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doi:10.3906/yer-1003-42

Carbon Isotope and Stomatal Data of Late Pliocene Betulaceae Leaves from SW China: Implications for Palaeoatmospheric CO$_2$-levels

BAI-NIAN SUN[1], SU-TING DING[1], JING-YU WU[2], CHONG DONG[3],
SANPING XIE[3] & ZHI-CHENG LIN[3]

[1] Lanzhou University, College of Earth and Environmental Sciences, Key Laboratory of Western China's Environmental Systems of the Ministry of Education, Lanzhou 730000, China; State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, CAS, Nanjing 210008, China
(E-mail: bnsun@lzu.edu.cn)
[2] Lanzhou University, College of Earth and Environmental Sciences, Lanzhou 730000, China; State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, CAS, Nanjing 210008, China
[3] Lanzhou University, College of Earth and Environmental Sciences, Lanzhou 730000, China

Received 23 April 2010; revised typescripts received 28 October 2010 & 10 December 2010; accepted 16 December 2010

Abstract: The cuticular δ^{13}C values and stomatal parameters (stomatal density and stomatal index: SD and SI) of two Betulaceae species, *Betula mioluminifera* Hu et Chaney and *Carpinus miofangiana* Nathorst, from a suite of superposed horizons in West Yunnan, southwestern China, were measured in order to recover Late Pliocene CO$_2$ levels. Correlations are given for δ^{13}C, SD, epidermal cell density (ECD), and SI. δ^{13}C reveals a positive trend with the SD and SI in the two species, and such a positive correlation can also be observed between the δ^{13}C and ECD in *C. miofangiana*. However, δ^{13}C has a slightly negative correlation with the ECD in *B. mioluminifera* (R^2= 0.06), possibly influenced by their different genotypes. Reflecting the changes through time, the δ^{13}C values of *B. mioluminifera* and *C. miofangiana* significantly increase with high determination coefficients (R^2= 0.67 and R^2= 0.65, respectively), as do SD (R^2= 0.66 and R^2= 0.51, respectively) and SI (R^2= 0.50 and R^2= 0.79, respectively). Research on extant *B. luminifera* and *C. fangiana* shows that the SD and especially SI, exhibit a prominent negative correlation with CO$_2$ concentration. Pliocene CO$_2$ levels are reconstructed as 381.5–439.4 ppmv and 377.8–472.3 ppmv, respectively, based on comparisons of the two fossil species with their nearest living equivalent (NLE) species. The significant positive trends of the δ^{13}C, SD and SI with ascending position of the fossils in the section indicate that the atmospheric CO$_2$ levels declined in the Late Pliocene (3.30–2.83 Ma). Furthermore, the calculated CO$_2$ levels are higher than in other studies and probably demonstrate that local CO$_2$ enrichment can be caused by frequent volcanic eruptions over a long time scale

Key Words: δ^{13}C value, Betulaceae, stomatal parameters, atmospheric CO$_2$ concentration, Late Pliocene, Southwest China

Güneybatı Çin Geç Pliyosen Betulaceae Yapraklarının Karbon İzotop ve Stomal Verileri: Paleoatmosferik CO$_2$ Düzeyleri İçin Öneriler

Özet: Geç Pliyosen CO$_2$ seviyesini yeniden elde etmek için, Batı Yunnan'dan, güneybatı Çin, üst üste gelen düzeylerin bir takımından, kutiküler δ^{13}C değerleri ve iki Betulaceae türü, *Betula mioluminifera* Hu et Chaney and *Carpinus miofangiana* Nathorst'in stomal parametreleri (stomal yoğunluğu ve stomal index: SD ve SI) ölçüldü. Korelasyonlar, δ^{13}C, SD, epidermal hücre yoğunluğu (ECD) ve SI için verilmektedir. δ^{13}C, iki türde SD ve SI için pozitif bir gidiş ortaya koymaktadır ve böyle bir pozitif korelasyon *C. miofangiana*'da δ^{13}C ve ECD arasında da gözlenebilmektedir. Ancak, *B. mioluminifera* (R^2= 0.06)'da δ^{13}C, ECD ile olasılıkla farklı genotiplerden etkilenmiş kısmen negatif bir korelasyona sahiptir. Zaman içinde değişiklikleri yansıtan, *B. mioluminifera* ve *C. miofangiana*'nın δ^{13}C değerleri, yüksek determinasyon katsayıları (R^2= 0.67 ve R^2= 0.65, sırasıyla) önemli ölçüde artar. Ayrıca SD (R^2= 0.66 ve R^2= 0.51, sırasıyla) ve SI (R^2= 0.50 ve R^2= 0.79, sırasıyla)'nın artışı da gözlenmektedir. Mevcut *B. luminifera* ve *C. fangiana* üzerindeki araştırma, SD ve SI'nın, özellikle ikincisinin, CO$_2$ konsantrasyonu ile belirgin bir negative korelasyon sergilemekte olduğunu göstermektedir. En yakın yaşayan akraba (NLE) türleri ile iki fosil türünün karşılaştırılmasına dayanarak, Pliyosen CO$_2$ seviyeleri, sırasıyla 381.5–439.4 ppmv ve 377.8–472.3 ppmv olarak yeniden değerlendirilmektedir.. Kesitteki fosillerin izleyen pozisyonu ile δ13C, SD ve SI 'nın anlamlı pozitif gidişleri atmosferik CO$_2$ seviyelerinin Geç Pliyosen (3.30–2.83

My önce) den kaynaklandığını göstermektedir. Ayrıca, hesaplanmış CO_2 seviyeleri diğer çalışmalardakilerden daha yüksektir ve olasılıkla yersel CO_2 zenginleşmesine, uzun bir zaman ölçeği üzerinde sık volkanik patlamaların neden olabileceğini göstermektedir.

Anahtar Sözcükler: $\delta^{13}C$ değeri, Betulaceae, stomal parametreleri, atmosferik CO_2 konsantrasyonu, Geç Pliyosen, Güneybatı Çin

Introduction

The mean global surface temperature increased by 0.74±0.18°C during the 20th century and is likely to rise a further 1.1 to 6.4°C (2.0 to 11.5°F) during the 21st century (IPCC 2007). Atmospheric CO_2 is one of the major greenhouse gases that greatly influences global climate change. The predicted continued increase in atmospheric CO_2 concentration in the near future is forcing scientists to concentrate their efforts on how the biosphere operates under elevated (relative to pre-industrial) CO_2 levels (Royer 2001).

The negative correlation between stomatal frequency and atmospheric CO_2 level, as well as the use of the carbon isotopic discrimination model in C3 plants forms the foundation for palaeoatmospheric CO_2 concentration reconstruction (Woodward 1987; Farquhar *et al.* 1982a, b; Sun *et al.* 2007). Stable carbon isotope analysis can be performed on fossil leaves, together with assessment of stomatal characters (stomatal density and stomatal index: SD and SI) to reveal information on CO_2 changes in the past (e.g., Van der Burgh *et al.* 1993; Beerling & Woodward 1995; Kürschner *et al.* 1996; Lockheart *et al.* 1998). However, plants interact with the environment directly by adapting to global change in CO_2 level and to other environmental factors such as air humidity, soil moisture, salinity, temperature, and irradiance (Farquhar *et al.* 1982a, b; Ehleringer & Cerling 1995; Aucour *et al.* 2008). Therefore, deciphering the relative effect of variations in global atmospheric CO_2 level and other environmental factors on plants is an important aspect of carbon cycle research (Ehleringer & Cerling 1995; Beerling & Woodward 1997; Feng 1999; Aucour *et al.* 2008). The carbon isotope fractionation of C3 plants has been clearly described by Farquhar *et al.* (1982a, b), and later widely utilized with stomatal number counts to show environmental relationship (e.g., Lockheart *et al.* 1998; Beerling & Royer 2002; Kürschner 2002; Van de Water *et al.* 2002; Jahren *et al.* 2004; Tu *et al.* 2004; Peters-Kottig *et al.* 2006).

Based on a detailed comparison of leaf architectural and cuticular characters, Wu (2009) reported 37 plant species based on leaf fossils from the upper unit of the Mangbang Formation at Tuantian, Tengchong, Yunnan Province, belonging to 28 genera within 20 families. Using the Coexistence Approach (CA), Sun *et al.* (2011) reconstructed the Late Pliocene climate in that region as having a mean annual temperature (MAT) of 16.3–20.8°C and a mean annual precipitation (MAP) of 1225.7–1695.4 mm. The Tuantian flora consists mainly of Lauraceae, Fagaceae, Betulaceae, Hamamelidaceae, Leguminosae, Myricaceae, Ulmaceae and Cupressaceae, of which *Alseodaphne, Machilus, Ormosia, Rhodoleia, Exbucklandia, Myrica* and *Calocedrus* indicate a humid subtropical climate (Sun *et al.* 2011).

In the present study we select two fossil species of Betulaceae (birches), *Betula mioluminifera* Hu et Chaney and *Carpinus miofangiana* Nathorst from the Late Pliocene of Tengchong in western Yunnan to analyse the variations in $^{13}C/^{12}C$ ratios and stomatal numbers of cuticles. Leaf fossils of *B. mioluminifera* were reported by Tao & Du (1982), Sun *et al.* (2003) and Wu (2009), and *C. miofangiana* was reported by Dai *et al.* (2009). Using detailed comparisons of cuticular characteristics, as well as the ecological tolerances between the fossil leaves and their nearest living equivalent (NLE) species, *B. luminifera* Winkler and *C. fangiana* Hu, Sun *et al.* (2003) and Dai *et al.* (2009) concluded that the stomatal parameters (SD and SI) of the two Betulaceous species are sensitive to CO_2 changes and can be used as good bioindicators for the reconstruction of palaeoatmospheric CO_2-concentrations.

Our objectives are to use the carbon isotope ratio (CIR), SD and SI in plants from consecutive horizons to estimate trends of the CO_2 level in the Late Pliocene of West Yunnan, southwestern China.

Material and Methods

A total of 28 specimens of the two fossil species (*B. mioluminifera* and *C. miofangiana*), collected from five consecutive lithologic units, were selected for stomatal and carbon isotope analysis. For comparison, modern leaves of *B. luminifera* and *C. fangiana*, collected from 1959 to 2009 that grew under different atmospheric CO_2 concentrations (CO_2 levels increased from 315.98 ppmv to 387.35 ppmv), were subjected to comparative stomatal analysis.

The fossil leaves were collected from an open-cast diatomite mine about 1 km west of the town of Tuantian (24° 41′ N, 98° 38′ E), Tengchong County, Yunnan Province, Southwest China (Figure 1a). The fossil-bearing diatomite belongs to the Upper Pliocene Mangbang Formation, which is subdivided into three lithological units. The lower unit consists of siltstones, claystones, sandy conglomerates and tuffs; the middle unit has basalts yielding an age of 3.297±0.040 Ma based on K-Ar dating (Li *et al.* 2000), and the upper unit comprises siltstones, mudstones, claystones and coal seams (Ge & Li 1999; Shang 2003). The andesitic rocks of the unconformably overlying Mingguang Formation (Figure 1b) have been dated at 2.322±0.036 Ma (Li *et al.* 2000).

The studied fossil plant specimens were recovered from five consecutive layers in the upper unit of the Mangbang Formation (Figure 1b, c). Based on sedimentary facies and geodynamic analysis, Li & Xue (1999) estimated that the average deposition rate for the Pliocene in the studied region is 0.09 mm/a. If so, the approximately 42-m-thick fossil horizons of the Mangbang Formation in the studied section represent an interval of ca. 0.47 million years within the Late Pliocene. Considering the break in the succession overlying the Mangbang Formation, the fossil-bearing deposits in the upper unit of the Mangbang Formation studied here are undoubtedly of Late Pliocene age (3.30–2.83 Ma).

The exposed sections (Figure 2) were mapped in planar view by using a quadrat grid system on which the accurate positions of all the fossil specimens were charted in the field; photographs were taken in the laboratory. Then fragments from the middle part of the leaf compressions were sampled for cuticular analysis (Figure 3). The cuticular and $\delta^{13}C$ data were calculated using the Microsoft Excel Chart (XY Scatterplot) to create the correlative plots (Figures 4 & 5). The sample information and raw data of carbon isotope and stomatal values are listed in Tables 1–3.

All fossil specimens and cuticle slides are housed in the Institute of Palaeontology and Stratigraphy, Lanzhou University, China (LUP).

Stomatal Analysis

The leaf fragments were immersed in 10% HCl solution for ca. 10 h, 50% HF solution for ca. 24–48 h, 30% HNO_3 solution for ca. 8–10 h and 5% NH_4OH for ca. 10 min, respectively. The lower and upper cuticles were isolated under a stereomicroscope (Leica M420) and separated into two parts. One part was stained with Safranine solution and mounted on slides for stomatal analysis, and the other was weighed for carbon isotope analysis. Stomatal and epidermal cell counts were made using a microscope (Leica DM4000B) linked to a computer with an image analyzer (Leica QWin V3). The experimental treatments for the fossil and extant cuticles have been well described by Sun *et al.* (2003) and the measurements of stomatal parameters followed Poole & Kürschner (1999). The SI was calculated using the equation of Salisbury (1927):

$$SI\ (\%) = \frac{SD}{SD + ECD} \times 100 \qquad (1)$$

where *SI*(%) represents the stomatal index, *SD* the stomatal density per unit leaf area and *ECD* the epidermal cell density per unit leaf area.

The palaeo-CO_2 was then calculated using the stomatal ratio (SR) method (McElwain & Chaloner 1995, 1996; McElwain 1998), which is defined as:

$$C_a(past) = \frac{SI(e)}{SI\ (f)} \times C_a(present) \qquad (2)$$

where *SI(e)* is the Stomatal Index of the extant plant, *SI(f)* is the Stomatal Index of the fossil plant, $C_a(past)$ is the palaeo-CO_2 and $C_a(present)$ is the current atmospheric CO_2 (McElwain 1998; Beerling & Royer 2002).

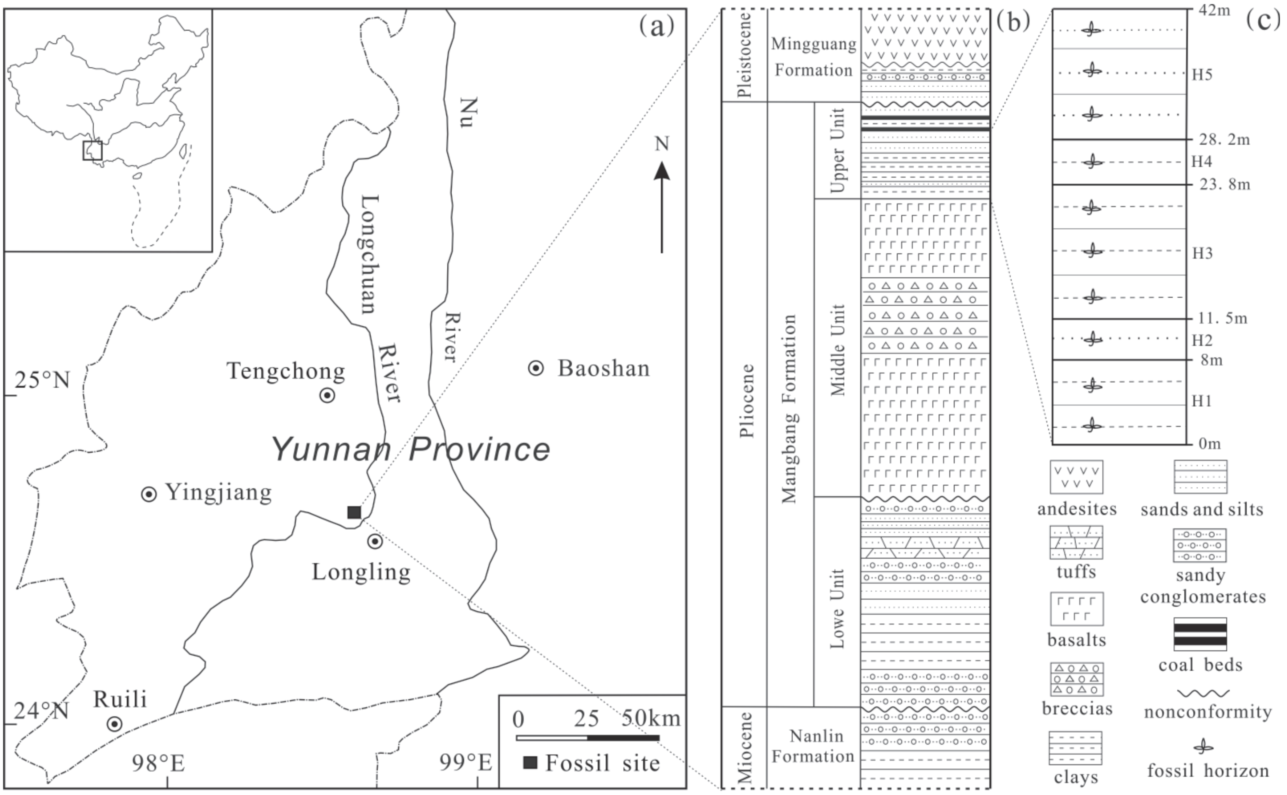

Figure 1. Geographic map and stratigraphic section of the sample location. **(a)** Sketch map of Tengchong County, Yunnan Province, China. **(b)** Stratigraphic section of the Pliocene Mangbang Formation. **(c)** Lithological column and fossil horizons of the upper unit of the Mangbang Formation.

Stable Carbon Isotope Analysis

The 3–5 mg cuticular samples were weighed and put into a sealed evacuated tube for combustion at 850°C for 1 h with copper oxide (CuO) wire as oxidant. The $^{13}C/^{12}C$ ratio was measured on the resultant CO_2 using a MAT–252 mass spectrometer, the precision associated with measurements being within ±0.1‰ in all cases. The carbon isotopic values are expressed according to the following equation (Craig 1953) including the Peedee belemnite standard (PDB) (Farquhar *et al.* 1989):

$$\delta(‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000 \qquad (3)$$

Results

On Figure 4a–c, the $\delta^{13}C$ data of the cuticles of each fossil sample are shown together with their SDs (Figure 4a), ECDs (Figure 4b), and SIs (Figure 4c). The mean and standard deviation of $\delta^{13}C$, SD, ECD and SI are listed in Table 2. In general, the cuticles of *B. mioluminifera* have slightly more negative $\delta^{13}C$ values, lower SDs and SIs than those of *C. subcordata*. The $\delta^{13}C$ values of *B. mioluminifera* and *C. subcordata* increase with rising SI (Figure 4c) as well as with an increase of SD (Figure 4a). However, the trends of $\delta^{13}C$ and ECD in the two species are opposite to each other (Figure 4b), but the correlation is very ambiguous in *C. subcordata* due to a low determination coefficient (R^2= 0.06).

Figure 4d–f also shows that the $\delta^{13}C$ (Figure 4d), SD (Figure 4e) and SI (Figure 4f) vary between the fossils from consecutive positions in the upper unit of the Mangbang Formation; all data are also listed in Table 2. The $\delta^{13}C$ values of the fossil cuticles from *B. mioluminifera* and *C. miofangiana* reveal a significant positive trend with decreasing specimen age (R^2= 0.67 and R^2= 0.65, respectively). A significant trend in the two species can also be observed as the SD

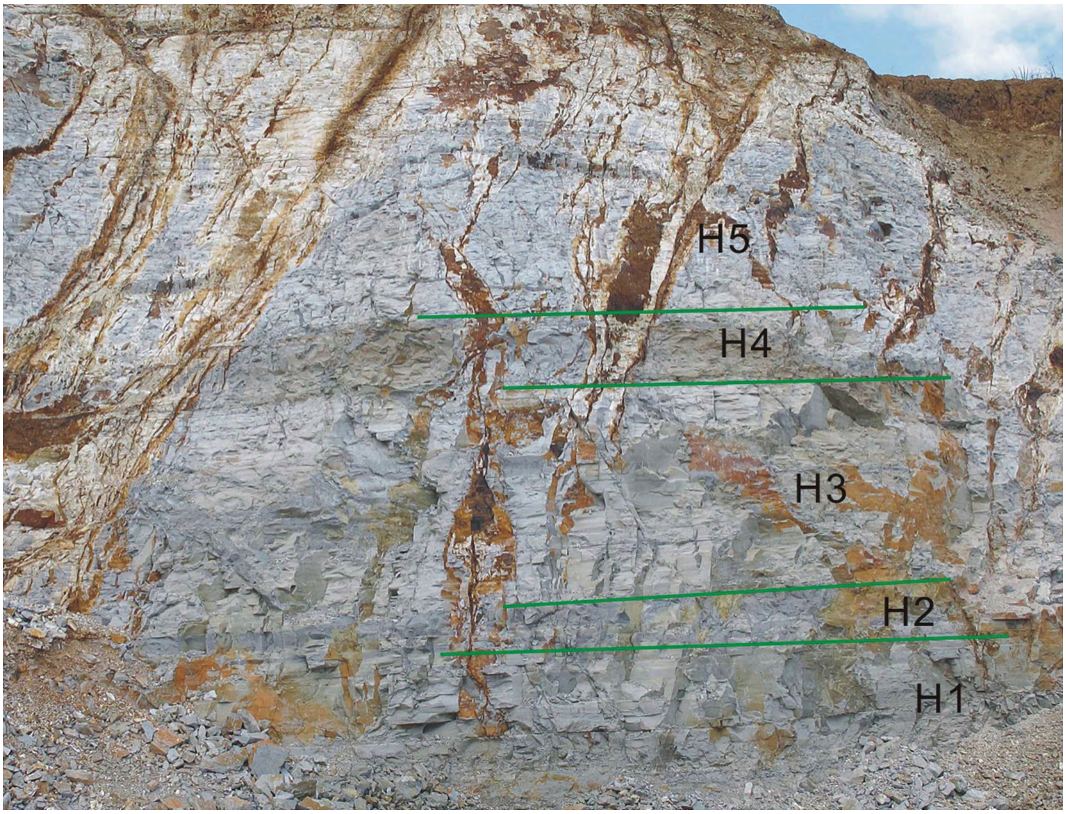

Figure 2. Upper unit of the Mangbang Formation in the open-cast diatomite mine at Tuantian Town, Tengchong County, Yunnan Province, China.

increases with the position of the fossils in the section (R^2= 0.66 and R^2= 0.51, respectively), accompanied by a distinct increase of SI (R^2= 0.50 and R^2= 0.79, respectively).

Figure 5 shows that SD (Figure 5a) and SI (Figure 5b) of the leaves of extant *B. luminifera* and *C. fangiana* change with the CO_2 concentration (Table 3). The SD and SI in the two NLE species have negative trends with increased CO_2. However, the correlation between the SD and CO_2 in both *B. luminifera* and *C. fangiana* has a lower determination coefficient (R^2= 0.3510 and R^2= 0.4268, respectively) than that of the SI (R^2= 0.8601 and R^2= 0.9070, respectively).

Discussion

The $\delta^{13}C$ of atmospheric CO_2 decreases with a rising concentration of atmospheric CO_2 (Francey *et al.* 1999; Chen *et al.* 2009; Minami *et al.* 2010); at the same time there is a decrease of $\delta^{13}C$ in plant bodies (Keeling *et al.* 1979; Polley *et al.* 1993; Tang & Qian 2000). Stomata provide an essential connection between the internal air spaces of plants and the external atmosphere. The most important characteristic of stomata is that they open and close, and the change in size of their aperture controls gas exchange, especially CO_2 uptake as necessary for photosynthesis and H_2O loss by transpiration (Franks & Farquhar 2007). An inverse relationship between the stomatal parameters of leaves and the ambient CO_2 pressure has been established in several gymnosperm and angiosperm species (e.g., Woodward 1987; Beerling *et al.* 1998; Retallack 2001; Royer 2001).

The relationships observed between the $\delta^{13}C$ values and the SDs, ECDs and SIs of several conifer morphotypes were discussed by Aucour *et al.* (2008). Their results indicate that the SD and SI decrease or do not significantly change with increasing $\delta^{13}C$ values. Furthermore, the $\delta^{13}C$ values of C3

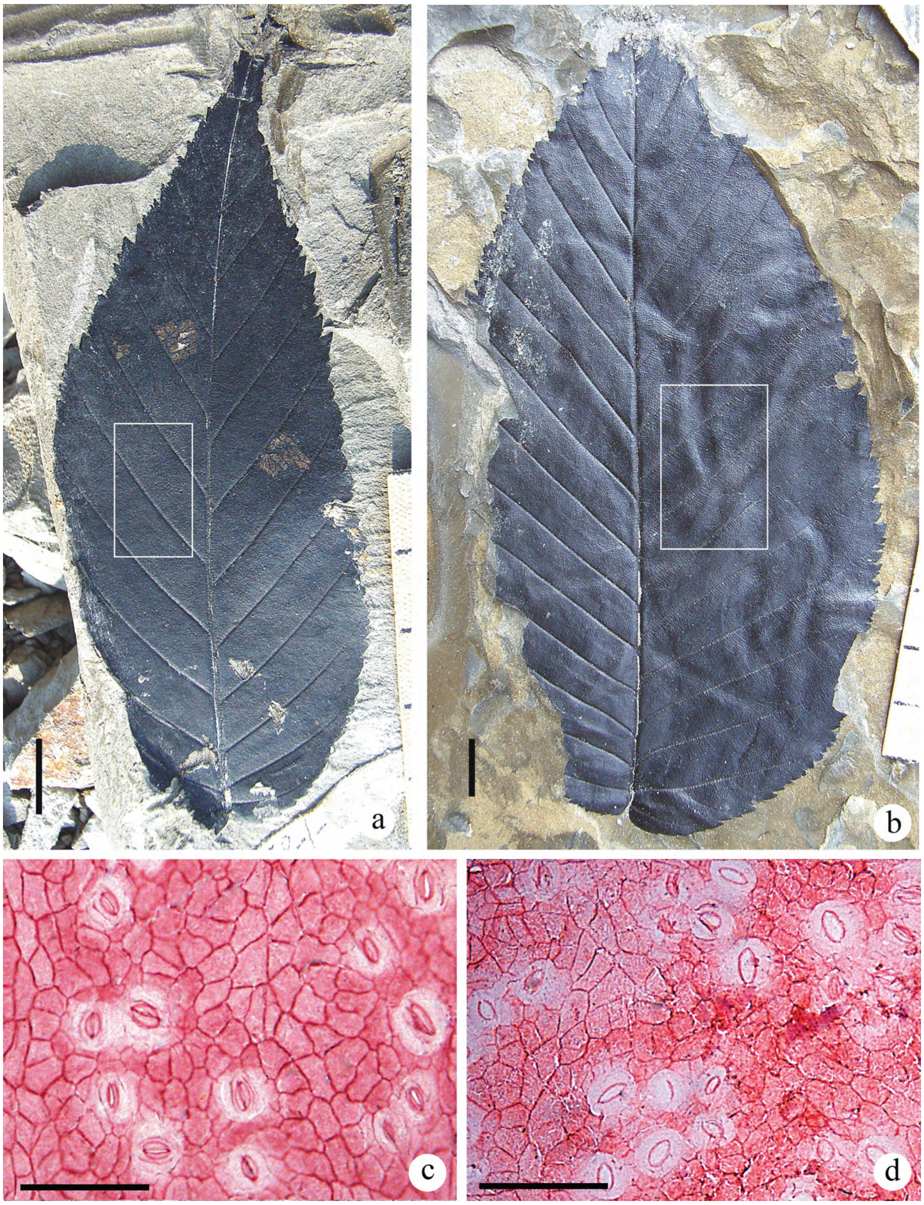

Figure 3. Examples of hand specimens and cuticles of *Betula mioluminifera* Hu et Chaney and *Carpinus miofangiana* Nathorst as used for stomatal and stable carbon isotope analysis. The white frames show the sampling sites for cuticular analysis. **(a)** Fossil leaf of *B. mioluminifera*, scale bar– 1 cm. **(b)** Fossil leaf of *C. miofangiana*, scale bar– 1 cm. **(c)** Lower epidermis of *B. mioluminifera*, scale bar– 100 µm. **(d)** Lower epidermis of *C. miofangiana*, scale bar- 100 µm.

plants may vary with a number of environmental factors such as light, temperature, water pressure, gravity, etc. (Ehleringer *et al.* 1986; Zimmerman & Ehleringer 1990; Anderson *et al.* 1996; Tans & White 1998; Gebrekirstos *et al.* 2009; Rajabi *et al.* 2009; Zhu *et al.* 2009). However, the Pliocene climate of West Yunnan was humid and subtropical (Xu *et al.* 2004, 2008; Wu *et al.* 2009; Sun *et al.* 2011) without major fluctuations of temperature and precipitation. Accordingly, all the fossiliferous horizons of the upper unit of the Mangbang Formation show similar associations of plant fossils with similar leaf-size

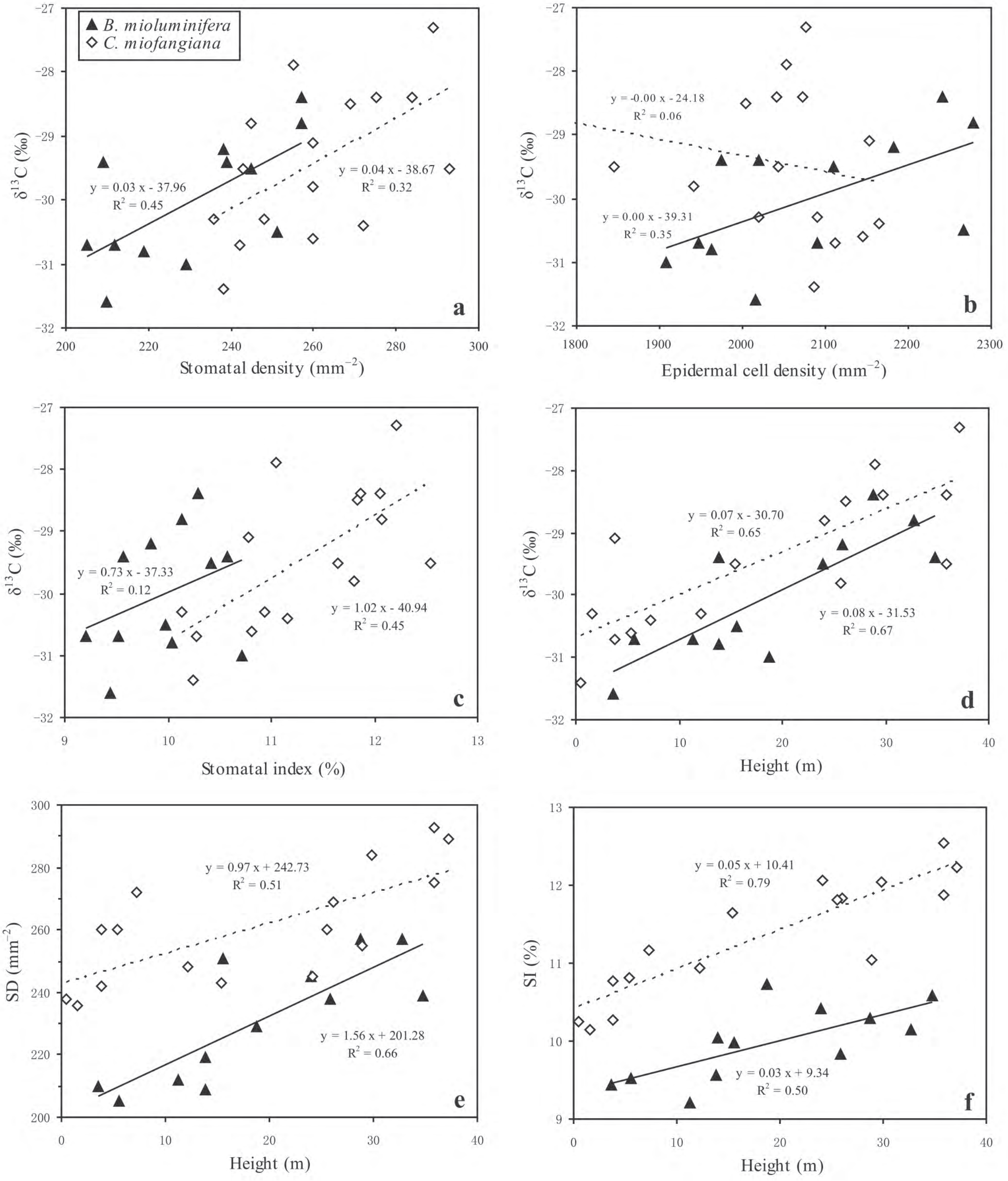

Figure 4. Plot of cuticular δ^{13}C as a function of **(a)** stomatal density, **(b)** epidermal cell density, and **(c)** stomatal index for *B. mioluminifera* and *C. miofangiana*. **(d–f)** Trends of (d) δ^{13}C, (e) SD and (f) SI with ascending position of samples in the section from H1 to H5. Each data point represents one leaf sample.

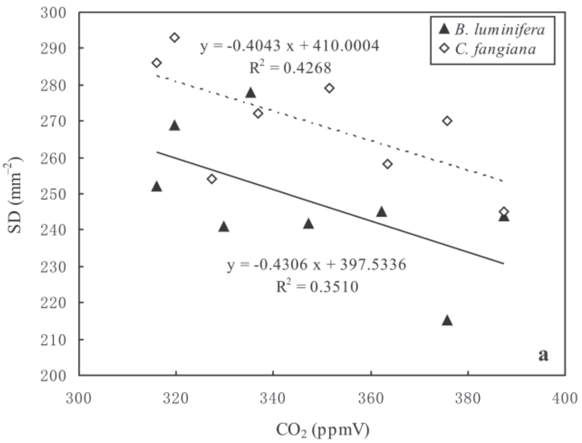

Figure 5. Correlation between **(a)** stomatal density and **(b)** stomatal index versus CO_2 concentration for *B. luminifera* and *C. fangiana*.

class and percentages of entire margined species (Wu 2009; Sun *et al.* 2011). Even though there was a slight temperature drop in the Late Pliocene as shown here, the $\delta^{13}C$ and the stomatal parameters of the two fossil plants studied here appear to be primarily related to the atmospheric CO_2 concentrations.

The $\delta^{13}C$ values have a positive correlation with SD and SI in the cuticles of both *B. mioluminifera* and *C. miofangiana* (Figure 3a, c). The $\delta^{13}C$ values of the cuticles of *B. mioluminifera* range from –31.6‰ to –28.4‰, and also reveal a positive correlation with the ECD (R^2= 0.35). Furthermore, the $\delta^{13}C$ values of *C. miofangiana* are slightly higher than those of *B. mioluminifera*, and a slight negative correlation between the $\delta^{13}C$ and the ECD with a very low determination coefficient (R^2= 0.06) can also be observed. These differences between the cuticles of *B. mioluminifera* and *C. miofangiana* were probably influenced by their genotypes rather than by environmental factors, which remained almost stable during the Late Pliocene.

The SD and SI of *B. mioluminifera* reveal a significant positive correlation with ascending position in the section through horizons H1 to H5 (Figure 3e, f), and the same trends can also be observed for the cuticles of *C. miofangiana.* Conformably, the $\delta^{13}C$ values in both *B. mioluminifera* and *C. miofangiana* show an actual positive correlation with their ascending positions in the section (Figure 3d).

In order to confirm the correlation between SD, SI, and the CO_2 concentration, the leaves of the NLEs (*B. luminifera* and *C. fangiana*) of the two fossil species were selected for comparative stomatal studies. The modern trees were chosen based on their distribution in subtropical regions with a MAT of 14.9–22.0°C, a MAP of 1070.5–1730 mm, and an altitude of 1200–1500 m (Table 1). The ecological requirements of these modern trees are close to those of the fossil species. Therefore, the influence of climate and altitude can be effectively excluded. The results show that SD and SI of the extant leaves have a prominent negative correlation with the CO_2 concentration, which is essential to the discussion of CO_2 trends in the Late Pliocene with regard to stomatal characters of fossil cuticles.

Figure 5 illuminates the fact that the SIs of *B. luminifera* and *C. fangiana* have a more prominent negative correlation with the CO_2 concentration (R^2= 0.8601 and R^2= 0.9070, respectively) relative to that of SD (R^2= 0.3510 and R^2= 4268, respectively). Although Woodward (1987) was the first to document that the SD and SI in extant plants are negatively correlated with atmospheric CO_2, environmental and biological factors such as natural variability, water stress, irradiance, and temperature can influence SD and SI (Royer 2001). The SD is influenced by many environmental factors such as e.g. genotype, ambient CO_2 concentration, light intensity, humidity and soil salinity (McElwain & Chaloner 1996). For example, in high-insolation or humid environments the SDs tend to be greater than those from shady or arid environments (Gay & Hurd 1975; Sharma & Dunn 1968). However, the variability of SD can be

Table 1. Source of *Betula luminifera* and *Carpinus fangiana* from China.

Species	Locality	Collecting year	Global CO_2 level (ppmv)[1]	Altitude (m a.s.l.)	MAT (°C)	MAP (mm)	Herbarium and Voucher
B. luminifera	Yinjiang, Guizhou	1959	315.98	1350	16.8	1189.0	PE (800784)
	Qianshan, Jiangxi	1964	319.62	1350	17.9	1730.0	PE (800198)
	Ruyuan, Guangdong	1973	329.68	1200	17.8	1650.0	IBSC (383098)
	Daoxian, Hunan	1978	335.41	1350	18.5	1506.7	IBSC (450195)
	Tujia, Guizhou	1986	347.19	1300	15.3	1500.0	PE (800822)
	Wuxi, Chongqian	1996	362.36	1280	18.2	1104.5	PE (800345)
	Kunming, Yunnan	2003	375.78	1300	14.9	1011.3	LUP (30021)
	Kunming, Yunnan	2009	387.35	1450	14.9	1011.3	LUP (90031)
C. fangiana	Leishan, Guizhou	1959	315.98	1480	15.3	1117.7	PE (777897)
	Jiangkou, Guizhou	1964	319.62	1200	15.2	1342.0	PE (1282360)
	Leibo, Sichuan	1972	327.45	1400	17.8	1063.1	PE (1802356)
	Longlin, Guangxi	1979	336.78	1330	22.0	1070.5	IBK (197642)
	Leibo, Sichuan	1988	351.45	1500	17.8	1063.1	PE (777796)
	Jingan, Jiangxi	1997	363.47	1320	17.6	1624.4	IBSC (650802)
	Kunming, Yunnan	2003	375.78	1300	14.9	1011.3	LUP (30028)
	Kunming, Yunnan	2009	387.35	1400	14.9	1011.3	LUP (90034)

* The global mean atmospheric carbon dioxide in the same year of collecting time is from measurements at the Mauna Loa Observatory in Hawaii (ftp://ftp.cmdl.noaa.gov/ccg/co2/trends/co2_annmean_mlo.txt).

minimized by use of the SI for comparison in the analysis (McElwain & Chaloner 1996). Although the proportion of SD and SI responses inversely relate to CO_2, the SI is mainly controlled by the genotype and ambient CO_2 concentration and thus SI-based CO_2 reconstructions are probably more accurate (Royer 2001).

Atmospheric CO_2 partial pressure generally decreases with altitude. McElwain (2004) presented a novel palaeoaltimetry method using leaf stomatal frequency response to the decline in CO_2 partial pressure with altitude. Later, new data detailing the influence of other climatic variables on leaf stomatal frequency changes with altitude were also presented (Kouwenberg *et al.* 2007). In the study of Kouwenberg *et al.* (2007), a clear increase in SD and SI was observed with increasing elevation for two plants growing on the slope of Mt. Ruapehu (New Zealand). Therefore, it seems possible that the increase of SD and SI in *B. mioluminifera* and *C. miofangiana* could have been affected by rapid tectonic uplift of West Yunnan. However, there is a consensus among many geologists that the Tibetan Plateau attained its considerable altitude before the Late Miocene (at 8 Ma) (e.g., Quade *et al.* 1989; Molnar & England 1990; Harrison *et al.* 1992, 1995; Cerling *et al.* 1993; Sun & Zheng 2003). Sun *et al.* (2011) also indicated that West Yunnan had approached its highest altitude before the Late Pliocene based on the vegetation and climatic changes. In our opinion, CO_2 concentration

Table 2. Stable carbon isotope composition, stomatal and epidermal cell numbers of cuticles in *Betula mioluminifera* and *Carpinus miofangiana* (*x*– mean, *σ*– standard deviation, *n*– number of cuticles).

Species	Specimen No.	Position (m)[1]	$\delta^{13}C$ (‰)			SD (mm^{-2})			ED (mm^{-2})			SI (%)		
			x	*σ*	*n*	*x*	*σ*	*n*	*x*	*σ*	*n*	*x*	*σ*	*n*
B. mioluminifera	LUP-H5-3	34.8	−29.4	0.5	3	239	8	8	2020	87	8	10.6	0.4	8
	LUP-H5-2	32.7	−28.8	0.8	3	257	12	8	2278	121	8	10.1	0.6	8
	LUP-H5-1	28.8	−28.4	0.3	3	257	12	8	2241	132	8	10.3	0.8	8
	LUP-H4-3	25.8	−29.2	0.5	3	238	21	8	2183	98	8	9.8	0.5	8
	LUP-H4-2	24	−29.5	0.5	3	245	14	8	2109	102	8	10.4	1.1	8
	LUP-H3-6	18.8	−31.0	1.0	3	229	12	8	1907	115	8	10.7	0.8	8
	LUP-H3-5	15.6	−30.5	0.3	3	251	14	8	2267	186	8	10.0	1.1	8
	LUP-H3-4	13.9	−30.8	0.8	3	219	16	8	1962	176	8	10.0	0.9	8
	LUP-H3-1	13.8	−29.4	0.4	3	209	8	8	1975	203	8	9.6	1.2	8
	LUP-H2-1	11.3	−30.7	0.5	3	212	11	8	2090	102	8	9.2	0.5	8
	LUP-H1-2	5.6	−30.7	0.4	3	205	10	8	1948	78	8	9.5	0.5	8
	LUP-H1-1	3.6	−31.6	0.6	3	210	16	8	2015	145	8	9.4	0.3	8
C.miofangiana	LUP-H5-6	37.2	−27.3	0.4	3	289	25	8	2076	156	8	12.2	0.6	8
	LUP-H5-5	35.9	−29.5	0.8	3	293	14	8	2044	231	8	12.5	0.8	8
	LUP-H5-3	35.9	−28.4	0.6	3	275	20	8	2042	256	8	11.9	0.6	8
	LUP-H5-2	29.8	−28.4	1.0	3	284	10	8	2073	142	8	12.1	0.5	8
	LUP-H5-1	28.9	−27.9	1.0	3	255	11	8	2053	106	8	11.1	0.8	8
	LUP-H4-4	26.1	−28.5	0.5	3	269	21	8	2003	98	8	11.8	0.6	8
	LUP-H4-3	25.6	−29.8	0.8	3	260	16	8	1942	206	8	11.8	0.9	8
	LUP-H4-2	24.1	−28.8	1.0	3	245	14	8	1785	156	8	12.1	0.8	8
	LUP-H3-2	15.4	−29.5	0.3	3	243	13	8	1845	142	8	11.6	1.1	8
	LUP-H3-1	12.2	−30.3	0.4	3	248	16	8	2019	189	8	10.9	0.6	8
	LUP-H1-16	7.3	−30.4	0.4	3	272	9	8	2165	124	8	11.2	0.7	8
	LUP-H1-12	5.4	−30.6	1.2	3	260	7	8	2145	219	8	10.8	1.3	8
	LUP-H1-8	3.8	−29.1	1.1	3	260	11	8	2152	203	8	10.8	0.8	8
	LUP-H1-7	3.8	−30.7	0.8	3	242	10	8	2112	289	8	10.3	1.0	8
	LUP-H1-4	1.6	−30.3	0.6	3	236	15	8	2091	215	8	10.1	0.8	8
	LUP-H1-3	0.5	−31.4	1.0	3	238	14	8	2086	156	8	10.2	0.6	8

* The data represent the position of fossil leaves in the ascending order from the H1 to H5 in the Upper Unit of Mangbang Formation (see Figure 1c).

Table 3. Stomatal and epidermal cell numbers of cuticles in *Betula luminifera* and *Carpinus fangiana* (*x*– mean, *σ*– standard deviation, *n*– number of cuticles).

Species	Collecting year	CO_2 (ppmv)	SD (mm^{-2})			ED (mm^{-2})			SI (%)		
			x	*σ*	*n*	*x*	*σ*	*n*	*x*	*σ*	*n*
B. luminifera	1959	315.98	252	12	10	1741	124	10	12.6	0.4	10
	1964	319.62	269	17	10	1918	98	10	12.3	0.6	10
	1973	329.68	241	15	10	1809	89	10	11.8	0.5	10
	1978	335.41	278	12	10	2055	106	10	11.9	0.4	10
	1986	347.19	242	14	10	1893	78	10	11.3	0.7	10
	1996	362.36	245	21	10	1859	125	10	11.6	0.6	10
	2003	375.78	215	10	10	1827	112	10	10.5	0.3	10
	2009	387.35	244	14	10	2041	97	10	10.7	0.6	10
C. fangiana	1959	315.98	286	15	10	1725	107	10	14.2	0.4	10
	1964	319.62	293	18	10	1818	115	10	13.9	0.4	10
	1972	327.45	254	22	10	1617	120	10	13.6	0.6	10
	1979	336.78	272	17	10	1759	117	10	13.4	0.5	10
	1988	351.45	279	11	10	1781	118	10	13.5	0.6	10
	1997	363.47	258	19	10	1711	98	10	13.1	0.6	10
	2003	375.78	270	15	10	1895	87	10	12.5	0.5	10
	2009	387.35	245	16	10	1781	103	10	12.1	0.6	10

is therefore probably the sole factor determining $\delta^{13}C$, SD and SI.

In this paper, the Late Pliocene CO_2 level was reconstructed based on the stomata ratio method (McElwain & Chaloner 1995, 1996). The SI of fossil *B. mioluminifera* ranges from 9.2 to 10.7, and the palaeo-CO_2 was calculated as 381.5–439.4 ppmv, using a correlation of the SI of extant *B. luminifera* to the recent CO_2 level. Similarly, the palaeo-CO_2 was calculated as 377.8–472.3 ppmv, based on the SRs of *C. fangiana* and *C. miofangiana*.

One possible reason for the changing trends of $\delta^{13}C$, SD and SI in our results is that the global atmospheric CO_2 level decreased in the Late Pliocene. In a recent study, Pagani *et al.* (2010) demonstrated that the atmospheric CO_2 levels peaked at about 4.5 million years ago and dwindled gradually afterwards. Tripati *et al.* (2009) also pointed out that pCO_2 decreased with the major episodes of glacial expansion during the Late Pliocene (~3.3 to 2.4 Mya). Retallack (2001) presented a continuous 300 Ma record of stomatal abundance from *Ginkgo*-related fossil leaves to reconstruct past atmospheric CO_2 concentrations. He indicated that the SI of fossil *Ginkgo* leaves was high and the reconstructed palaeo-CO_2 concentrations decreased during the Neogene.

The palaeo-CO_2 levels reconstructed in our study are slightly higher than those from other studies based on fossil plants (Kürschner *et al.* 1996; Berner & Kothavala 2001; Royer 2001; Royer *et al.* 2001; Berner 2006). We consider that higher CO_2 levels at the Tuantian fossil locality could have been caused by volcanic activity which was common in the region during the Late Pliocene (e.g., Jiang 1998; Guo & Lin 1999; Li *et al.* 2000; Shang 2003). Volcanic eruptions can produce large amounts of gases, especially CO_2, which may potentially lead to local enrichment of CO_2 in the respective area for 10–10^5 years (Wignall 2001). Therefore, the Pliocene flora of Tuantian may have grown in a volcanically perturbed atmosphere with locally raised levels of CO_2.

Conclusions

Based on the studies of cuticular $\delta^{13}C$ values and stomatal parameters of two Late Pliocene plants within the Tuantian flora of West Yunnan, *B. mioluminifera* and *C. miofangiana*, and their NLE species, *B. luminifera* and *C. fangiana*, we conclude: (1) The $\delta^{13}C$, SD and SI of *B. mioluminifera* and *C. miofangiana* from Tengchong, West Yunnan show an increase in the Late Pliocene (3.30–2.83 Mya). In contrast, the SD and SI of *B. luminifera* and *C. fangiana*, especially the SI, show a prominent negative correlation with the CO_2 concentration. Based on the stomatal ratio method, the CO_2 concentration was reconstructed from the two fossil species as 381.5–439.4 ppmv and 377.8–472.3 ppmv, respectively. (2) The increase of $\delta^{13}C$, SD and SI in the Late Pliocene corresponds clearly to a decrease in atmospheric CO_2 in this time interval rather than to the tectonic uplift of West Yunnan, especially since the regional uplift had approached its peak before the Late Pliocene. (3) Slightly higher levels of CO_2 in the Late Pliocene of West Yunnan may have been caused by frequent volcanic activity, which is also known to cause local CO_2 enrichment.

Acknowledgements

We are grateful to the reviewers for their significant suggestions for this paper. We also thank Dr. Susan Turner, Queensland Museum, Australia, for her help with the English. This research was conducted under the National Natural Science Foundation of China (Nos. 40772012 & 40802008), the Specialized Research Fund for the Doctoral Program of Higher Education (Nos. 20100211110019 & 20100211120009) and the Foundation of the State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology, CAS (No. 103108).

References

Anderson, J.E., Williams, J., Kriedemann, P.E., Austin, M.P. & Farquhar, G.D. 1996. Correlations between carbon isotope discrimination and climate of native habitats for diverse eucalypt taxa growing in a common garden. *Australian Journal of Plant Physiology* **23**, 311–320.

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., 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.

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. & Woodward, F.I. 1995. Stomatal responses of variegated leaves to CO_2 enrichment. *Annals of Botany* **75**, 507–511.

Beerling, D.J. & Woodward, F.I. 1997. Changes in land plant function over the Phanerozoic: reconstructions based on the fossil record. *Botanical Journal of the Linnean Society* **124**, 137–153.

Berner, R.A. 2006. GEOCARBSULF: A combined model for Phanerozoic atmospheric O_2 and CO_2. *Geochimica et Cosmochimica Acta* **70**, 5653–5664.

Berner, R.A. & Kothavala, Z. 2001. Geocarb III: A revised model of atmospheric CO_2 over Phanerozoic time. *American Journal of Science* **301**, 182–204.

Cerling, T.E., Wang, Y. & Quade, J. 1993. Expansion of C4 ecosystems as an indicator of global ecological change in the late Miocene. *Nature* **361**, 344–345.

Chen, P.N., Wang, G.A., Han, J.M., Liu, X.J. & Liu, M. 2009. $\delta^{13}C$ difference between plants and soil organic matter along the eastern slope of Mount Gongga. *Chinese Science Bulletin* **55**, 55–62.

Craig, H. 1953. The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta* **3**, 53–92.

Dai, J., Sun, B., Xie, S., Wu, J. & Li, N. 2009. *Carpinus miofangiana* from the Pliocene of Tengchong in Yunnan Province and its palaeoclimatic significance. *Advances in Earth Science* **24**, 1024–1032 [in Chinese, with English Abstract].

Ehleringer, J.R. & Cerling, T.E. 1995. Atmospheric CO_2 and the ratio of intercellular to ambient CO_2 concentration in plants. *Tree Physiology* **15**, 105–111.

Ehleringer, J.R., Field, C.B. & Lin, Z.F. 1986. Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. *Oecologia* **70**, 520–526.

Farquhar, G.D., Ball, M.C., Von Caemmerer, S. & Roksandic, Z. 1982a. Effect of salinity and humidity on δ^{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 during photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 503–37.

Feng, X. 1999. Trends in intrinsic water–use efficiency of natural trees for the past 100–200 years: a response to atmospheric CO_2 concentration. *Geochimica et Cosmochimica Acta* **63**, 1891–1903.

Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M., Langenfelds, R.L., Michel, E. & Steele, L.P. 1999. A 1000–year high precision record of δ^{13}C in atmospheric CO_2. Tellus B **51**, 170–193.

Franks, P.J. & Farquhar, G.D. 2007. Gas exchange, stomatal behavior, and δ^{13}C values of the *flacca* Tomato Mutant in relation to Abscisic Acid. *Plant Physiology* **143**, 78–87.

Gay, A.P. & Hurd, R.G. 1975. The infuence of light on stomatal density in the tomato. *New Phytologist* **75**, 37–46.

Ge, H.R. & Li, D.Y. 1999. Cenozoic Coal-bearing basins and coal forming regularity in west Yunnan. *Yunnan Science and Technology Press,* Kunming, China, 20–85 [in Chinese].

Gebrekirstos, A., Worbes, M., Teketay, D., Fetene, M. & Itlöhner, R. 2009. Stable carbon isotope ratios in tree rings of co-occurring species from semi-arid tropics in Africa: Patterns and climatic signals. *Global and Planetary Change* **66**, 253–260.

Guo, G.Y. & Lin, Z.H. 1999. Discussion on late Cenozoic volcanic activities in Tengchong area, Yunnan Province, China. *Contributions to Geology and Mineral Resources Research* **14**, 8–15 [in Chinese, with English abstract].

Harrison, T.M., Copeland, P., Kidd, W.S.F. & Lovera, O.M. 1995. Activation of the Nyainqentanglha Shear Zone, applications for uplift of the southern Tibet Plateau. *Tectonics* **14**, 658–676.

Harrison, T.M., Copeland, P., Kidd, W.S.F. & Yin, A. 1992. Raising Tibet. *Science* **255**, 1663–1670.

Ipcc (Intergovernmental Panel on Climate Change) 2007. Climate Change 2007: The Physical Science Basis. Summary for Policymakers. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC WGI Fourth Assessment Report, 1–26.

Jahren, A.H., Lepage, B.A. & Werts, S.P. 2004. Methanogenesis in Eocene Arctic soils inferred from δ^{13}C of tree fossil carbonates. *Palaeogeography, Palaeoclimatology, Palaeoecology* **214**, 347–358.

Jiang, C.S. 1998. Period division of volcano activities in the Cenozoic era of Tengchong. *Journal of Seismological Research* **21**, 320–329 [in Chinese, with English abstract].

Keeling, C.D., Mook, W.M. & Tans, P. 1979. Recent trends in the ^{13}C/^{12}C ratio of atmospheric carbon dioxide. *Nature* **277**, 121–123.

Kouwenberg, L.L.R., Kürschner, W.M. & Mcelwain, J.C. 2007. Stomatal frequency change over altitudinal gradients: prospects for paleoaltimetry. *Reviews in Mineralogy and Geochemistry* **66**, 215–241.

Kürschner, W.M. 2002. Carbon isotope composition of fossil leaves – revealing ecophysiological responses to past environmental change. *New Phytologist* **155**, 199–201.

Kürschner, W.M., Van Der Burgh, J., Visscher, H. & Dilcher, D.L. 1996. Oak leaves as biosensors of late Neogene and early Pleistocene paleoatmospheric CO_2 concentrations. *Marine Micropaleontology* **27**, 299–312.

Li, D.M., Li, Q. & Chen, W.J. 2000. Volcanic activities in the Tengchong volcano area since Pliocene. *Acta Petrologica Sinica* **16**, 362–370 [in Chinese, with English Abstract].

Li, F., & Xue, C.D. 1999. Geodynamic setting since the Cenozoic and its environmental effects in Northwest Yunnan, China. *Geotectonica et Metallogenia* **23**, 115–122 [in Chinese, with English abstract].

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.

McElwain, J.C. 1998. Do fossil plants signal palaeoatmospheric CO_2 concentration in the geological past? *Philosophical Transactions of the Royal Society B* **353**, 83–96.

McElwain, J.C. 2004. Climate-independent paleoaltimetry using stomatal density in fossil leaves as a proxy for CO_2 partial pressure. *Geology* **32**, 1017–1020.

McElwain, J.C. & Chaloner, W.G. 1995. Stomatal density and index of fossil plants track atmospheric carbon dioxide in the Paleozoic. *Annals of Botany* **76**, 389–395.

McElwain, J.C. & Chaloner, W.G. 1996. The fossil cuticle as a skeletal record of environmental change. *Palaios* **11**, 376–388.

Minami, M., Goto, A.S., Omori, T., Ohta, T. & Nakamura, T. 2010. Comparison of δ^{13}C and ^{14}C activities of CO_2 samples combusted in closed-tube and elemental-analyzer systems. *Nuclear Instruments and Methods in Physics Research Section B* **268**, 914–918.

Molnar, P. & England, P. 1990. Late Cenozoic uplift of mountain ranges and global climate change: chicken or egg? *Nature* **346**, 29–34.

Pagani, M., Liu, Z., Lariviere, J. & Ravelo, A.C. 2010. High Earth-system climate sensitivity determined from Pliocene carbon dioxide concentrations. *Nature Geoscience* **3**, 27–30.

Peters-Kottig, W., Strauss, H. & Kerp H. 2006. The land plant δ^{13}C record and plant evolution in the Late Palaeozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology* **240**, 237–252.

Polley, H.W., Johnson, H.B., Marino B.D. & Mayeux, H.S. 1993. Increase in C3 plant water-use efficiency and biomass over Glacial to present CO_2 concentrations. *Nature* **361**, 61–63.

Poole, I. & Kürschner, W. M. 1999. Stomatal density and index: the practice. *In*: Jones, T.P. & Rowe, N.P. (eds), *Fossil Plant and Spores: Modern Techniques*. The Geological Society, London, UK, 257–260.

Quade, J., Cerling, T.E. & Bowman, J.R. 1989. Development of Asian monsoon revealed by marked ecological shift during the latest Miocene in northern Pakistan. *Nature* **342**, 163–166.

Rajabi, A., Ober, E.S. & Griffiths, H. 2009. Genotypic variation for water use efficiency, carbon isotope discrimination, and potential surrogate measures in sugar beet. *Field Crops Research* **112**, 172–181.

Retallack, G.J. 2001. A 300-million-year record of atmospheric carbon dioxide from fossil plant cuticles. *Nature* **411**, 287–290.

Royer, D.L. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO_2oncentration. *Review of Palaeobotany and Palynology* **114**, 1–28.

Royer, D.L., Berner, R.A. & Beerling, D.J. 2001. Phanerozoic atmospheric CO_2 change: evaluating geochemical and paleobiological approaches. *Earth–Science Reviews* **54**, 349–392.

Salisbury, E.J. 1927. On the causes and ecological significance of stomatal frequency, with special reference to the Woodland flora. *Philosophical Transactions of the Royal Society of London B* **216**, 1–65.

Shang, Y.L. 2003. Tengchong diatomite deposit and its genesis. *Yunnan Geology* **22,** 418–425 [in Chinese, with English abstract].

Sharma, G.K. & Dunn, D.B. 1968. Effect of environment on the cuticular features in Kalanchoe fedschenkoi. *Bulletin of the Torrey Botanical Club* **95**, 464–473.

Sun, B.N., Cong, P.Y., Yan, D.F. & Xie, S.P. 2003. Cuticular structure of two angiosperm fossils in Neogene from Tengchong, Yunnan Province and its palaeoenvironmental significance. *Acta Palaeontologica Sinica* **42**, 216–222 [in Chinese, with English abstract].

Sun, B.N., Xiao, L., Xie, S.P., Deng, S.H., Wang, Y.D., Jia, H. & Turner, S. 2007. Quantitative analysis of paleoatmospheric CO_2 level based on stomatal characters of fossil *Ginkgo* from Jurassic to Cretaceous in China. *Acta Geologica Sinca* **81**, 931–939.

Sun, B.N., Wu, J.Y., Liu, Y.S., Ding, S.T., Li, X.C., Xie, S.P., Yan, D.F. & Lin, Z.C. 2011. Reconstructing Neogene vegetation and climates to infer tectonic uplift in western Yunnan, China. *Palaeogeography, Palaeoclimatology, Palaeoecology* **304**, 328–336.

Sun, H.L. & Zheng, D. 2003. *Formation, Environment and Development of Qinghai–Xizang (Tibetan) Plateau*. Shijiazhuang: Hebei Science & Technology Press, Shijiazhuang, China [in Chinese].

Tang, J. & Qian, J. 2000. Restructing CO_2 concentration by the tree-ring carbon isotopic ratios of West Tianmu Mountain. *Journal of Nanjing Forestry University* **24**, 45–48 [in Chinese, with English abstract].

Tans, P.P. & White, J.W.C. 1998. In balance, with a little help from the plants. *Science* **281**, 183–184.

Tao, J.R. & Du, N.Q. 1982. Neogene flora of Tengchong basin in western Yunnan, China. *Journal of Integrative Plant Biology* 2**4**, 273–281 [in Chinese, with English Abstract].

Tripati, A.K., Roberts, C.D. & Eagle, R.A. 2009. Coupling of CO_2 and ice sheet stability over major climate transitions of the last 20 million years. *Science* **326**, 1394–1397.

Tu, T.T.N., Kürschner, W.A., Schouten, S. & Van Bergen, P.F. 2004. Leaf carbon isotope composition of fossil and extant oaks grown under differing atmospheric CO_2 levels. *Palaeogeography, Palaeoclimatology, Palaeoecology* **212**, 199–213.

Van Der Burgh, J., Vissher, H., Dilcher, D.L. & Kürschner, W.M. 1993. Paleoatmospheric signatures in Neogene fossil leaves. *Science* **260**, 1788–1790.

Van De Water, P.K., Leavitt, S.W. & Betancourt, J.L. 2002. Leaf $\delta^{13}C$ variability with elevation, slope aspect, and precipitation in the southwest United States. *Oecologia* **132**, 332–343.

Wignall, P.B. 2001. Large igneous provinces and mass extinctions. *Earth-Science Reviews* **53**, 1–33.

Woodward, F.I. 1987. Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. *Nature* **327**, 617–18.

Wu, J.Y. 2009. *The Pliocene Tuantian Flora of Tengchong, Yunnan Province and its Paleoenvironmental Analysis*. PhD. Thesis, Lanzhou University, Lanzhou, China.

Wu, J.Y., Sun, B.N., Liu, Y.S., Xie, S.P. & Lin, Z.C. 2009. A new species of *Exbucklandia* (Hamamelidaceae) from the Pliocene of China and its paleoclimatic significance. *Review of Palaeobotany and Palynology* **155**, 32–41.

Xu, J.X., Ferguson, D.K., Li, C.S., Wang, Y.F. & Du, N.Q. 2004. Climatic and ecological implications of Late Pliocene Palynoflora from Longling, Yunnan, China. *Quaternary International* **117**, 91–103.

Xu, J.X., Ferguson, D.K., Li, C.S. & Wang, Y.F. 2008. Late Miocene vegetation and climate of the Lühe region in Yunnan, southwestern China. *Review of Palaeobotany and Palynology* **148**, 36–59.

Zhu, L., Liang, Z.S., Xu, X., Li, S.H. & Monneveux, P. 2009. Evidences for the association between carbon isotope discrimination and grain yield — Ash content and stem carbohydrate in spring wheat grown in Ningxia (Northwest China). *Plant Science* **176**, 758–767.

Zimmerman, J.K. & Ehleringer, J.R. 1990. Carbon isotope ratios are correlated with irradiance levels in the Panamanian orchid catasetum viridiflavam. *Oecologia* **83**, 247–249.