The impact of Miocene atmospheric carbon dioxide fluctuations on climate and the evolution of terrestrial ecosystems

Wolfram M. Kürschner*†, Zlatko Kváček‡, and David L. Dilcher§

*Faculty of Science, Institute of Environmental Biology, Department of Palaeoecology, Laboratory of Palaeobotany and Palynology, Utrecht University, Budapestlaan 4, 3584 CD, Utrecht, The Netherlands; ‡Faculty of Natural Sciences, Charles University, Albertov 6, 128 43 Prague 2, Czech Republic; and §Florida Museum of Natural History, University of Florida, Gainesville, FL 32611-7800

The Miocene is characterized by a series of key climatic events that led to the founding of the late Cenozoic icehouse mode and the dawn of modern biota. The processes that caused these developments, and particularly the role of atmospheric CO2 as a forcing factor, are poorly understood. Here we present a CO2 record based on stomatal frequency data from multiple tree species. Our data show striking CO2 fluctuations of ~600–300 parts per million by volume (ppmv). Periods of low CO2 are contemporaneous with major glaciations, whereas elevated CO2 of 500 ppmv coincides with the climatic optimum in the Miocene. Our data point to a long-term coupling between atmospheric CO2 and climate. Major changes in Miocene terrestrial ecosystems, such as the expansion of grasslands and radiations among terrestrial herbivores such as horses, can be linked to these marked fluctuations in CO2.

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The Miocene is distinguished by extreme climatic optima alternating with major long-term climatic cooling, which together mark the founding of the modern late Cenozoic ice mode and the evolution of modern terrestrial biomes (1). Grass-dominated ecosystems became established in the low and middle latitudes of many parts of the world, such as North America, Eurasia, Africa, and Australia (2). Major radiations in large mammalian herbivores have been attributed to changes in the distribution of vegetation and terrestrial primary productivity (3–5). A significant change in dental morphology from low-to high-crowned toothed horses occurs during the middle Miocene, whereas a transition from a C3 plant to C4 plant diet did not take place before the late Miocene (6).

Both Cenozoic climate trends and changes in terrestrial ecosystems have been thought to be influenced by long-term CO2 fluctuations (6–8). Before marine pCO2 proxy records were available, Cenozoic CO2 trends were inferred from carbon-isotope records of paleosols (9) and from carbon cycling models (10), which indicated a long-term decrease from ~1,000 to <500 parts per million by volume (ppmv) throughout the Cenozoic. Approximately a decade later, CO2 reconstructions based on marine geochemical proxies indicated consistently low late Pleistocene (glacial-like) CO2 values of ~200–280 ppmv (11, 12). Consequently, the Miocene has been regarded as a geological period in which climate and the carbon cycle were essentially decoupled. Because of this alleged decoupling, the role of atmospheric CO2 as a climate forcing factor has been disputed (13–15). However, a permanently low CO2 scenario has been challenged because photosynthetic models predict that plant life would not have thrived under such conditions (16). Climate models showed the importance of atmospheric CO2 as a fundamental boundary condition for Cenozoic climate change (17). In fact, a coupling between atmospheric CO2 and glacial–interglacial cycles over the past 600,000 years is well documented by ice core analysis (18). Understanding the long-term perspective beyond the Pleistocene is essential because climate and CO2 fluctuations on progressively shorter time scales are ultimately dependent on the evolution of the global carbon cycle and earth’s climate on longer time scales. Recently, refined geochemical studies of marine and terrestrial sedimentary records suggest a coupling between atmospheric CO2 and temperature over Phanerozoic time scales (19–21).

In addition to geochemical CO2 proxies, stomatal frequency analysis on fossil leaf remains represents a terrestrial proxy for CO2 that is based on the inverse relationship between atmospheric CO2 and stomatal frequency (22). In the present study, stomatal frequency is expressed as the stomatal index (SI), which is calculated as $SI (\%) = (SD/SD + ED) \times 100$, where SD is the stomatal density and ED is the epidermal cell density. Because SI normalizes for leaf expansion, it is largely independent of plant water stress and is primarily a function of CO2 (22, 23). Calculation of SI provides a robust method for estimating CO2 levels on short (24) and geologically long time scales (25, 26). Because the stomatal frequency response to CO2 is species-specific, quantitative estimates of CO2 are limited to extant species. Here we present a CO2 reconstruction based on a multiple-species stomatal frequency record from leaf remains of two extant lineages of laurel species (the Laurus abchasica and Laurus nobilis lineage and the Ocotea hradenskis and Ocotea foetens lineage), maidenhair tree (Ginkgo biloba), and an extinct laurel species (Laurophyllum pseudoprincipes). For each extant species, the stomatal frequency response has been independently calibrated based on historical sets of herbarium leaf material, using standard protocols (27). The training datasets and CO2 inference models, respectively, for L. nobilis and O. foetens are shown in Figs. 1 and 2 (for details, see Material and Methods). The SI calibration for the extinct species L. pseudoprincipes has been established by cross-calibration with known CO2 levels from those stratigraphic units in which it occurs together with the extant laurel species and Ginkgo.

We address two main questions in this article: (i) What was the relationship between the long-term CO2 fluctuations and climate evolution during the Miocene? and (ii) Was CO2 an environmental stress factor that influenced the evolution of modern terrestrial ecosystems?

Results and Discussion

Stomatal SIs of all four species (L. pseudoprincipes, L. nobilis, O. foetens, and G. biloba) show pronounced fluctuations over the...
time interval studied (Fig. 3A). Three periods can be recognized in the present record. During the oldest period—the late Oligocene to early Miocene (25–20 Ma)—the SI of L. pseudoprin-ceps shows a significant increase from ≈9% to 15% and remains high during the early Miocene. During the middle period—the late early to early middle Miocene (20–16 Ma)—L. pseudoprin-ceps shows a decrease in SI from 15% to 12%. A simultaneous decline in SI is found for two other laurel species, L. nobilis and O. foetens, from 12% to 10% and from 12% to 8%, respectively. In the youngest period of the present study—the middle Miocene (16–12 Ma)—L. nobilis shows a marked increase from 10% to 20%, followed by a decrease to 14% during the late middle Miocene. The pronounced increase in the SI of L. nobilis during the early middle Miocene is confirmed by a sharp shift in the SI of G. biloba from 8% to 15%.

The SI record of the present study reveals prominent changes in CO₂ since the late Oligocene (Fig. 3B). CO₂ declines drastically, from ≈600 to ≈340 ppmv, during the Oligocene–Miocene transition. It remains low (at about this level) until the middle early Miocene. From ≈20 Ma, CO₂ increases again to reach a maximum of ≈400–500 ppmv in the early middle Miocene (15.5 Ma). Superimposed on this rising trend are several smaller, temporary CO₂ oscillations with amplitude of ≈50 ppmv. A second major CO₂ decrease to 280 ppmv occurs during the middle Miocene (15.5–14 Ma), followed by a slight increase to ≈340 ppmv during the late Miocene. A previous CO₂ reconstruction based on North American leaf remains (26) fit perfectly into the present study, but it represented only a few measurements at ≈15 Ma.

It should be noted that because of the nonlinear nature of stomatal frequency response, the sensitivity of this proxy decreases at high CO₂ levels (greater than or equal to ≈400 ppmv). Consequently, the high CO₂ levels during the climate optimum at ≈22 Ma and 15.5 Ma may represent a conservative estimation rather than the average CO₂ level. Despite the applied log-transformation, the CO₂ inference models (Eqs. 3 and 4 in Materials and Methods) underestimate the actual CO₂ levels at the calculated SI for the calibration interval. To estimate this uncertainty, the CO₂ levels predicted by the inference models are plotted vs. the input CO₂ values used to establish the transfer functions (Fig. 4). At ≈500 ppm CO₂, the inference models of L. nobilis and O. foetens underestimate the CO₂ value by ≈150 and 200 ppmv, respectively. Consequently, the atmospheric CO₂ levels during the middle Miocene climatic optimum would have been closer to ≈650–700 ppmv than to the uncorrected value of ≈500 ppmv. During the late Oligocene, climatic optimum CO₂ levels would have been 800–900 ppmv, rather than the uncorrected 600 ppmv, which is intriguingly close to the estimates provided by the alkenone-based CO₂ proxy record (14). Moreover, well preserved leaf material is rare, and the number of observations per individual sample is (at the present stage) in some cases rather low. However, the largest portion, 70%, of the CO₂ record is based on stomata counts from the three extant species that are independently calibrated. Different species show the same trends, which reinforces the fidelity of the reconstructed CO₂ trends through the Miocene. All in all, our data do not show any signs of a prolonged Miocene low-CO₂ scenario, as suggested by geochemical proxy records (11, 12, 14).

If such a scenario had existed, it should also be evident in the present study because the botanical proxy used is sensitive to low CO₂ levels (22).

The present leaf-based CO₂ reconstruction shows a similar CO₂ decline at the Oligocene–Miocene transition as in the marine proxy records (12, 14), but it deviates significantly throughout the course of the Miocene. The alkenone-based CO₂ record indicates consistently low Pleistocene-like CO₂ levels (200–280 ppmv) and shows hardly any covariation with Miocene climate fluctuations. The boron isotope-based CO₂ reconstruction shows a decrease in atmospheric CO₂ (≈15 Ma), which is in the order of the present study (≈150 ppmv), although the absolute values are significantly lower (by ≈200 ppmv) than in our study. However, our results provide evidence from the terrestrial fossil record that CO₂ had a profound influence on the Miocene long-term climate evolution as recorded in marine records (1) (Fig. 3C). Despite the limited temporal resolution of the present study, CO₂ fluctuations correlate with some of the climate events (Mi1/1b, Mi2, and Mi3/4) known from the marine oxygen isotope curve (1). In the future, leaf-based CO₂ reconstructions with a finer time resolution may could potentially reveal patterns of higher order fluctuations that are coupled to orbital cycles, as suggested by marine proxy records (28). The global mean temperature model shows a 2–3°C decline during the early Miocene and middle Miocene climate events (Fig. 3C).
Our data confirm modeling experiments that show that Cenozoic CO2 must decline below a threshold of \( \sim 500 \) ppmv to induce significant buildup of Antarctic ice sheets (17). Unlike the geochemical proxy records (11, 12, 14), our data indicate that elevated CO2 levels contributed to the middle Miocene climatic optimum. Several lines of evidence from the fossil and geological record (15, 29–32) suggest that this time interval (\( \sim 14.5–17 \) Ma) was the warmest period of the past 35 Ma. Our results demonstrate that this climate optimum was forced significantly by elevated CO2 levels similar to those, for example, during the early Eocene (12, 14, 26). A likely source of the late, early, and middle Miocene CO2 increases was extensive volcanic activity during the Columbia River Flood Basalt volcanism and the Central European volcanism (33). The marked CO2 drop during the Miocene, in turn, may be the result of increased Corg burial resulting from the Himalayan uplift (34) and/or of enhanced marine productivity in the Pacific ocean (7) and the global occurrence of vast brown-coal-forming basins (35).

The marked Miocene CO2 variations may have directly impacted the structure and productivity of terrestrial biomes by affecting plant photosynthetic performance. In sensitivity tests at 280 and 560 ppmv for Miocene global vegetation models (36), CO2 shows pronounced changes related to vegetation distribution with regard to the degree of tree coverage for the seasonal dry tropics. However, evidence from the fossil record for the global distribution pattern of these biomes in the Miocene is rather scattered because fossil plant assemblages are highly influenced by taphonomical processes. Phytolith studies from the North American continental interior have shed new light on Miocene vegetational history (37). They reveal that pronounced changes in vegetation took place at the Oligocene–Miocene transition. Late Oligocene vegetation was a closed forest with palm and bamboo understory, whereas early Miocene plant communities were characterized by a mix of C3 grasses and
herbs, forming savannas or open woodland habitats (37). Recently, phytolith studies from the eastern Mediterranean region reveal that relatively open, grass-dominated habitats were established by at least the early Miocene (38). Intriguingly, these examples from the fossil record are similar to changes in vegetation distributions that occur in the Miocene global vegetation models as the result of CO₂ sensitivity runs (36). Growth experiments and vegetation models with modern mixed tree/grass ecosystems show that changes in atmospheric CO₂ directly affect tree cover by modifying water relationships in herbaceous vs. woody plants (39) or by modifying the (re)growth rates of plants recovering after a disturbance such as wildfire (40). Therefore, the opening of the forest vegetation and proliferation of open grassland habitat may have been influenced by the initial CO₂ drawdown to ~300 ppm during the early Miocene (Fig. 3D). After a continuous CO₂ increase up to ~550 ppm between 20 and 16 Ma, a second major drop in CO₂ at ~12 Ma down to ~280 ppm may have increased the environmental stress induced by CO₂ starvation. We hypothesize that under these CO₂-limiting conditions, in concert with an open habitat, exposure of grasses to high light intensity and water-stressed environments, combined with the coevolution of herbivores, may have facilitated the first radiation of C₄ grasses. To date, the fossil record of C₄ plants is still rather enigmatic. A few paleobotanical studies reveal evidence for C₄ plants in the middle Miocene (41, 42), whereas geochemical data suggest their presence since the early Miocene (43). Another line of evidence on the origin of C₄ plants comes from the molecular clock approach, which estimates an approximate maximum age for the origin of modern C₄ plants of 25 Ma (44). These data coincide approximately with the CO₂ drop in the early Miocene. Carbon isotope records of paleosols and fossil tooth enamel indicate, however, that the major proliferation of C₄ grasslands did take place during the late Miocene, ~8 Ma ago (6). The later vegetation change has been attributed to an increased seasonality and increased fire frequency (45).

Changes in the productivity and species richness of terrestrial vegetation must have affected herbivore communities. Hooved herbivorous large mammals on the North American continent show a maximum diversity during the middle Miocene climatic optimum (4, 5) (Fig. 3D). This maximum in both local and regional diversity greatly exceeds the diversity of ungulates in any present-day habitat, which implies a greater primary productivity than is seen today. Preliminary review data suggest that the pattern of elevated ungulate diversity is a global phenomenon, and, therefore, a global driving force is the most likely explanation. CO₂ fertilization during the middle Miocene climatic optimum may have made possible the expansion of high-productivity terrestrial biomes that supported high-diversity browser communities.

As a result of climatic changes such as increased seasonality and cooling, as well as the decline in primary productivity due to the marked post-middle Miocene CO₂ crisis, woodland biomes retreated, the species richness of browsing mammals declined, and grazers increased their diversity (3). A large number of terrestrial mammalian herbivores, such as equids, camels, antilocaprids, rhinos, and proboscideans show dramatic morphological changes of their dentitions during the Miocene (3–5). In particular, early Cenozoic horses are characterized by brachyodont (short-crowned) dentitions but show, from the late middle Miocene on (~15 Ma), a rapid diversification of hypsodont (high-crowned) taxa, which has been attributed as an adaptation to include more fibrous and abrasive material, such as grasses, in the diet (3). This development implies the coevolution of large herbivores and plants in response to Miocene climate and atmospheric CO₂ fluctuations.

Materials and Methods

Standardized computer-aided determinations of epidermal parameters were performed on an AnalySIS image analysis system (Soft Imaging Systems) software. All statistics and graphs were made with Sigmaplot version 9.1 (Systat Software). On average, 5–10 stomata-bearing alveoles per leaf sample were measured for ED (n/mm²) and SD (n/mm²). From SD and ED, the area-independent SI was calculated: SI (%) = [SD(SD + ED)] × 100. Most of the cuticle preparations (stored at Charles University) were collected from several brown-coal basins in the Czech Republic (East and Vitava basins, South Bohemia), supplemented with some material from Austria (Parschlag Basin, Styria) and Germany (Lower Rhine Embayment, Lausitz Basin). References for the age assessments of the individual fossil leaf samples are given in supporting information (SI) Table 3.

A total of 68 herbaceous leaf samples (see SI Tables 1 and 2) and 36 fossil leaf samples (SI Table 3) were studied for their epidermal cell properties. The data shown in SI Tables 1–3 represent means with standard deviation per herbaceous leaf or fossil leaf remains, respectively. The species included are the extant taxa L. abachisca, O. hradekensis, and G. biloba, as well as one extinct species, L. pseudoprinceps. To convert the SI values from the extant species L. abachisca and O. hradekensis to atmospheric CO₂ levels, CO₂ inference models were established from their living equivalents, L. nobilis and O. foetens (Figs. 1 and 2), from historical herbarian sheets collected over the 19th and 20th centuries, covering the CO₂ increase since the industrial revolution. Historical atmospheric CO₂ concentrations used for calibration are annual means as measured on Mauna Loa, HI, since 1952 (47), supplemented by CO₂ measurements from Antarctic ice cores (Siple Station) (48). The taxonomical relationships have been established by extensive comparative studies on leaf morphology and cuticle anatomy (49–51). CO₂ estimates from G. biloba were based on calibration data by Royer et al. (26). In the absence of a modern equivalent for the extinct species L. pseudoprinceps, the SI response of this species has been cross-calibrated by using Miocene CO₂ levels inferred from the three extant species.

The SI calibration of L. nobilis to atmospheric CO₂ concentrations results in a linear relationship, with

\[ SI = -0.059 \times \text{CO}_2 + 35.876 \]  

and a coefficient of determination (R²) of 0.56.

The SI calibration of O. foetens to atmospheric CO₂ concentrations results in a linear relationship, with

\[ SI = -0.0552 \times \text{CO}_2 + 30.245 \]  

and an R² of 0.5.

To account for the nonlinear response of SI to changing CO₂ concentrations, both herbarium SI data and the historical CO₂ concentrations are log-transformed before fitting a linear response curve through the datasets. For L. nobilis, this results in a relationship of

\[ \text{CO}_2 = 10^{3.173 - 0.5499 \times \log(SI_{\text{fossil}})} \]  

with an R² of 0.78 between measured and inferred CO₂ values and a root mean square error (RMSE) of 13.5 ppm CO₂.

For O. foetens, the regression curve (Fig. 2) and statistics are

\[ \text{CO}_2 = 10^{2.9567 - 0.4284 \times \log(SI_{\text{fossil}})} \]  

with an R² of 0.5 between measured and inferred CO₂ values and an RMSE of 16 ppm CO₂.

In the absence of a modern equivalent for the extinct species L. pseudoprinceps, the SI response of this species has been cross-calibrated by using Miocene CO₂ levels inferred from the three extant species. This results in a relationship of

\[ \text{CO}_2 = -46.011 \times SI_{\text{fossil}} + 993.37 \]  

with an R² of 0.68.

The multiple-species SI record based on three extant species allows that CO₂ values are independently inferred from the individual species-specific CO₂ inference models and verified for interspecific coherence.

O. foetens is an endemic tree from Madeira and the Canary Islands. It occurs over a wide range of altitudes in valleys and hills of the interior of the islands and is the highest tree of the laurel forest (Larissilva). Because the historical herbarium leaves were collected at different altitudes from approximately sea level up to 1,000 m, the stomatal frequency response is expressed against the
To calculate CO₂ estimates in mixing ratios (ppmv), the altitude-corrected CO₂ partial pressure values of the O. foetens training set were reconverted into mixing ratios at sea level with

\[ C_{CO_2} = \left( \frac{P_{CO_2, sea\ level}}{101,325} \right) \times P_{CO_2, sea\ level} \]

where \( C_{CO_2} \) is the mixing ratio in ppmv, and \( P_{CO_2, sea\ level} \) is the partial pressure at sea level.

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