., GIBBS, S. J., HUBER,

M., SCHOUTEN, S., ZACHOS, J. C. & PANCOST, R. D. (2009): Tropical sea temperatures in the high-latitude South Pacific during the Eocene. – Geology, **37**: 99–102.

ISHIDA, A., TOMA, T., MATSUMOTO, Y., YAP, S. K. & MARUYAMA, Y. (1996): Diurnal changes in leaf gas exchange characteristics in the uppermost canopy of a rain forest tree, *Dryobalanops aromatica* Gaertn. f. – Tree Physiology, **16**: 779–785.

JAHREN, A. H. (2007): The arctic forest of the Middle Eocene. – Annual Review of Earth and Planetary Sciences, **35**: 509–540.

KEELING, R. F., PIPER, S. C., BOLLENBACHER, A. F. & WALKER, S. J. (2010): Monthly atmospheric $^{13}C/^{12}C$ isotopic ratios for 11 SIO stations. – In: Trends: A Compendium of Data on Global Change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn., U.S.A. (http://cdiac.ornl.gov/trends/co2/iso-sio/iso-sio.html, Version: 16.02.2010).

KINGSTON, J. D. & HARRISON, T. (2007): Isotopic dietary reconstructions of Pliocene herbivores at Laetoli: Implications for early hominin paleoecology. – Palaeogeography, Palaeoclimatology, Palaeoecology, **243**: 272–306.

KÜRSCHNER, W. M. (1996): Leaf stomata as biosensors of palaeoatmospheric CO_2 levels. – PhD thesis, Laboratory of Palaeobotany and Palynology, Utrecht University, LPP contributions series, **5**: 153 pp.

LARCHER, W. (2003): Physiological plant ecology: Ecophysiology and stress physiology of functional groups. 4th ed. 513 pp.; Berlin (Springer).

LEAVITT, S. W. & NEWBERRY, T. (1992): Systematics of stable-carbon isotopic differences between gymnosperm and angiosperm trees. – Plant Physiology (Life Science Advances), **11**: 257–262.

LLOYD, J. & FARQUHAR, G. D. (1994): ^{13}C discrimination during CO_2 assimilation by the terrestrial biosphere. – Oecologia, **99**: 201–215.

LLOYD, J., SYVERTSEN, J. P., KRIEDEMANN, P. E. & FARQUHAR, G. D. (1992): Low conductances for CO_2 diffusion from stomata to the sites of carboxylation in leaves of woody species. – Plant, Cell and Environment, **15**: 873–899.

LOCKHEART, M. J., POOLE, I., VAN BERGEN, P. F. & EVERSHED, R. P. (1998): Leaf carbon isotope compositions and stomatal characters: important considerations for palaeoclimate reconstructions. – Organic Geochemistry, **29**: 1003–1008.

MERTZ, D. F. & RENNE, P. R. (2005): A numerical age for the Messel fossil deposit (UNESCO World Heritage Site) derived from $^{40}Ar/^{39}Ar$ dating on a basaltic rock fragment. – Courier Forschungsinstitut Senckenberg, **255**: 67–75.

MORA, C. I., DRIESE, S. G. & COLARUSSO, L. A. (1996): Middle to Late Paleozoic atmospheric CO_2 levels from soil carbonate and organic matter. – Science, **271**: 1105–1107.

NGUYEN TU, T. T., KÜRSCHNER, W. M., SCHOUTEN, S. & VAN BERGEN, P. F. (2004): Leaf carbon isotope compositon of fossil and extant oaks grown under differing atmospheric CO_2 levels. – Palaeogeography, Palaeoclimatology, Palaeoecology, **212**: 199–213.

O'LEARY, M. H. (1995): Environmental effects on carbon isotope fractionation in terrestrial plants. – In: WADA, E., YONEYAMA, T., MINIGAWA, M., ANDO, T. & FRY, B. D. (eds.): Stable Isotopes in the Biosphere: 78–91; Kyoto (Kyoto University Press).

PROTHERO, D. R., IVANY, L. C. & NESBITT, E. A. (2003): From greenhouse to icehouse: the marine Eocene-Oligocene transition. 541 pp.; New York (Columbia University Press).

SCHAARSCHMIDT, F. (1988): Der Wald, fossile Pflanzen als Zeugen eines warmen Klimas. – In: SCHAAL, S. & ZIEGLER W. (eds.): Messel – Ein Schaufenster in die Geschichte der Erde und des Lebens: 27–52; Frankfurt am Main (Waldemar Kramer).

SCHWEIZER, M. K., STEELE, A., TOPORSKI, J. K. W. & FOGEL, M. L. (2007): Stable isotopic evidence for fossil food webs in Eocene Lake Messel. – Paleobiology, **33**: 590–609.

SHERIFF, D. W. (1992): Roles of carbon gain and allocation in growth at different nitrogen nutrition in *Eucalyptus camaldulesis* and *Eucalyptus globulus* seedlings. – Australian Journal of Plant Physiology, **19**: 637–652.

TIESZEN, L. L. (1991): Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. – Journal of Archaeological Science, **18**: 227–248.

WILDE, V. (1989): Untersuchungen zur Systematik der Blattreste aus dem Mitteleozän der Grube Messel bei Darmstadt (Hessen, Bundesrepublik Deutschland). – Courier Forschungsinstitut Senckenberg, **115**: 1–213.

WILDE, V. (2005): The green Eocene. The diverse flora of a paratropical climate. – Vernissage UNESCO World Heritage Series, **21**: 14–19.

WILDE, V. & MICKLICH, N. (2007): Lebensraum Messel-See. Der See und seine Uferzonen. – In: GRUBER, G. & MICKLICH, N. (eds.): Messel – Schätze der Urzeit (Begleitbuch zur Ausstellung "Messel on Tour"): 52–55; Hessisches Landesmuseum Darmstadt (Theiss).

WILF, P., WING, S. L., GREENWOOD, D. R. & GREENWOOD, C. L. (1998): Using fossil leaves as paleoprecipitation indicators: An Eocene example. – Geology, **26**: 203–206.

ZACHOS, J. C., STOTT, L. D. & LOHMANN, K. C. (1994): Evolution of early Cenozoic marine temperatures. – Paleoceanography, **9**: 353–387.

ZACHOS, J. C., PAGANI, M., SLOAN, L., THOMAS, E. & BILLUPS, K. (2001): Trends, rhythms, and aberrations in global climate 65 Ma to present. – Science, **292**: 686–693.

Addresses of the authors:

Dr. MICHAELA GREIN, University of Tübingen, Institute for Geosciences, Sigwartstr. 10, 72076 Tübingen, Germany / State Museum of Natural History Stuttgart (SMNS), Rosenstein 1, 70191 Stuttgart, Germany
E-mail: michaela.grein@smns-bw.de

PD Dr. ANITA ROTH-NEBELSICK, State Museum of Natural History Stuttgart (SMNS), Rosenstein 1, 70191 Stuttgart, Germany
E-mail: anita.rothnebelsick@smns-bw.de

PD Dr. VOLKER WILDE, Senckenberg Forschungsinstitut und Naturmuseum, Sektion Paläobotanik, Senckenberganlage 25, 60325 Frankfurt am Main, Germany
E-mail: volker.wilde@senckenberg.de

Manuscript received: 30 April 2010, accepted: 30 September 2010.