



Increased interglacial atmospheric CO₂ levels followed the mid-Pleistocene Transition

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Atmospheric CO₂ and polar ice volume have been strongly coupled over the past 805,000 years. However, the prior extent of coupling, during times of lower-amplitude ice-volume variability, is unknown because continuous high-resolution CO₂ records are lacking. We reconstructed the past 1,460,000 years of atmospheric CO₂ (~1,700 year sample resolution) by taking advantage of the unique relationship between CO₂ concentration and leaf-wax $\delta^{13}\text{C}$ resulting from changes in the extent of C₃ and C₄ vegetation in East India. Notably, reconstructed interglacial CO₂ concentrations were lower before the transition to large volume variability during the mid-Pleistocene Transition (900,000 years ago). Prior to the mid-Pleistocene Transition, CO₂ exhibited a secular trend similar to that of deep-ocean carbon isotopes. At orbital time scales, phase analysis indicates that the CO₂ lead relative to ice volume changed to a lag during the mid-Pleistocene Transition. Combined, these findings suggest that deep-ocean circulation controlled the long-term CO₂ trend, and that interaction between CO₂, continental ice and deep-ocean circulation was reorganized during the mid-Pleistocene Transition, involving a decrease in carbon storage in the deep Pacific.

Earth's atmospheric carbon dioxide (CO₂) concentration is now >415 ppm, far exceeding the highest interglacial values (299 ppm) during the past 805,000 years (805 kyr) as documented in Antarctic ice cores^{1–4} (Fig. 1a). Before the past 805 kyr, marine sediment foraminifera $\delta^{11}\text{B}$, alkenone $\delta^{13}\text{C}$, terrestrial pedogenic carbonate $\delta^{13}\text{C}$ and C₃ plant $\delta^{13}\text{C}$ proxies have been used to reconstruct CO₂ (refs. 5–12). However, they do not yield long continuous records sufficient to resolve orbital-scale cycles, except for some specific periods^{6,7,12}. Blue ice yielded spot data at ~1 million years ago (Ma)¹³, 1.5 Ma and 2 Ma (ref. 14). Reported values range from 214 to 279 ppm. Compared with blue-ice measurements, other proxies indicate far wider ranges over the same interval of time^{5–11}. There are disagreements among existing proxies, highlighting the need for more efforts for CO₂ reconstruction.

From 1,200 to 700 thousand years ago (ka), the dominant 41 kyr cycles of benthic marine carbonate $\delta^{18}\text{O}$ transitioned to dominant quasi-100 kyr cycles, accompanied by increased amplitude and a sawtooth pattern (rapid deglaciations followed by slower glacial build-up). This transition is called the mid-Pleistocene Transition (MPT)¹⁵. Numerous hypotheses have been proposed to explain the cause of the MPT, such as a gradual lowering of CO₂ (ref. 16), an increase in ice-sheet stability^{17–19}, their combination^{7,20}, an abrupt decrease in CO₂ around 900 ka (refs. 6,21), phase locking of bipolar ice sheets²², changes in self-sustained oscillation of global carbon cycles²³ and extremely low Southern Hemisphere summer insolation at marine isotope stage (MIS) 23 as the trigger²⁴. The extended CO₂ reconstruction presented here is highly relevant to this discussion.

In this study, we analyse the $\delta^{13}\text{C}$ of leaf-wax long-chain *n*-fatty acids ($\delta^{13}\text{C}_{\text{FA}}$) in sediments from International Ocean Discovery Program (IODP) site U1446 (19.08°N, 85.73°E) on the Indian margin, Bay of Bengal (Extended Data Fig. 1). We generate a record of CO₂ spanning the last 1.46 million years (Myr), with ~1.7 kyr sample resolution. We discuss the use of $\delta^{13}\text{C}_{\text{FA}}$ as a CO₂ proxy (CO₂^{FA}), compare our results with those of other independent

reconstructions and discuss changes in the relationship among CO₂, continental ice and deep-ocean circulation over the MPT.

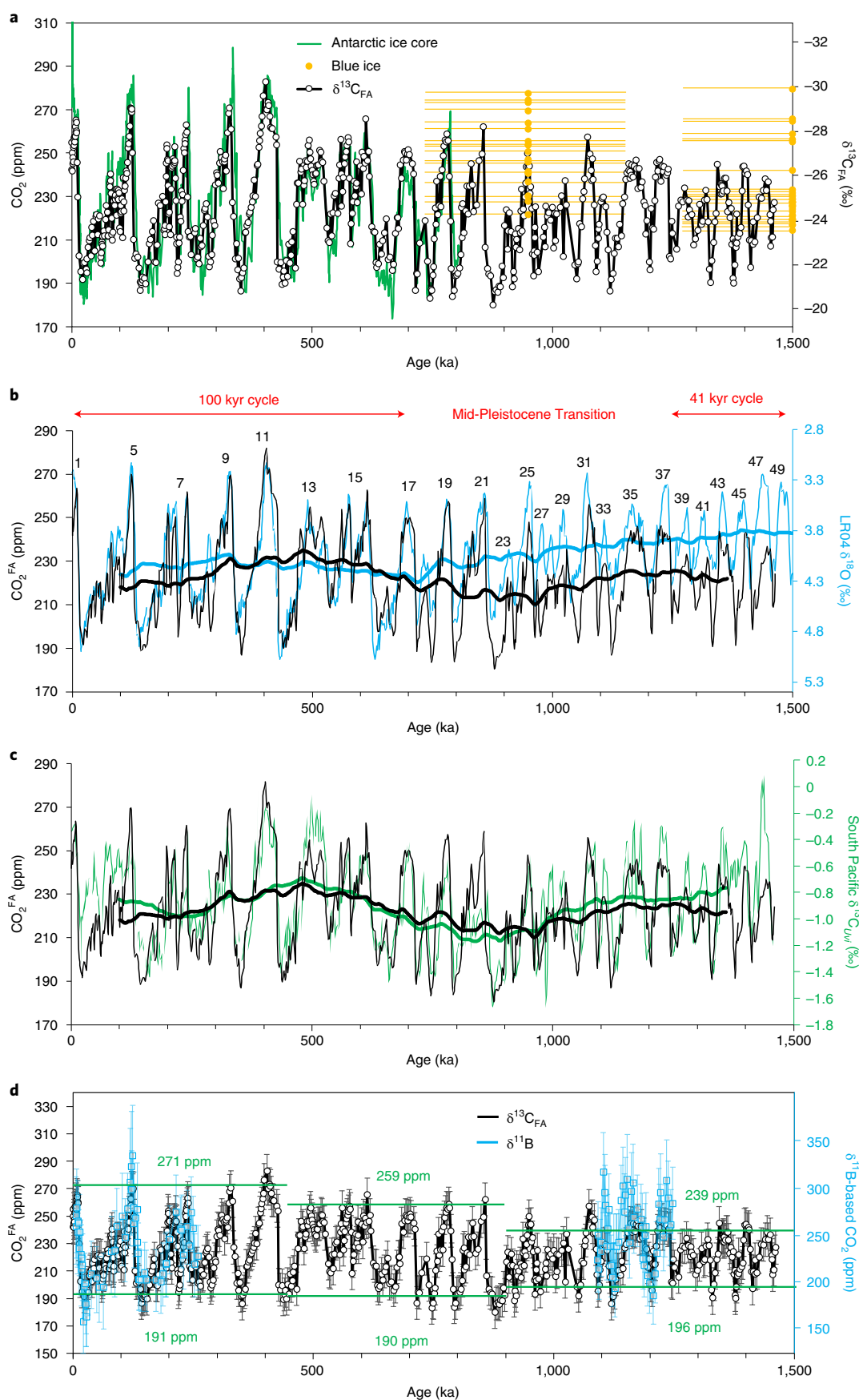
Carbon isotopic ratio of long-chain *n*-fatty acids

The average $\delta^{13}\text{C}$ value of *n*-C₂₆, C₂₈, C₃₀ and C₃₂ fatty acids ($\delta^{13}\text{C}_{\text{FA}}$), leaf wax derived from terrestrial higher plants, ranges from −30.3‰ to −20.3‰ Vienna Pee Dee Belemnite (Fig. 1a; data are partly presented²⁵). The $\delta^{13}\text{C}_{\text{FA}}$ is higher during glacial and lower during interglacials (Fig. 1b). The variation is positively correlated with that of benthic foraminifera $\delta^{18}\text{O}$, a proxy of global ice volume and bottom-water temperature ($r=0.76$ for 0–1,460 ka). Of particular interest, the variation of $\delta^{13}\text{C}_{\text{FA}}$ mirrors that of the Antarctic ice-core CO₂ concentration over the past 805 kyr (Fig. 1a). The $\delta^{13}\text{C}_{\text{FA}}$ in site U1446 sediments ranges between the end-members of C₃ and C₄ plants (Fig. 2a and Methods), indicating that it predominantly reflects the relative contributions of C₃ and C₄ plants from the sediment source area, that is, the Mahanadi River and adjacent coastal regions (Methods and Extended Data Fig. 1).

Effects of CO₂, temperature and precipitation on $\delta^{13}\text{C}_{\text{FA}}$

The proportion of C₃ and C₄ vegetation is affected by three factors: CO₂ concentration, water availability and air temperature²⁶. C₃ plants fix carbon from CO₂ by photosynthesis using ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) in mesophyll cells. At the same time, the RuBisCO fixes oxygen and loses carbon through photorespiration. In low-CO₂ conditions, photosynthesis of C₃ plants is suppressed relative to photorespiration. C₄ plants convert CO₂ to C₄ diacids in mesophyll cells, transfer it to bundle-sheath cells and fix reconverted CO₂ using RuBisCO. Because the oxygen content in bundle-sheath cells is low, RuBisCO can fix carbon efficiently. Thus, C₄ plants have an advantage over C₃ plants in low-CO₂ environments²⁷. Meta-analysis studies of many open-air CO₂ enrichment experiments for native plants indicate that CO₂ enrichment to 550–600 ppm enhances the production rate

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of C_3 plants relative to C_4 plants by $\sim 25\%$ (ref. ²⁸). To further test these inferences, a series of experiments were run using a vegetation model to assess the sensitivity of regional vegetation functional

types to changes in CO_2 (Fig. 3a), showing that changing CO_2 from 285 to 185 ppm increases the $\text{C}_4/(\text{C}_3 + \text{C}_4)$ vegetation coverage ratio by 0.15 in the sediment source area (Fig. 3b).

Fig. 1 | Reconstructed CO₂ concentration during the last 1.46 Myr. **a**, Reconstructed atmospheric CO₂ concentration (CO₂^{FA}) based on fatty acid δ¹³C (δ¹³C_{FA}) with a calibration error of 12 ppm at site U1446 during the last 1.46 Myr (black) with Antarctic ice-core CO₂ concentration (green)⁴ and CO₂ concentration in Allan Hills blue ice with age uncertainty ±213 kyr (orange)^{13,14}. **b**, The benthic foraminifera LR04 δ¹⁸O stack⁴⁰ with MIS. **c**, Benthic foraminifera *Uvigerina* δ¹³C at ODP site 1123 of the deep South Pacific²⁴. **d**, Average CO₂ concentrations of interglacials and glacials (green lines) and δ¹¹B-based CO₂ concentration (blue, with 2σ interval)⁷. Thick lines in **b** and **c** indicate the 200 kyr running means. The scale of the δ¹¹B-based CO₂ concentration in **d** is adjusted to the range of Antarctic ice-core CO₂ during the last two glacial cycles following the regression equation [Ice-core CO₂] = 0.678 × [δ¹¹B-based CO₂] + 67 (*r* = 0.87, *n* = 57).

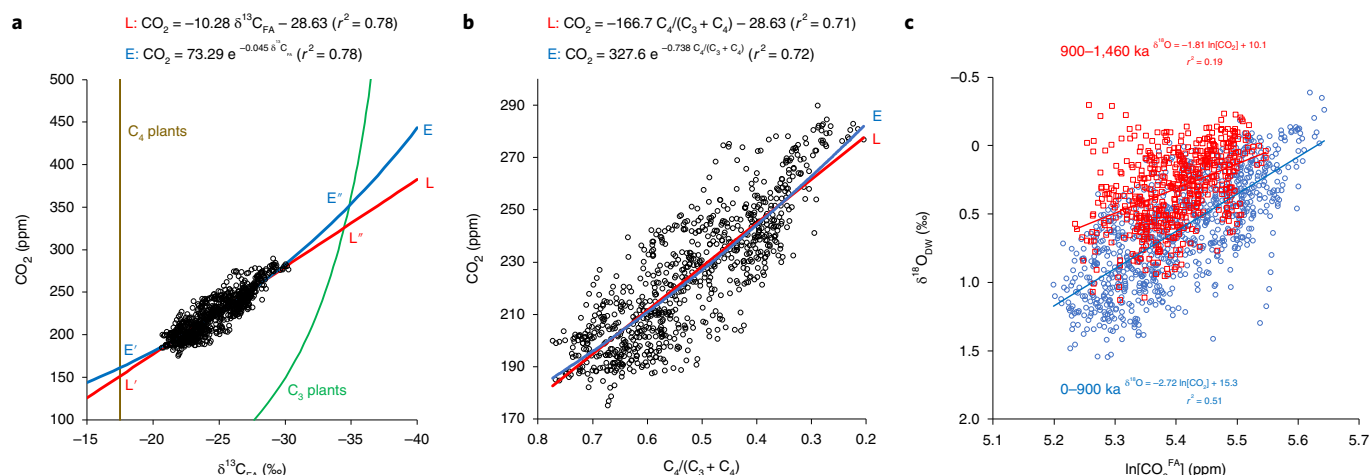


Fig. 2 | δ¹³C_{FA}, C₄/(C₃ + C₄) plant ratio, CO₂ concentration and ice volume. **a**, δ¹³C_{FA} (axis reversed) and the Antarctic ice-core CO₂ concentration⁴, and end-member values of δ¹³C_{FA} of C₃ and C₄ plants predicted based on modern δ¹³C values^{46,47}, the empirical relationship between the isotope fractionation of δ¹³C of C₃ plants and CO₂ concentration and the Suess effect (Methods). The Antarctic CO₂ record⁴ was tuned to the δ¹³C_{FA} record, as shown in Extended Data Fig. 6 (Methods). The letters L and E indicate the linear and exponential regression lines, respectively. The letters L' and L'' indicate the lower and upper ends (151 and 325 ppm), respectively, of the δ¹³C_{FA}–CO₂ calibration in the linear regression model. The letters E' and E'' indicate the lower and upper ends (161 and 357 ppm), respectively, in the exponential regression model. **b**, The C₄/(C₃ + C₄) plant ratio and the ice-core CO₂ concentration, **c**, ln[CO₂^{FA}] and the δ¹⁸O of deep water in the South Pacific (δ¹⁸O_{DW})²⁴ before (red dots) and after 900 ka (blue dots). δ¹⁸O_{DW} reflects the volume of the continental ice sheets²⁴.

The δ¹³C_{FA} variation during glacial–interglacial cycles reflected the physiological effects of CO₂ on the carbon isotope fractionation of C₃ plants (Fig. 2a, green curve)³⁹, in addition to the relative abundance of C₃ and C₄ vegetations. A CO₂ reduction from 280 to 190 ppm induced a change in isotopic fractionation in C₃ plants that resulted in an increase of 2.2‰ in δ¹³C_{FA}. This implies that about one-fourth of the δ¹³C_{FA} variation can be attributed to the CO₂ effect on C₃ plant δ¹³C_{FA}. The C₄/(C₃ + C₄) plant ratio and the ice-core CO₂ concentration showed good correlation (*r*² = 0.71; Fig. 2b), but this correlation was slightly weaker than the correlation between δ¹³C_{FA} and the ice-core CO₂ concentration (*r*² = 0.78; Fig. 2a). This suggests that the CO₂ effect on C₃ plant δ¹³C_{FA} enhances the correlation between δ¹³C_{FA} and ice-core CO₂, although by only a small amount.

Precipitation is the second factor determining C₃/C₄ vegetation, with C₄ plants having an advantage in drier conditions²⁶. δ¹³C_{FA} has a different structure compared with precipitation reconstructions based on regional river runoff into the Bay of Bengal (seawater δ¹⁸O_{sw})²⁵ from the study site, and other Indian summer monsoon records (Extended Data Fig. 2). The δ¹⁸O_{sw} variation shares 12% of the total δ¹³C_{FA} variation deduced from *r*² = 0.12 in Extended Data Fig. 3. This relevance is minor compared with that of the CO₂ concentration (78% of total δ¹³C_{FA} variation; Extended Data Fig. 3). The results of nine different general circulation models (GCMs) (Extended Data Fig. 4) show that precipitation decreased by 9 ± 23% from interglacial to glacial conditions (Fig. 3d)^{30–32}. The dynamic vegetation model shows that the 9% precipitation decrease at the 285 ppm CO₂ level increases the C₄/(C₃ + C₄) vegetation coverage ratio by 0.02 in the sediment source area (Fig. 3b). This result demonstrates that the impact of precipitation on the C₃/C₄ vegetation

structure (0.02) is much smaller than that of the atmospheric CO₂ concentration (0.15) in the sediment source region over glacial–interglacial time scales.

Temperature is the third factor determining C₃/C₄ vegetation, with C₄ plants having an advantage in warmer conditions²⁶. However, the data from site U1446 indicate that C₄ plants were more abundant (δ¹³C_{FA} was higher) when the sea surface temperature (SST) decreased by ~3 °C during glacials (Extended Data Figs. 2 and 4). This is inconsistent with the physiology of C₄ plants as advantaged in warmer conditions. Higher CO₂ increases C₃ plants relative to C₄ plants according to the difference in physiological responses, but it also decreases C₃ plants relative to C₄ plants under warmer conditions induced by the greenhouse effect. Because these two factors shift δ¹³C_{FA} in the opposite direction, the temperature effect works against the shift in δ¹³C_{FA} caused by CO₂ change. The negative correlation between δ¹³C_{FA} and the European Project for Ice Coring in Antarctica (EPICA) CO₂ concentration (Fig. 2a) indicates that the effect of CO₂ concentration on C₃/C₄ vegetation is much stronger than the effect of temperature in the sediment source region.

These considerations, and the strong correspondence of δ¹³C_{FA} with the ice-core CO₂ concentration, indicate that CO₂ is the predominant factor determining δ¹³C_{FA} in the sediment source area and, hence, in U1446 sediments. Our dynamic vegetation model demonstrates that the vegetation in the sediment source area (within the range of mean annual temperatures >18 °C and annual precipitation of 1,000–1,500 mm) changed from predominantly C₄ during the last glacial maximum (LGM) to C₃ in the preindustrial (PI) period (Fig. 3c). A few other regions exhibit this same response

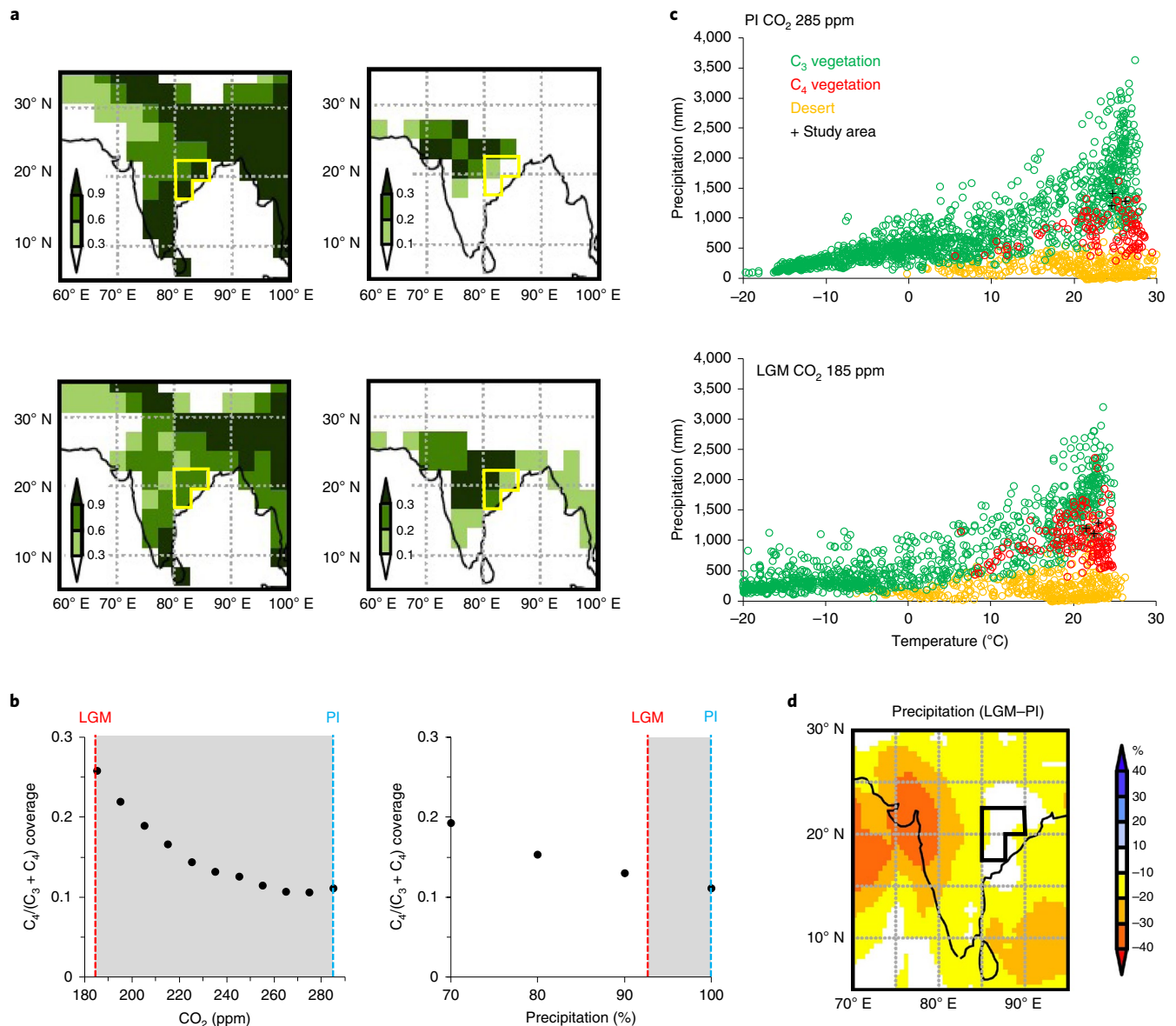


Fig. 3 | Dynamic vegetation model results. a, Fractional coverages of C₃ (left) and C₄ (right) vegetation at PI temperature and precipitation conditions for CO₂ concentration of 185 ppm (lower) and 285 ppm (upper). **b**, Changes in the coverage of C₄ and C₃ plants in the sediment source area (grids with yellow border in **a**) as the CO₂ concentration decreases from 285 to 185 ppm under PI precipitation and temperature^{30–32}, and as precipitation decreases from 100% (PI condition) to 70% under PI CO₂ and temperature^{30–32}. **c**, World C₃/C₄ vegetation under PI and LGM conditions^{30–32} estimated with the LPJ-DGVM⁴⁸. **d**, Difference in annual mean precipitation between LGM and PI conditions (LGM – PI), estimated with nine different general circulation models (Extended Data Fig. 4)^{30–32}.

(Extended Data Fig. 5), characterized by hot and seasonally dry (savannah) climates, and could serve as targets for replicating our CO₂ reconstruction.

$\delta^{13}\text{C}_{\text{FA}}$ as a proxy of CO₂

The $\delta^{13}\text{C}_{\text{FA}}$ in site U1446 sediments shows a variation that is nearly identical to that of Antarctic ice-core CO₂ (Fig. 1a). However, age model differences between the marine and ice-core records are somewhat relevant to this comparison. In this study, the linear regression equation after the tuning is used to convert $\delta^{13}\text{C}_{\text{FA}}$ to CO₂ concentration (Methods and Extended Data Figs. 6 and 7). The calibration error (root-mean-square error) of $\delta^{13}\text{C}_{\text{FA}}$ -based CO₂ reconstruction (CO₂^{FA}) is 12 ppm, including an analytical error of 1 ppm. The upper limit of the CO₂^{FA} estimation is 325 ppm as C₃ plants

attain 100%, while the lower limit is 151 ppm as C₃ plants attain 0% (Fig. 2a). The calibration stability during the last 800 kyr showed that it remained essentially unchanged and within the range of the above calibration error (Extended Data Fig. 8). The calibration is likely to be site specific and may not be directly applicable to other sites with different climate and environmental settings (Extended Data Fig. 5).

We should keep in mind that precipitation and temperature potentially affect $\delta^{13}\text{C}_{\text{FA}}$ if the background condition changes substantially. Proxy records of temperature and precipitation from the study site show that the ranges of variations are not different before and after the MPT (Extended Data Fig. 2). The mineral, major, trace and rare-earth element compositions at National Gas Hydrate Program (NGHP) sites 18A and 19A near the study site were almost

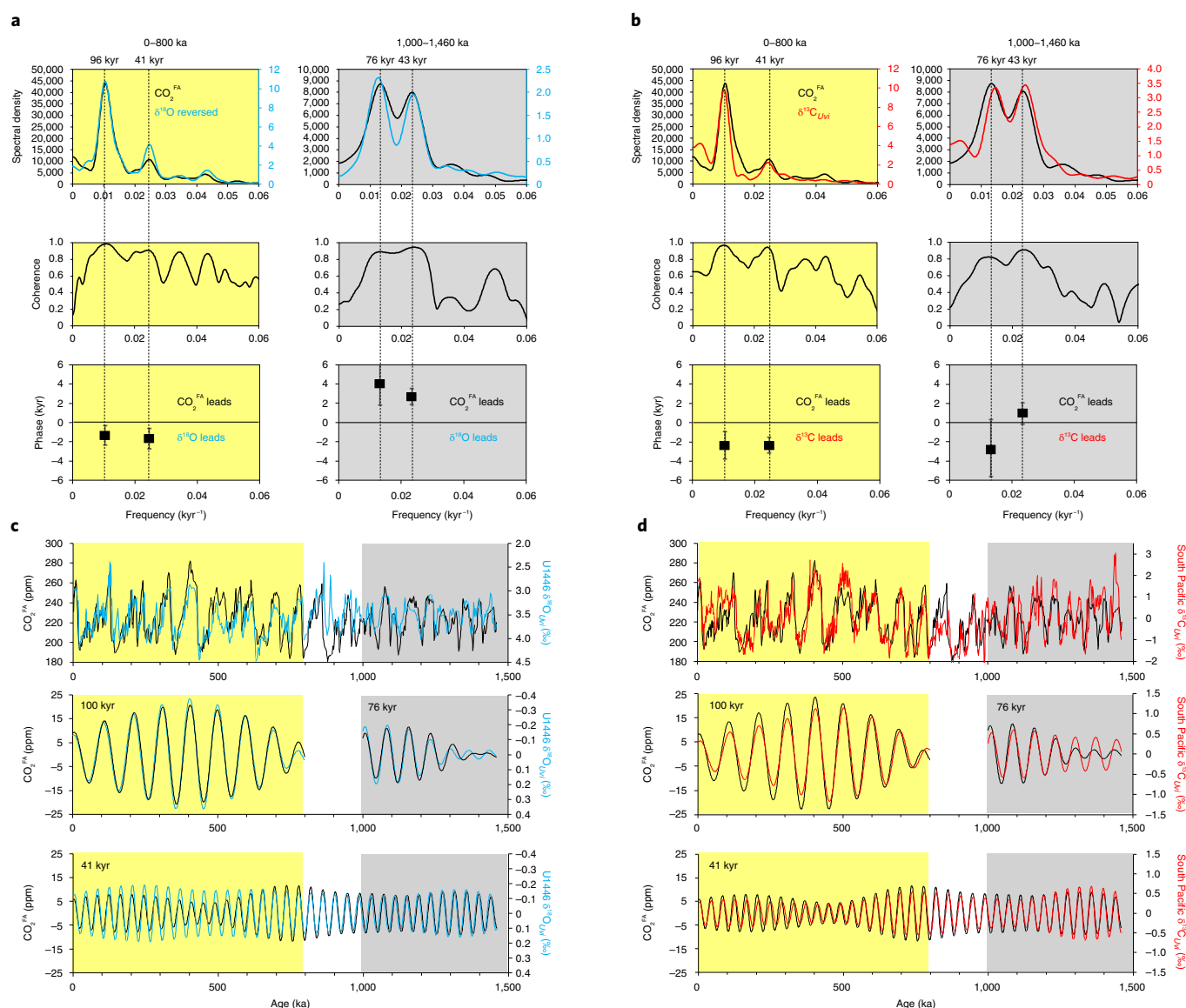


Fig. 4 | Phase lead and lag of CO_2^{FA} , ice volume and deep-ocean circulation. **a,b**, Power spectra, coherence and phase differences with 80% confidence level (vertical bars) of the variation in CO_2^{FA} , benthic foraminifera *Uvigerina* $\delta^{18}\text{O}$ at the study site (U1446 $\delta^{18}\text{O}$ reversed, reflecting global ice volume and bottom-water temperature; **a**)²⁵ and *Uvigerina* $\delta^{13}\text{C}$ at ODP site 1123 in the deep South Pacific (South Pacific $\delta^{13}\text{C}_{\text{Uwi}}$ reflecting deep-ocean circulation; **b**)²⁴. **c,d**, Variation in CO_2^{FA} , U1446 $\delta^{18}\text{O}$ (axis reversed; **c**), South Pacific $\delta^{13}\text{C}_{\text{Uwi}}$ (**d**) and their 100, 76, and 41 kyr filtered components before and after the MPT (1,460 to 1,000 ka and 800 to 0 ka, respectively). Cross-spectral analysis was performed with the AnalySeries package⁴⁹.

constant in Miocene to Pleistocene sediments, suggesting no substantial change in sediment provenance^{33,34}. These do not ideally guarantee but encourage us to apply the $\delta^{13}\text{C}_{\text{FA}}$ to CO_2 reconstruction prior to the MPT.

A comparison with blue-ice values show that the $\delta^{13}\text{C}_{\text{FA}}$ -based CO_2^{FA} around 1 Ma (~180 to ~262 ppm) overlap 18 of 23 values for ~1 Ma blue ice (222–277 ppm)¹³, and the average value is 31 ppm lower than that of blue ice (Extended Data Fig. 9). The CO_2^{FA} values around 1.5 Ma (~190 to ~250 ppm) overlap 27 of the 33 values for ~1.5 Ma blue ice (214–279 ppm)¹⁴, and the average value is 10 ppm lower than that of blue ice. Foraminifera $\delta^{11}\text{B}$ -based CO_2 values show a wider range than that of ice-core measurements⁸; the lower values overlap blue-ice values and CO_2^{FA} (Extended Data Figs. 9 and 10). Despite the difference in their absolute concentrations, the *Globigerinoides ruber* $\delta^{11}\text{B}$ -based CO_2 variation is consistent with the CO_2^{FA} variation in the pre- and post-MPT

periods (Fig. 1d). This correspondence suggests that both the $\delta^{11}\text{B}$ and $\delta^{13}\text{C}_{\text{FA}}$ proxies reflect the atmospheric CO_2 concentration, but the absolute concentrations remain a calibration issue for converting $\delta^{11}\text{B}$ into the CO_2 concentration³⁵. Alkenone $\delta^{13}\text{C}$ analysis gave higher CO_2 concentrations than blue ice⁹, which could be explained by a calibration issue in the physiological parameter³⁶. The pedogenic carbonate $\delta^{13}\text{C}$ and C_3 plant $\delta^{13}\text{C}$ reconstructions showed wider CO_2 values than that of blue ice (Extended Data Figs. 9 and 10)^{10,11}.

The CO_2^{FA} varied between 180 and 283 (± 12) ppm during the last 900 ka. The highest CO_2^{FA} concentrations appear in MIS 5, 9 and 11, as is also the case for the PI ice-core CO_2 data³⁴. The lowest CO_2^{FA} concentration appears in MIS 22 (Fig. 1b). Prior to the MPT, CO_2^{FA} varied between 188 and 257 ppm, averaging 5 ppm lower than post-MPT values (Fig. 1b). The interglacial values in the pre-MPT period were 20 ppm lower than those of the lukewarm interglacial

periods (~900 to 450 ka) and 32 ppm lower than those of the last five strong interglacials (~450 ka to present) (Fig. 1d).

The CO_2^{FA} trend is consistent with the SST variations in the western Pacific warm pool (WPWP) region across the MPT (Extended Data Fig. 2d). The SSTs in the WPWP are assumed to directly reflect the greenhouse effect because of the remote location from the ice sheets²³. On the other hand, the glacial CO_2^{FA} trend differs from the glacial cooling SST trends in regions other than the WPWP³⁷. In those regions, the glacial SSTs are assumed to be influenced by additional factors, such as ice-driven cooling, albedo feedback and upwelling. The CO_2^{FA} record seems to be beneficial to understand the greenhouse effect on the climate before the MPT.

It is thus important to test the robustness of our reconstruction. This could be done using future blue-ice drilling, vegetation modelling studies, intercomparison with other CO_2 proxies and $\delta^{13}\text{C}_{\text{FA}}$ -based reconstructions at other sites (Extended Data Fig. 5).

CO_2 variability through the MPT

The variation in CO_2^{FA} shows 96 kyr (eccentricity), 41 kyr (obliquity), 23 kyr and 19 kyr (precession) cycles during 800 to 0 ka, while it shows 76 and 43 kyr cycles during 1,460 to 1,000 ka (Fig. 4a,b). The variation in CO_2^{FA} is highly coherent at all significant periodicities with variations in benthic foraminifera Uvigerina $\delta^{18}\text{O}$ at the study site (U1446 $\delta^{18}\text{O}$ reversed)²⁵ as well as Uvigerina $\delta^{13}\text{C}$ at ODP site 1123 in the deep (3,300 m) South Pacific (South Pacific $\delta^{13}\text{C}$)²⁴ (Fig. 4a,b). They show similar amplitude variations with CO_2 in each cycle (Fig. 4c,d). These striking similarities indicate that CO_2 , ice volume and deep-ocean circulation are tightly coupled on orbital time scales.

Time-series analysis shows a small but robust change in the relative phase of CO_2^{FA} and benthic $\delta^{18}\text{O}$ variations over the MPT. On a 41 kyr cycle, the CO_2^{FA} preceded benthic $\delta^{18}\text{O}$ by 2.7 ± 0.9 kyr during 1,460 to 1,000 ka, whereas CO_2^{FA} was delayed by 1.7 ± 1.1 kyr during 800 to 0 ka (Fig. 4a). On the other hand, CO_2^{FA} was synchronous with South Pacific $\delta^{13}\text{C}$ during 1,460 to 1,000 ka, whereas it was delayed by 2.3 ± 0.8 kyr during 800 to 0 ka on a 41 kyr cycle (Fig. 4b). Thus, the shifts of the relative phase of CO_2^{FA} relative to benthic $\delta^{18}\text{O}$ and South Pacific $\delta^{13}\text{C}$ (4.4 and 3.3 kyr, respectively) between 1,000 and 800 ka are statistically significant. These results imply that the interaction between CO_2 , continental ice and deep-ocean circulation changed across the MPT. Because the long-chain *n*-fatty acids were stored in continental reservoirs (for example, for ~1 kyr (ref. ³⁸) and ~2.7 kyr (ref. ³⁹) in the Ganges–Brahmaputra River basin), the CO_2^{FA} variation is expected to be delayed by the contribution of older components (Methods). However, even taking this effect into account, the phase shift during the MPT described above is significant.

The long-term mean trends of the CO_2^{FA} and $\delta^{18}\text{O}$ records differ (Fig. 1b). The mean $\delta^{18}\text{O}$ shows a long-term drift in a positive direction until 700 ka (ref. ⁴⁰). This long-term shift is not seen in CO_2^{FA} (Fig. 1b). The offset suggests that reduced continental ice volume coexisted with lower CO_2 levels before the MPT compared with afterwards.

In contrast to foraminifera $\delta^{18}\text{O}$, South Pacific $\delta^{13}\text{C}$ (ref. ²⁴) shows a high correlation with CO_2^{FA} on their 200 kyr running means ($r=0.80$; Fig. 1c). The South Pacific $\delta^{13}\text{C}$ is interpreted to reflect changes in deep-ocean circulation coupled with changes in the Southern Ocean deep-water ventilation and marine carbon reservoir²⁴. Thus, the long-term covariation of CO_2^{FA} and South Pacific $\delta^{13}\text{C}$ suggests that the deep-ocean circulation changes were a major factor determining the long-term trend of the atmospheric CO_2 concentration via changes in ocean carbon storage through the MPT^{21,41–43}.

The mean values of CO_2^{FA} and South Pacific $\delta^{13}\text{C}$ (ref. ²⁴) decreased by 15 ppm and 0.3‰ on average, respectively, from 1,150 to 950 ka during the MPT (Fig. 1c). The decrease in CO_2 may

have driven the expansion of continental ice sheets by decreasing the greenhouse effect. However, from 950 to 700 ka, the ice sheets continued to expand, despite the increase in mean CO_2 (Fig. 1b). This peculiar change reflected a changing response of ice sheets to CO_2 forcing throughout 950 to 700 ka, as shown by the two distinct regression trends between CO_2^{FA} and the deep-water (3,300 m) $\delta^{18}\text{O}$ in the South Pacific (a proxy of continental ice volume)²⁴ before and after 900 ka (Fig. 2c). As described above, at a 41,000 year cycle, the CO_2 change precedes the benthic $\delta^{18}\text{O}$ change before the MPT, but the benthic $\delta^{18}\text{O}$ change slightly precedes CO_2 after 800 ka (Fig. 4a). These results suggest that the CO_2 –ice interaction was reorganized during the MPT. This reorganization co-occurred with the beginning of large ice-sheet development under relatively high CO_2 concentrations. The expansion of the North American ice sheet could have triggered the onset of the glacial 100 kyr cycle⁴⁴.

This study raises the new key question of which mechanism caused this reorganization. The close relationship between CO_2^{FA} and South Pacific $\delta^{13}\text{C}$ variability in both the long-term trend (Fig. 1c) and glacial–interglacial cycles (Fig. 4b,d) indicates that the process involved deep-ocean circulation. The Atlantic Meridional Overturning Circulation was consistently active during both glacials and interglacials before 900 ka (refs. ^{21,43}) while it was damped during glacials after 900 ka. In response to this change, carbonate preservation, with maxima at deglaciations and minima at glaciations, increased in the deep Pacific across the MPT⁴⁵. If the more severe carbonate dissolution before the MPT simply means that the abyssal Pacific or the Pacific sector of the Southern Ocean sequestered more carbon, the carbon storage in those areas would constitute key information for understanding the low CO_2 concentrations in the pre-MPT period. Elucidating this mechanism is critical for understanding past and future changes in the interactions between CO_2 , continental ice and deep-ocean circulation.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41561-022-00918-1>.

Received: 23 February 2021; Accepted: 25 February 2022;

Published online: 31 March 2022

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Methods

Samples. IODP site U1446 is located near the mouth of the Mahanadi River at 19.08°N, 85.73°E and 1,425 m water depth on the slope of the Indian margin of the Bay of Bengal (Extended Data Fig. 1). Sediments were drilled to 180 m below the seafloor and consist of calcareous microfossil-bearing clay and silt⁵⁰. Samples were taken every 30 cm (1,500–2,000 years) throughout and cover the last 1.46 Myr. The sediments at site U1446 were derived mainly from the Mahanadi River and the adjacent coastal rivers (Extended Data Fig. 1)^{33,34,51}, although the potential contribution of the Ganges–Brahmaputra River cannot be ignored³⁵. The mineral, major, trace and rare/earth element compositions at sites NGHP 18A and 19A were almost constant in Miocene to Pleistocene sediments, suggesting no significant change in sediment provenance^{34,51}. An age model in calendar years was created by oxygen isotope stratigraphy⁴⁰ of benthic foraminifera *Uvigerina* spp. (Supplementary Table 1)²⁵.

The modern vegetation of the Mahanadi and Godavari River basins consists mainly of tropical thorny vegetation, with tropical dry deciduous forest and tropical moist deciduous forest (a map by Gaussen et al. is shown in refs. ^{52,53}). A pollen assemblage in Holocene sediments at site NGHP-01-16, located ~400 km southwest of the study site, near the Godavari River mouth, demonstrates that Poaceae and Cyperaceae pollen account for 50% of the total pollen in the Holocene sediments. The content of Poaceae and Cyperaceae pollen reached 71% in Heinrich Stadial 2 (~26 ka) when the CO₂ concentration was ~200 ppm, which was ~80 ppm lower than the PI level. Because 62% of Poaceae and 16% of Cyperaceae species are C₃ plants⁵⁴, the increases in Poaceae and Cyperaceae in the glacial period are consistent with the expansion of C₄ plants in the sediment source area shown by the positive shift of δ¹³C_{FA} at site U1446 during glacial periods.

Fatty acids and their δ¹³C. Lipids were extracted (×3) from ca. 3 g of dried sediment using a DIONEX ASE-200 accelerated solvent extractor with dichloromethane–methanol (6:4). The extract was separated into neutral and acid fractions by aminopropyl silica gel column chromatography⁵⁵. The acid fraction was methylated by using methanol–acetyl chloride (95:5) and purified by SiO₂ column chromatography. The δ¹³C (‰ Vienna Pee Dee Belemnite) of the methylated *n*-fatty acid was analysed using an Agilent 6890 series gas chromatograph combined with a Finnigan MAT DELTAplus mass spectrometer. The δ¹³C values of fatty acids were obtained from the measured values of fatty acid–methyl esters by correcting methyl carbon (−34.1‰). The reproducibility of the measurement based on repeated analyses was better than ±0.1‰. δ¹³C_{FA} was calculated by averaging the δ¹³C of C₂₆, C₂₈, C₃₀ and C₃₂ *n*-fatty acids. The δ¹³C values of *n*-C₂₆, C₂₈, C₃₀ and C₃₂ fatty acids are within the range of 2‰ in most samples (Supplementary Table 2). The data of samples showing a standard deviation larger than 2‰ were removed from the dataset.

All samples from site U1446 show a bimodal homologous distribution of *n*-fatty acids with maxima at C₁₆/C₁₈ and C₂₆. The carbon number preference (4.4 ± 0.5) and averaged chain length (28.2 ± 0.3 in C₂₆–C₃₂) are nearly constant, indicating that the long-chain *n*-fatty acids are leaf wax derived from terrestrial higher plants.

Compound-specific radiocarbon analysis of surface sediments from the Santa Monica Basin indicated that the Δ¹⁴C values of long-chain *n*-fatty acids are the same as those of phytoplankton biomarkers⁵⁶. By contrast, the Δ¹⁴C values of long-chain *n*-alkanes are more depleted than those of phytoplankton biomarkers. This indicates that *n*-fatty acids are better for monitoring palaeovegetation changes than are *n*-alkanes because of their shorter continental retention time.

In the Bay of Bengal, ref. ³⁸ estimated the continental reservoir age of long-chain *n*-fatty acids based on the depth profile of the radiocarbon concentration in the Bengal shelf canyon. A two-end-member model indicated that 79–83% of the long-chain *n*-fatty acids were stored in continental reservoirs for an average of 1.0–1.2 kyr (slow-cycling component), and the remainder was stored for an average of 15 years (fast-cycling component). In the deep Bay of Bengal sediments, the age offset of long-chain *n*-fatty acids was estimated at 2.7 kyr on average³⁹. However, the millennial-scale retention time of the slow-cycling component does not markedly delay the timing of multi-millennial-scale δ¹³C_{FA} events because it represents a broad distribution of *n*-fatty acids with different ages that tend to smear each other out^{38,39}.

End-member δ¹³C_{FA} values of C₃ and C₄ plants. The δ¹³C_{FA} of the C₃ and C₄ plants sampled in the early 2000s (at CO₂ of ~370 ppm) average −37.1 ± 2.0‰ (*n* = 13) and −19.5 ± 1.8‰ (*n* = 9), respectively^{46,47}. However, these end-member values have varied in the past. Contemporary δ¹³C_{FA} values for both C₃ and C₄ plants are affected by the ¹³C Suess effect (decreasing by about 2‰ over the past 250 years)³⁷. The δ¹³C_{FA} values of C₃ plants have been diminished further by the increasing isotopic fractionation governed by the atmospheric CO₂ concentration²⁹. The isotopic fractionation is defined as

$$\Delta^{13}\text{C} = \left(\delta^{13}\text{C}_{\text{CO}_2} - \delta^{13}\text{C} \right) / \left(1 + \delta^{13}\text{C}/1,000 \right),$$

where δ¹³C_{CO₂} is the δ¹³C of the atmospheric CO₂ and δ¹³C is the δ¹³C of the bulk plant. The empirical relationship between the isotope fractionation of δ¹³C of

C₃ plants (Δ¹³C) and the CO₂ concentration [CO₂] was obtained from field and chamber experiments as²⁹

$$\begin{aligned} \Delta^{13}\text{C} &= (A \times B \times ([\text{CO}_2] + C)) / (A + B \times ([\text{CO}_2] + C)) \\ &= (6.22 \times ([\text{CO}_2] + 23.9)) / (28.26 + 0.22 \times ([\text{CO}_2] + 23.9)) \end{aligned}$$

where *A* = 28.26, *B* = 0.22 and *C* = 23.9 (ref. ²⁹). Thus, the Δ¹³C values at CO₂ concentrations of 370 and 280 ppm are 21.3 and 19.9‰, respectively (Supplementary Table 2). The difference is 1.4‰. As the δ¹³C_{FA} of C₃ plants at a CO₂ level of 370 ppm is −37.1‰ (ref. ⁴⁶) and the Suess effect is ~2‰ (ref. ³⁷), the δ¹³C_{FA} of C₃ plants (δ¹³C_{FA(C3)}) at CO₂ of 280 ppm before 1750 CE can be calculated as

$$\begin{aligned} \delta^{13}\text{C}_{\text{FA(C3)280 ppm}} &= \delta^{13}\text{C}_{370 \text{ ppm}} + \text{Suess effect} + \Delta^{13}\text{C}_{370 \text{ ppm}} - \Delta^{13}\text{C}_{280 \text{ ppm}} \\ &= -37.1 + 2.0 + 1.4 = -33.7 \end{aligned}$$

Similarly, the δ¹³C_{FA(C3)} at a given CO₂ concentration (ppm) is calculated by the formula

$$\begin{aligned} \delta^{13}\text{C}_{\text{FA(C3)}} &= \delta^{13}\text{C}_{370 \text{ ppm}} + \text{Suess effect} + \Delta^{13}\text{C}_{370 \text{ ppm}} - \Delta^{13}\text{C} \\ &= -37.1 + 2.0 + 21.3 - (6.22 \times ([\text{CO}_2] + 23.9)) / (28.26 \\ &\quad + 0.22 \times ([\text{CO}_2] + 23.9)) \end{aligned}$$

On the other hand, the δ¹³C value for C₄ plants is independent of the atmospheric CO₂ concentration. Because the Suess effect is ~2‰, the δ¹³C_{FA} of C₄ plants (δ¹³C_{FA(C4)}) before 1750 CE can be obtained from the δ¹³C_{FA} of −19.5‰ of modern C₄ plants¹⁷ as

$$\delta^{13}\text{C}_{\text{FA(C4)}} = -19.5 + 2.0 = -17.5$$

The calculated δ¹³C_{FA} values for C₃ and C₄ plants are shown in Fig. 2a and included in Supplementary Table 3.

Over the last 30 kyr, the δ¹³C value of trapped atmospheric CO₂ in Antarctic ice cores has varied by 0.7‰ (refs. ^{58,59}). However, this variation before 30 ka is unknown and thus not included in further calculations.

Precipitation also affects the isotopic fractionation of C₃ plants⁶⁰, but its effect is negligible in the range of glacial and interglacial mean annual precipitations (9% difference) estimated in nine GCM simulations (Fig. 3d).

Tuning the Antarctic CO₂ record to the δ¹³C_{FA} record. The ages of gas in ice cores have uncertainty of more than ±5 kyr (ref. ⁶¹), comparable to uncertainties in the marine record⁴⁰. The U1446 δ¹³C_{FA} record plotted on the LR04 marine chronology lags behind the EPICA CO₂ record on the AICC2012 chronology⁶² by a maximum ~10 kyr (Extended Data Fig. 6a). Tuning the Antarctic EPICA CO₂ record to the δ¹³C_{FA} record improves the correlation between the CO₂ concentration and δ¹³C_{FA} (linear regression; *r*² = 0.63 before and 0.78 after tuning) (Extended Data Fig. 6). Cross-spectral analysis confirmed that tuning increased coherences and reduced the lag between CO₂ and δ¹³C_{FA} variations at all orbital periods (Extended Data Fig. 7). The regression lines are similar and would not change the resulting CO₂ reconstruction to any practical degree (within 5 ppm) regardless of which regression is used, with the exception that the calibration error (root-mean-square error) is improved with tuning. The exponential regression after tuning gives a correlation of determination (*r*² = 0.78) as high as that of the linear regression (Fig. 2a), and the estimated CO₂ concentrations are nearly identical (within 4 ppm).

Model description. We performed 16 sensitivity experiments, examining vegetation in CO₂ levels from 185 to 285 ppm in 10 ppm steps (285 ppm being the control experiment) and precipitation from 70% to 100% in 10% steps (100% being the control experiment). We used the Lund–Potsdam–Jena Dynamic Global Vegetation Model (LPJ-DGVM)¹⁸, which predicts the distributions of ten vegetation types (plant functional types) based on bioclimatic limits, photosynthesis, respiration, carbon allocation, plant establishment, growth, turnover, mortality and competition among the plant functional types with a spatial resolution of ~2.8°. Temperature, precipitation and cloud coverage in the control experiment are taken from the European Centre for Medium-Range Weather Forecasts re-analysis (ECMWF EAR-40)⁶² and Climate Prediction Center Merged Analysis of Precipitation⁶³ datasets for 1979–1999, and were performed 50 times to integrate 1,000 years. The results from the last 20 years are analysed. The mean temperature and precipitation in the LGM and PI conditions were calculated from those predicted with nine different GCMs^{30–32} to reconstruct C₃/C₄ vegetation in the LGM and PI periods. The combination of PFTs was translated into the Minimal Advanced Treatments of Surface Interaction and Runoff vegetation classification according to reported methods^{32,64}.

Spectral analysis. Spectral analysis quantifies, as a function of frequency, the lead/lag (phase) and linear coherence (calculated with the phase set to zero) relationships between two time series and has been applied routinely to Pleistocene

reconstructions over orbital time scales since early work^{65,66}. Here, cross-spectral analysis was performed using the Blackman–Tukey method in the *AnalySeries* package⁴⁹ with a 30% lag. The bandwidth was 0.0034, the non-zero coherence was >0.3842 and the error estimation on the power spectrum was 0.6256. All data were interpolated to 1 kyr prior to analysis. Band-pass filtering was calculated using a Gaussian filter with the *AnalySeries* package and a bandwidth of 0.002.

Data availability

The data are available in supplementary tables and at https://www.ncei.noaa.gov/pub/data/paleo/paleocean/indian_ocean/yamamoto2021/yamamoto2021-u1446.txt. The CMIP5 and PMIP3 datasets are publicly available at <https://pcmdi.llnl.gov/mips/cmip5/> and <https://pmip3.lscce.ipsl.fr>, respectively. CMAP precipitation data are available at <http://www.cdc.noaa.gov/>. ECMWF ERA-40 data are available at <https://apps.ecmwf.int/datasets/>.

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Acknowledgements

This research used samples provided by IODP expedition 353. We thank Y. Sazuka, A. Muto and K. Ono for analytical assistance, and Y. Chikaraishi for providing the end-member value of wild C_4 plants. M.Y. was funded by JSPS grants JPMXS05R2900001 and 19H05595 and JAMSTEC Exp 353 post-cruise study. S.C.C. was supported by US NSF OCE1634774. A.A.-O. was supported by JSPS grant 17H06104, MEXT grant 17H06323 and JAMSTEC to use the Earth Simulator supercomputer. R.O. was supported by ArCS project JPMXD1300000000 (MEXT, Japan).

Author contributions

M.Y. designed the study. M.Y., O.S., Y.T. and Y.H. generated $\delta^{13}C_{PA}$ data. S.C.C. generated foraminifera $\delta^{18}O$ data and the age–depth model. M.Y. analysed data. R.O. and A.A.-O. performed model experiments. M.Y. wrote the manuscript with input from others.

Competing interests

The authors declare no competing interests.

Additional information

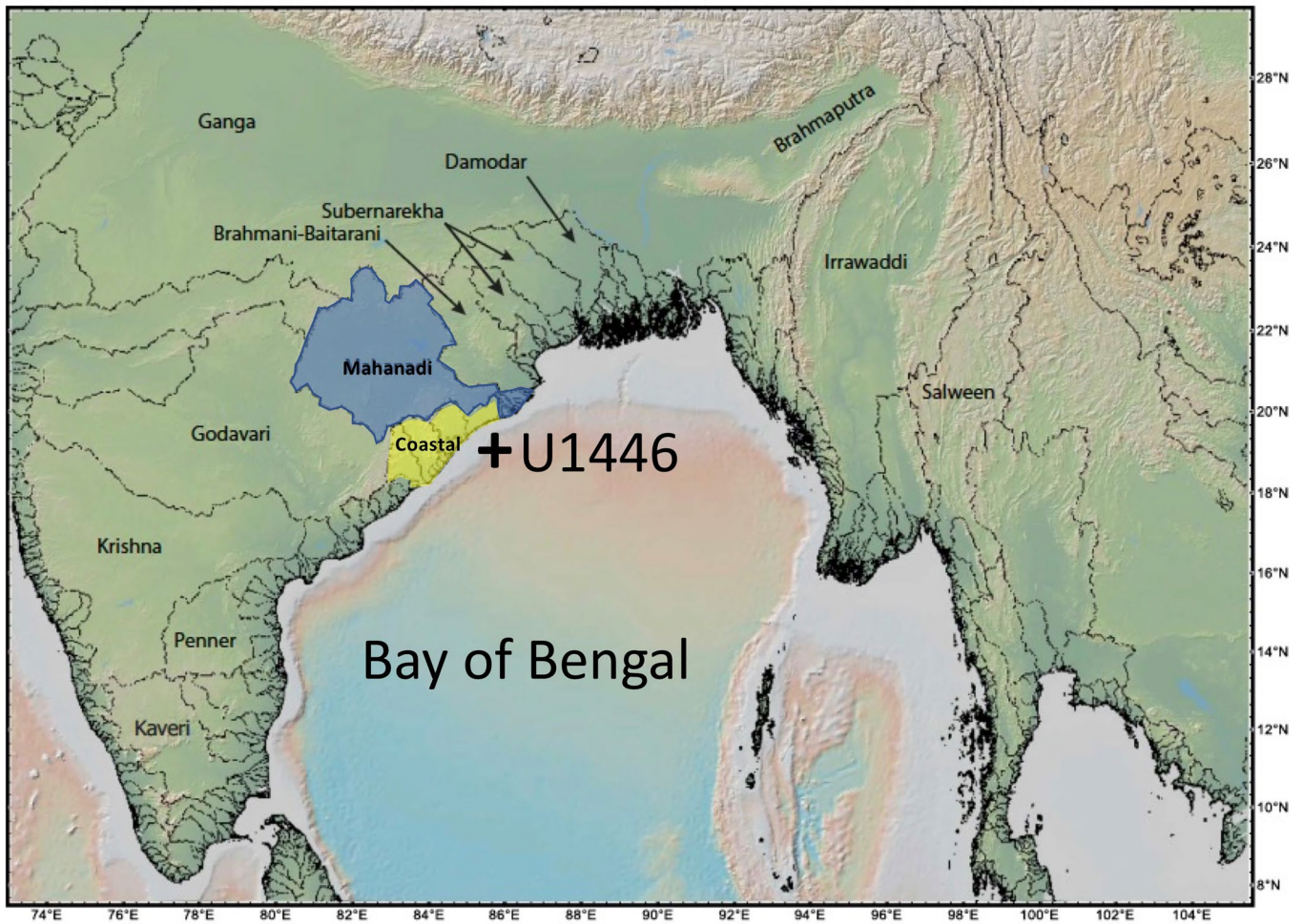
Extended data is available for this paper at <https://doi.org/10.1038/s41561-022-00918-1>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41561-022-00918-1>.

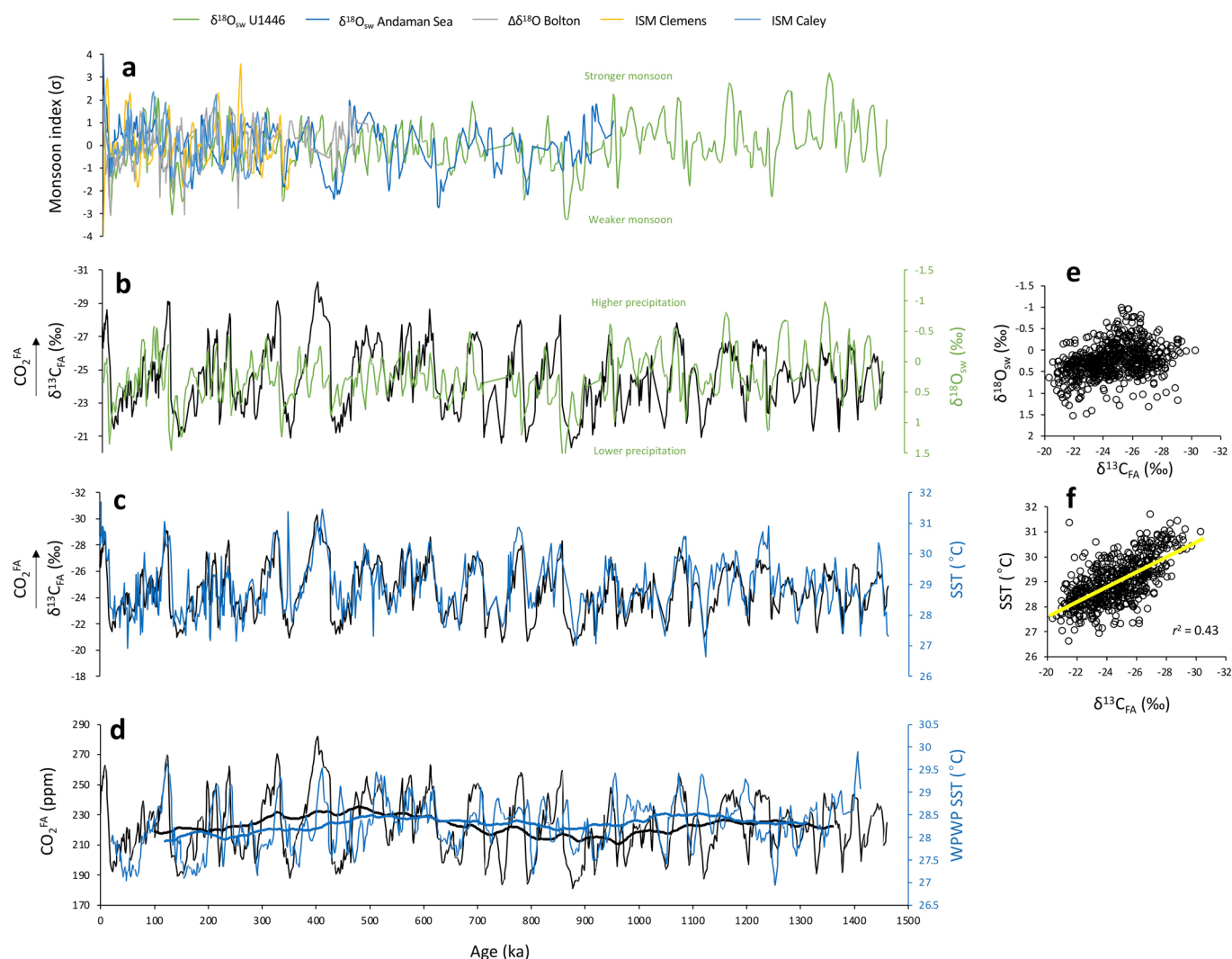
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Peer review information *Nature Geoscience* thanks Julio Sepulveda and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: James Super, in collaboration with the *Nature Geoscience* team.

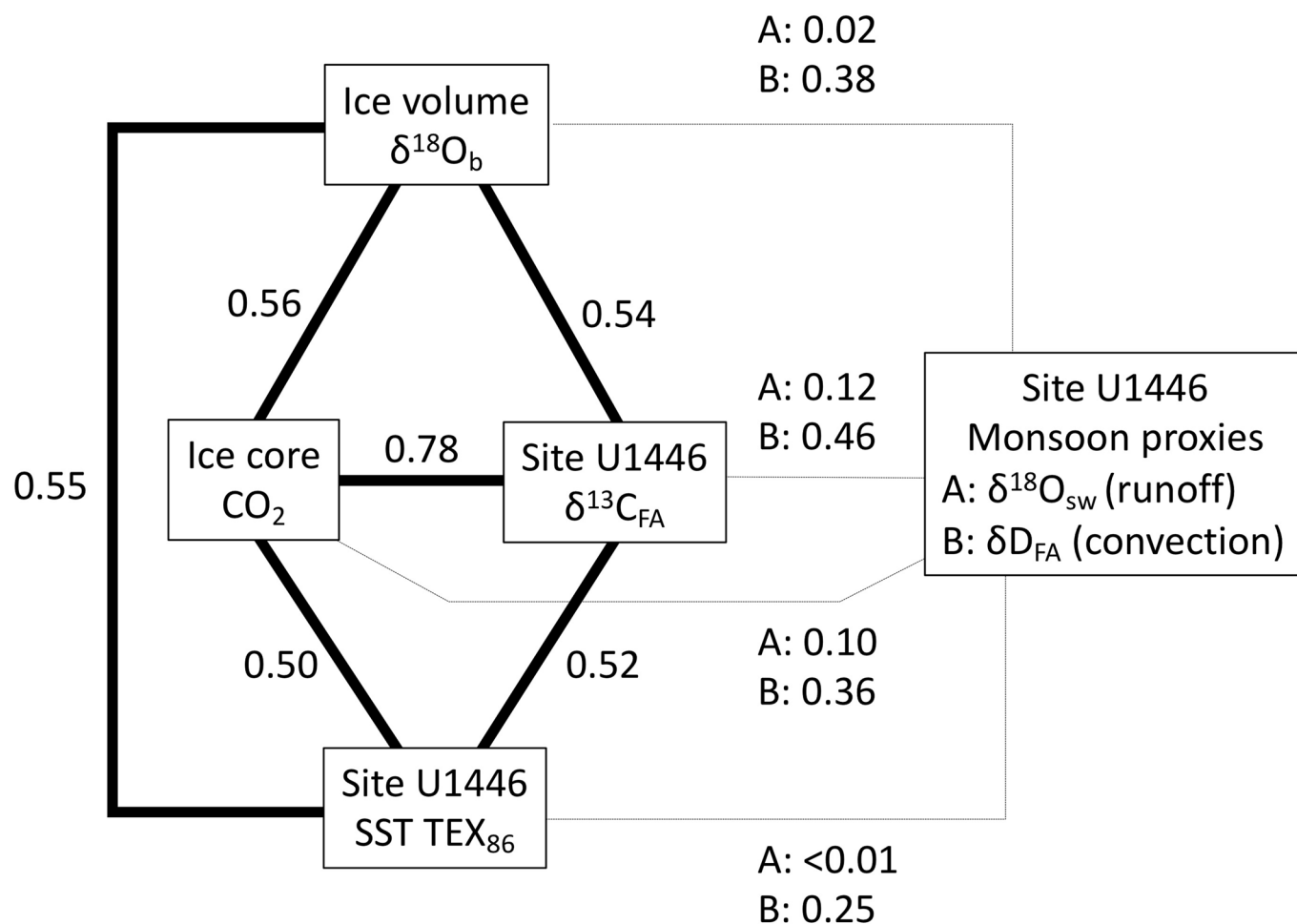
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Extended Data Fig. 1 | Location of IODP Site U1446. The sediments at Site U1446 were derived mainly from the Mahanadi River (blue) and the adjacent coastal rivers (yellow)^{33,34,51}.

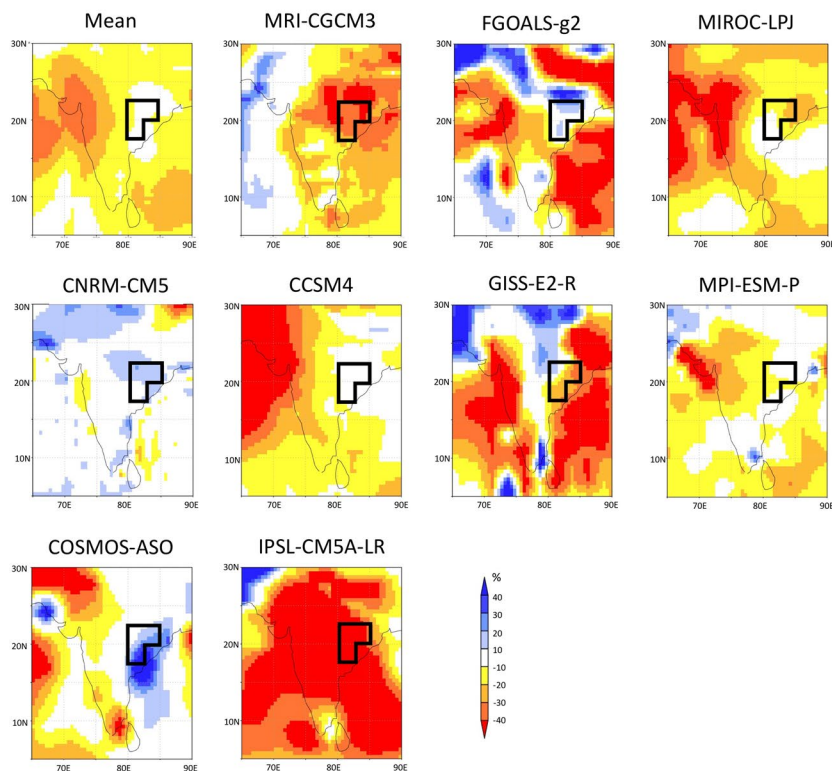


Extended Data Fig. 2 | $\delta^{13}\text{C}_{\text{FA}}$, monsoon indices, and sea surface temperature (SST) at Site U1446 during the last 1.46 Myr. **a**, Monsoon indices: normalized values of Indian summer monsoon (ISM) records based on the seawater $\delta^{18}\text{O}$ (reversed $\delta^{18}\text{O}_{\text{sw}}$ U1446)²⁵ at Site U1446, seawater $\delta^{18}\text{O}$ of the Andaman Sea (reversed $\delta^{18}\text{O}_{\text{sw}}$ Andaman Sea)⁶⁷, difference between surface and thermocline foraminifera $\delta^{18}\text{O}$ ($\Delta\delta^{18}\text{O}$) in the equatorial Indian Ocean⁶⁸, Indian summer monsoon (I.S.M.) stack of the Arabian Sea^{69,70}. **b** and **e**, $\delta^{13}\text{C}_{\text{FA}}$ and U1446 $\delta^{18}\text{O}_{\text{sw}}$. **c** and **f**, $\delta^{13}\text{C}_{\text{FA}}$ and the U1446 TEX₈₆^H-based SST²⁵. **d**, CO_2^{FA} and the western Pacific warm pool (WPWP) SST. The SSTs in the WPWP were averaged (Sites ODP Site 806^{23,71} and MD97-2140⁷²). Thick lines indicate the 200-kyr running mean.

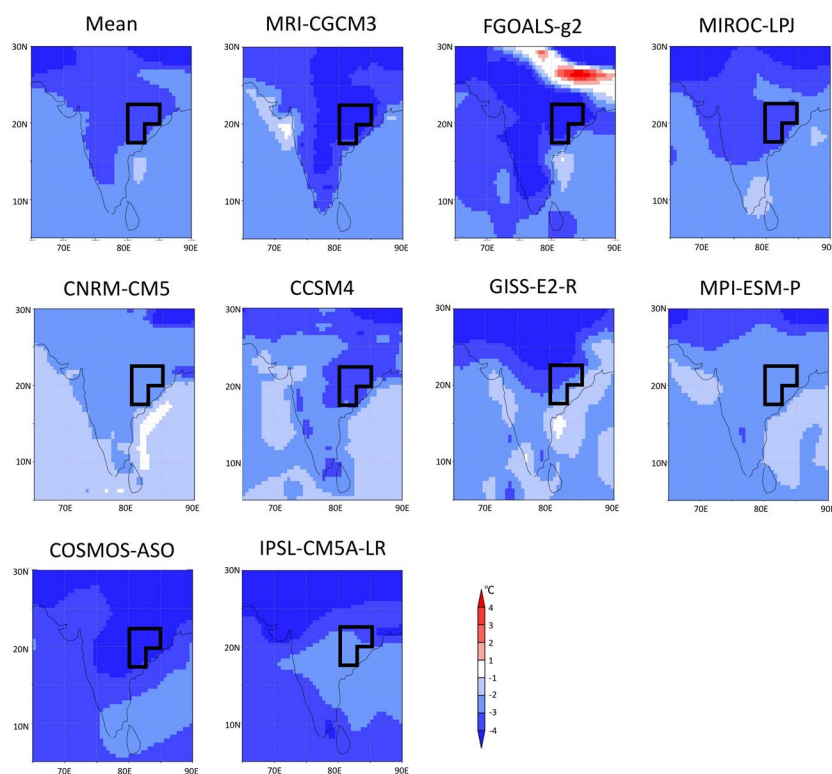


Extended Data Fig. 3 | Coefficients of determination (r^2) between $\delta^{13}\text{C}_{\text{FA}}$ and climate parameters for Site U1446 during the last 805 kyr. Monsoon proxies A and B respectively correspond to the seawater $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{sw}}$)²⁵, a proxy of salinity in the Bay of Bengal, and long-chain n-fatty acid δD (for the last 640 kyr: $\delta\text{D}_{\text{FA}}$)⁷³, a proxy of tropical convection activity⁷⁴, at Site U1446, respectively. The r^2 value between $\delta^{13}\text{C}_{\text{FA}}$ and the Antarctic ice core CO_2 concentration with tuned age⁴ is highest, indicating that atmospheric CO_2 concentration is a major factor determining $\delta^{13}\text{C}_{\text{FA}}$. The low r^2 value between $\delta^{13}\text{C}_{\text{FA}}$ and $\delta^{18}\text{O}_{\text{sw}}$ suggests that the influence of precipitation on $\delta^{13}\text{C}_{\text{FA}}$ is limited. The $\delta\text{D}_{\text{FA}}$ has higher coefficients with $\delta^{13}\text{C}_{\text{FA}}$, ice core CO_2 concentration, benthic foraminifera $\delta^{18}\text{O}_b$ ($\delta^{18}\text{O}_b$)²⁵ and SST²⁵. The higher correlation between $\delta^{13}\text{C}_{\text{FA}}$ and $\delta\text{D}_{\text{FA}}$ is attributable to the response of the tropical convection activity to CO_2 -induced global climate.

Precipitation

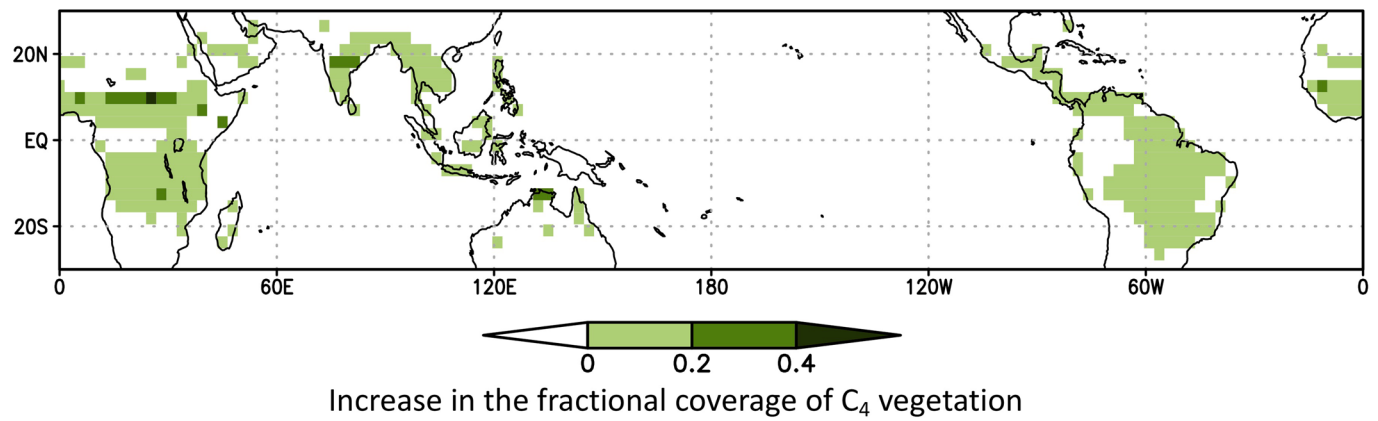


Temperature

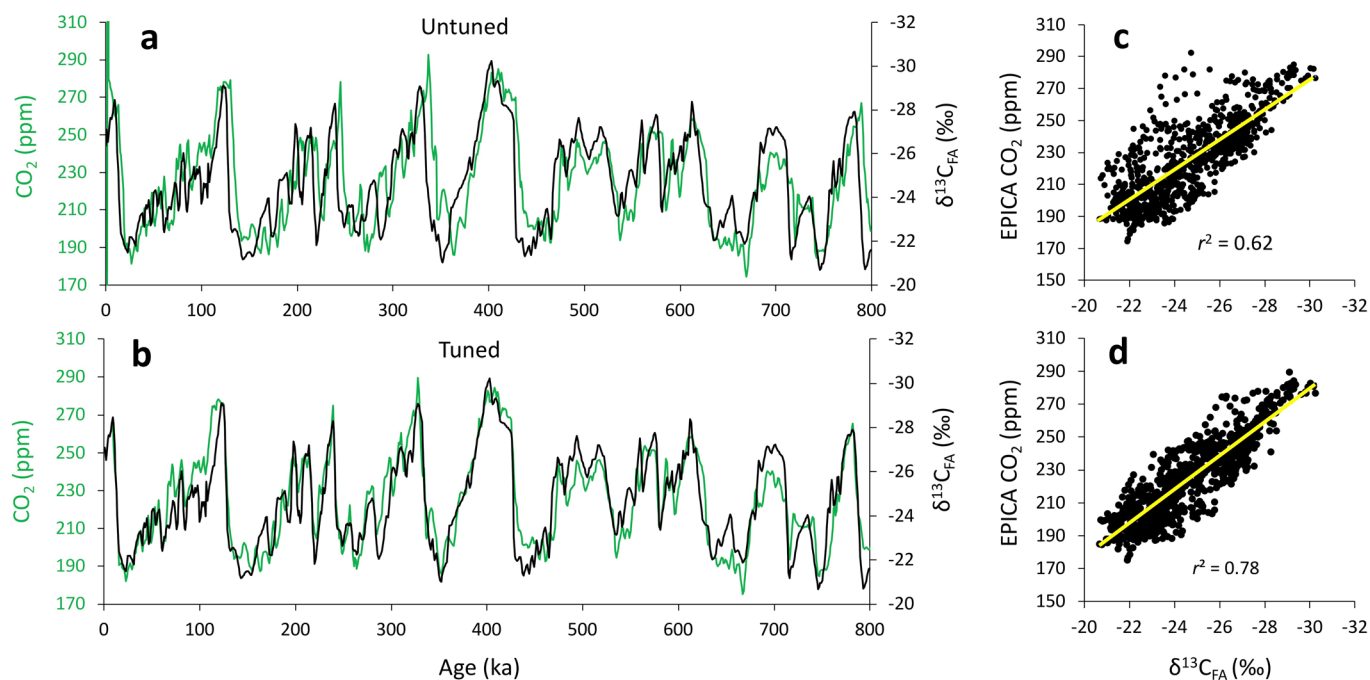


Extended Data Fig. 4 | See next page for caption.

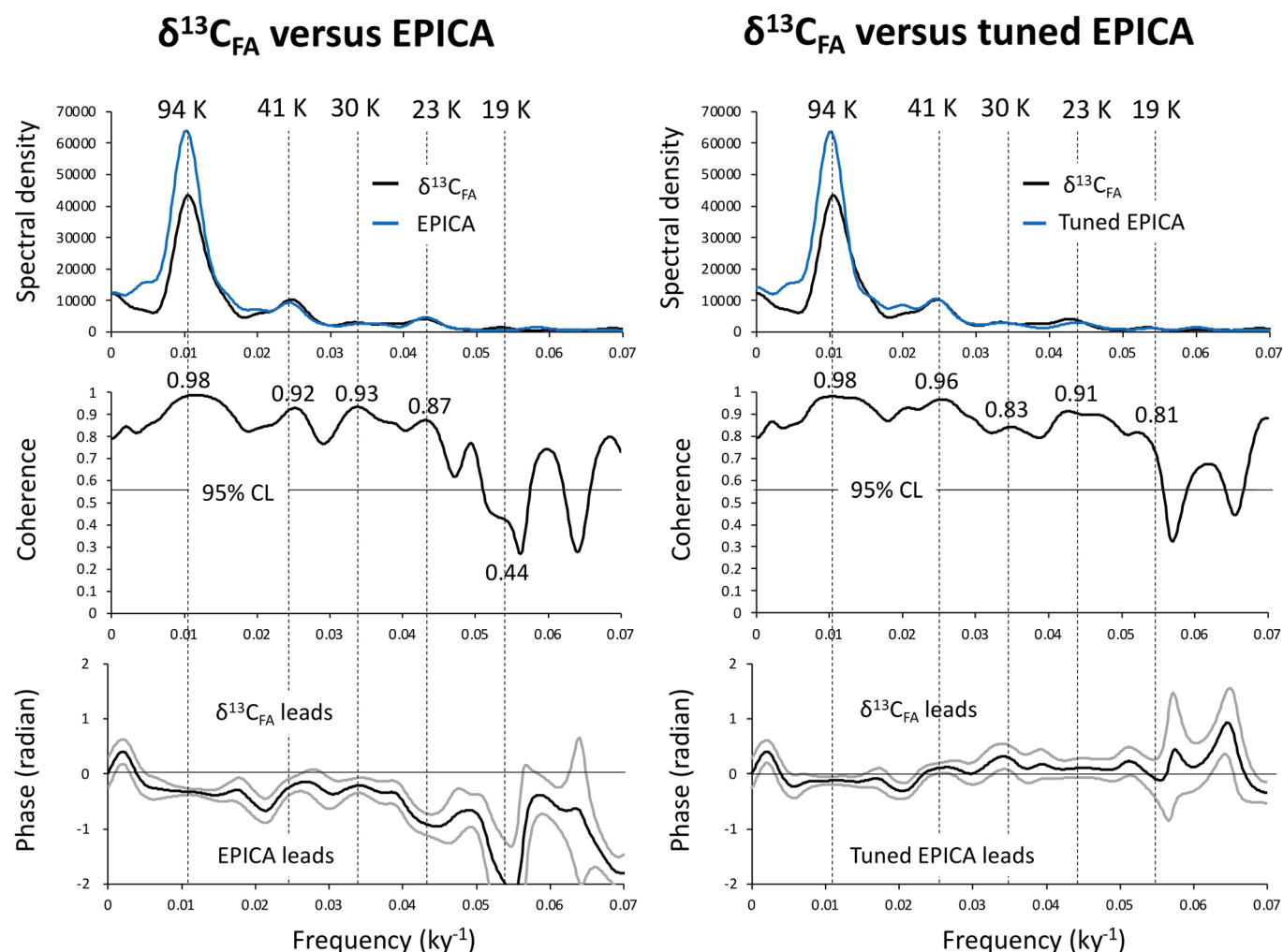
Extended Data Fig. 4 | The differences in annual mean precipitation and surface air temperature in the sediment source area between the LGM and preindustrial periods (LGM – PI). They were estimated with nine different GCMs^{30–32}. The decreases in annual mean precipitation and temperature in the sediment source area (grids of the black border) were $9 \pm 23\%$ and $3.4 \pm 0.8^\circ\text{C}$, respectively.



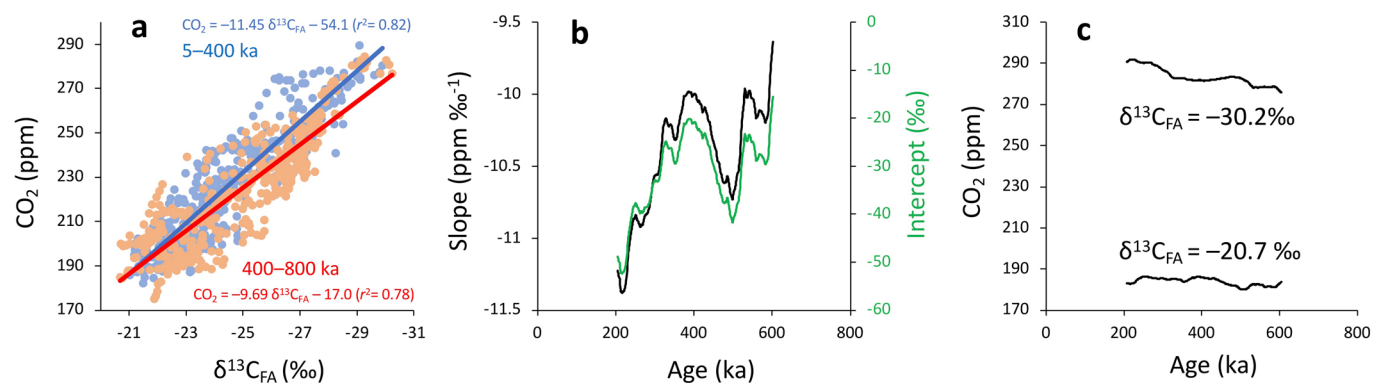
Extended Data Fig. 5 | The areas where C_3/C_4 vegetation is expected to respond primarily to CO_2 changes. The green grid shows the site where the increase in C_4 vegetation due to the decrease in CO_2 from 285 to 185 ppm is greater than the increase due to the decrease in precipitation and temperature from the PI level to the LGM level. Vegetation was predicted using LPJ-DGVM under the PI and LGM conditions^{30–32}. A very few regions where the increase in C_4 vegetation is significant show an empirical response of C_3/C_4 vegetation to CO_2 variation, characterized by hot and seasonally dry (savanna) climates, and could serve as targets for replicating our CO_2 reconstruction.



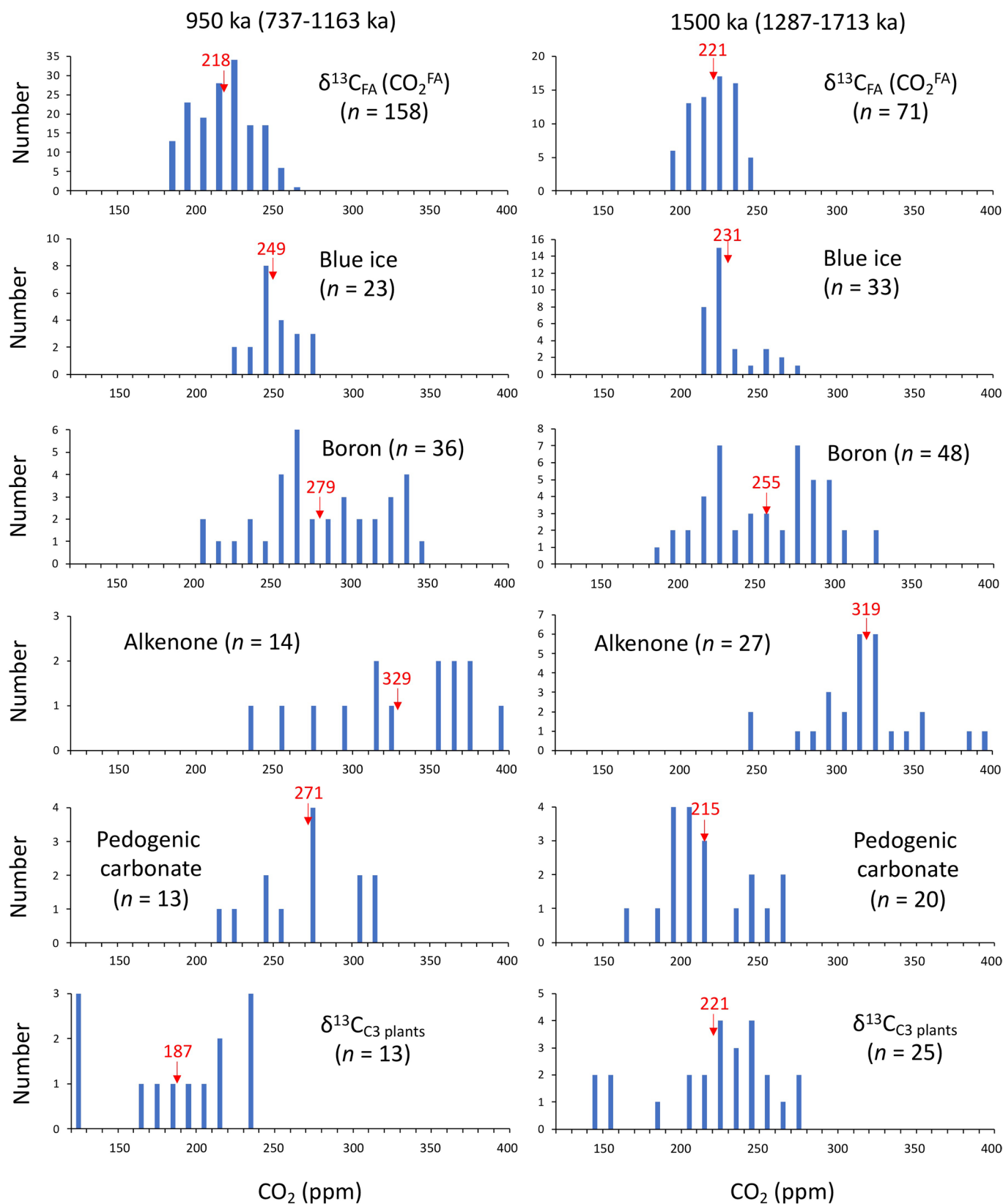
Extended Data Fig. 6 | Tuning of records. **a,b**, The δ¹³C_{FA} at Site U1446 and Antarctic ice core CO₂ concentrations⁴ during the last 805 kyr before and after tuning, and **c** and **d**, the plots of δ¹³C_{FA} and Antarctic ice core CO₂ concentration⁴ between 5 and 800 ka before and after tuning. The Antarctic CO₂ record⁴ was tuned to the δ¹³C_{FA} record.



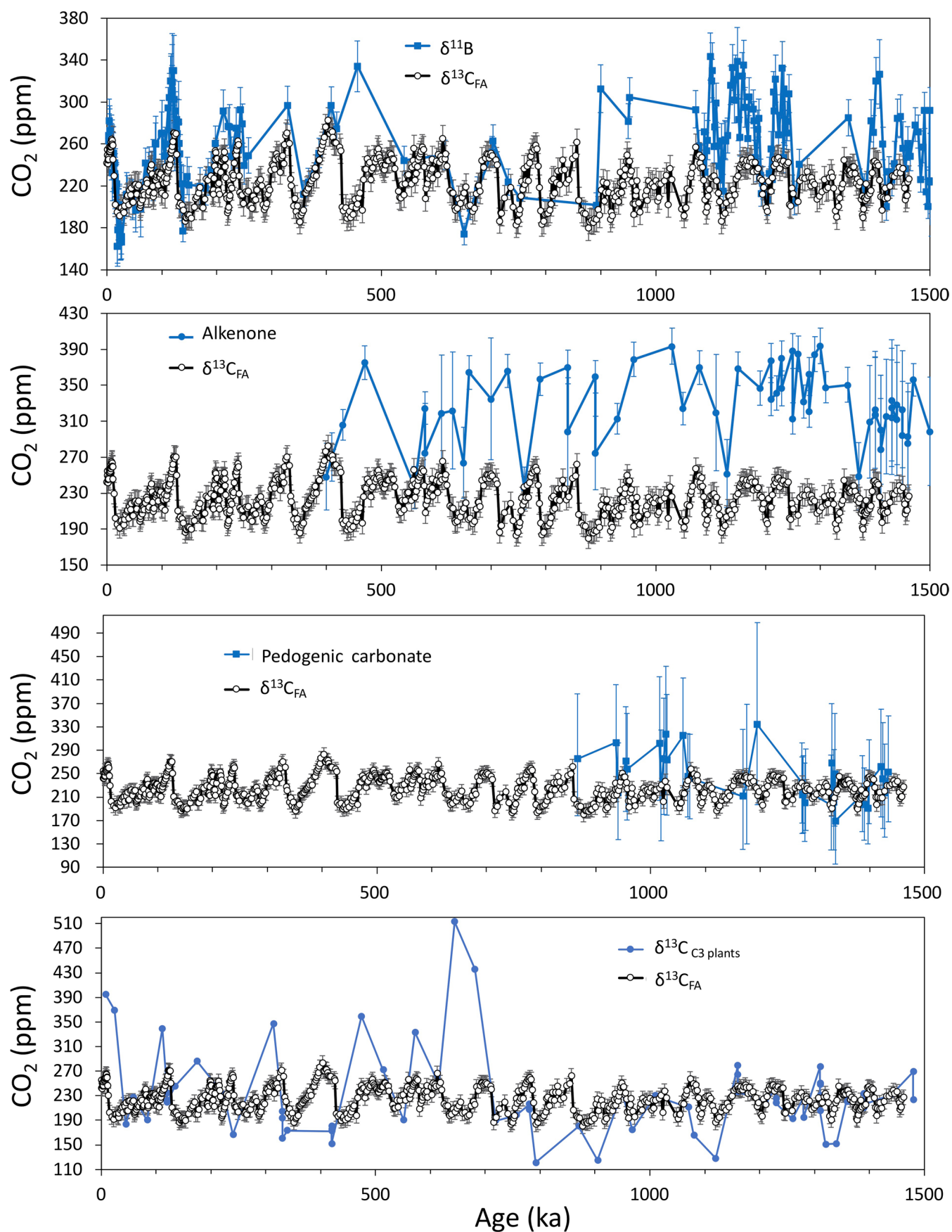
Extended Data Fig. 7 | The effect of tuning. Power spectra, coherence, and phase difference of the variation in $\delta^{13}\text{C}_{\text{FA}}$ -based CO_2 (CO_2^{FA}) and Antarctic EPICA CO_2 (ref. ⁴) before and after tuning the EPICA record to the $\delta^{13}\text{C}_{\text{FA}}$ -record in the period between 5 and 800 ka. The horizontal line in the coherence panel indicates the 95% confidence level. Faint lines in the phase difference panel indicate the upper and lower limits of the 80% confidence level. The $\delta^{13}\text{C}_{\text{FA}}$ and EPICA CO_2 records showed similar power spectra and high coherences at orbital cycles, implying that the $\delta^{13}\text{C}_{\text{FA}}$ reflects the atmospheric CO_2 concentration. The tuning increased coherence in the 41-, 23- and 19-kyr cycles, and reduced the phase lags.



Extended Data Fig. 8 | The calibration of $\delta^{13}\text{C}_{\text{FA}}$ to CO_2 concentration. **a**, Plot of $\delta^{13}\text{C}_{\text{FA}}$ (axis reversed) and the Antarctic ice core CO_2 concentration⁴ in the 5–400 and 400–800 ka periods, **b**, The slope and intercept of the linear regression equation between $\delta^{13}\text{C}_{\text{FA}}$ and ice core CO_2 variations in moving 400-kyr windows, **c**, The CO_2 concentrations estimated from constant $\delta^{13}\text{C}_{\text{FA}}$ values using the calibration equations of the panel b.



Extended Data Fig. 9 | Histograms showing the number of samples by CO_2 value during two distinct periods of 950 and 1,500 ka. The values were estimated by the $\delta^{13}\text{C}_{\text{FA}}$ at Site U1446 (CO_2^{FA}), blue ice^{13,14}, foraminiferal $\delta^{11}\text{B}$ (ref. ⁸), alkenone $\delta^{13}\text{C}$ (ref. ⁹), pedogenic carbonate $\delta^{13}\text{C}$ (ref. ¹⁰) and C_3 plant $\delta^{13}\text{C}$ (ref. ¹¹).



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | Estimates of CO₂ in various methods. CO₂ values estimated by the $\delta^{13}\text{C}_{\text{FA}}$ at Site U1446 (CO_2^{FA}) with a calibration error of 12 ppm, foraminiferal $\delta^{11}\text{B}$ (with 1 σ interval; ref. ⁸ data after refs. ^{5–7}), alkenone $\delta^{13}\text{C}$ (with the upper and lower estimates)⁹, pedogenic carbonate $\delta^{13}\text{C}$ (ref. ¹⁰) and $\delta^{13}\text{C}$ of C₃ plants¹¹ during the past 1.5 Myr.