nature geoscience SUPPLEMENTARY INFORMATION
DOI: 10.1038/NGEO1922

Independent variations of CH_4 emissions and isotopic composition over the past 160,000 years

Authors:

Lars Möller, Todd Sowers, Michael Bock, Renato Spahni, Melanie Behrens, Jochen Schmitt, Heinrich Miller, Hubertus Fischer*

*Correspondence and requests for materials should be addressed to H.F.

2. Supplementary Methods and technical descriptions

2.1 $\delta^{13}CH_4$ measurements performed at Alfred Wegener Institute (AWI)

EDML $\delta^{13}CH_4$ measurements were performed using a continuous flow gas chromatography combustion isotope ratio mass spectrometry system (GC/C/IRMS) with a preceeding purge and trap extraction and pre-concentration setup. A few modifications have been made compared to the published experimental and data processing procedure[1,2]. Sample sizes between 150-200 g of ice, equivalent to 15-20 ml of air at standard temperature and pressure (STP) were used after scraping off 1-2 mm at the surface with a microtome knife to minimize the risk of contamination e.g. by drill fluid. Sealed with a copper gasket in an ultra-high-vacuum stainless steel to glass sample vessel with an inner volume of 350 ml, the samples were melted after evacuation and the air stripped out of the water and vessel head space with a helium carrier gas stream (150 mL/min) for 90 minutes. The bulk of the entrained water vapor was removed by a cooled Nafion membrane and most of the easily condensable gases like CO_2 and N_2O are trapped in a ⅛ inch steel trap at -196 °C (liquid nitrogen, LN_2). While methane from the sample air was subsequently pre-concentrated on a ⅛" steel tubing filled with an adsorbent (Hayesep D 80/100 mesh, Supelco), bulk components of the air were vented at -140 °C. The retained sample methane and residual air components were focussed on a cryofocus trap and separated on a 30 m GC column (30 °C, CarbonPLOT, Agilent, Böblingen, Germany). The methane was then quantitatively combusted to CO_2 in a 940 °C oxidation furnace (Ni/CU/Pt wires in Al_2O_3 tubing, ThermoFinnigan, Bremen, Germany) and admitted to the IRMS (Isoprime, Elementar, Germany) using an open split.

A pure CO_2 working standard (ISO-TOP, Air Liquide, Germany) was admitted to the IRMS during each acquisition. Moreover, a pure methane standard (99.995 vol.-% purity; AirLiquide, Germany) admitted to the GC stream was used to monitor fractionation in the GC or the combustion oven. 20 ml STP synthetic atmospheric air standard injections from two different standard bottles ("SynthAir" with 1000 ppb CH4 (99.995 vol.-% purity), 250 ppm ISOTOP CO_2 and 250 ppb N_2O (99.999 vol.-% purity); "Crystal-Mix" with 1081 ± 22 ppb CH_4 4.5, 249.9 ± 5.0 ppm CO_2 and 259 ± 26 ppb; both AirLiquide, Germany) were used to correct for long-term systematic shifts and internal calibration. The mean $\delta^{13}CH_4$ value for "SynthAir" was -40.93 ± 0.10 ‰ (n=448) and -49.66 ± 0.11 ‰ (n=136) for "Crystal-Mix" after drift correction. The absolute accuracy of the system was determined by 16 measurements of a modern air sample from Neumayer Station, Antarctica. The target value ($\delta^{13}CH_4$: -46.97 ± 0.04 ‰ versus V-PDB (n=7)) was determined by off-line sample preparation and dual-inlet IRMS on a MAT252 mass spectrometer (ThermoFinnigan) at the Institut für Umweltphysik, Heidelberg[3]. A calculated machine offset of 0.02 ‰ of our GC/C/IRMS determined values after correction for Kr interference (see below) relative to the Heidelberg reference value was used to correct the EDML $\delta^{13}CH_4$ data of this study. Accordingly, our data are reported relative to the V-PDB scale as defined by the Heidelberg measurements[4].

The EDML $\delta^{13}CH_4$ time series includes 129 data points and 32 replicates from different depth intervals with a mean reproducibility of the replicates of ± 0.18 ‰. A total of 19 outliers, either caused by machine instabilities or other experimental problems, were excluded from the dataset and remeasured. Processing of the chromatogram for each measurement has been performed using a self developed, fully automated peak detection, integration and referencing script written in the Python programming language (www.python.org). This script allows uniform and comprehensible background and peak detection, genuine automation for post-processing (like e.g. long term trend corrections) and data archiving. Using this script we were able to reproduce published $\delta^{13}CH_4$ data for termination I[2], which were obtained with the software provided with the MS (Isoprime, Elementar, Germany) with an offset of 0.10 ‰ (1σ: 0.17€‰, n=34) without and 0.17 ‰ (1σ: 0.15€‰) with correction for Kr interference (see below). Note that both offsets are smaller than the measurement uncertainty of this earlier data set (0.3 ‰, 1σ). This re-evaluated record for termination I was used in Figure 1 and 2 in the main text, and is also shown in supplementary Figure S1 for the comparison with the unmodified, uncorrected data of our previous publication.

2.2 $\delta^{13}CH_4$ measurements performed at Pennsylvania State University (PSU)

For the Vostok ice core sample measurements at Penn State University, two different extraction methods were employed. A dry extraction system[5] was used to liberate gas from ice samples where N_2O measurements were needed[6,7]. Trapped gases were liberated from 1-1.5 kg ice samples using a "cheese grater" oscillator with immediate cryogenic freezing of liberated air into a 35 ml stainless steel sample tube immersed in liquid Helium. Once the air was transferred, the sample tube was isolated, removed from the Helium Dewar and equilibrated at room temperature before CH_4 and N_2O analyses were performed using standard GC techniques. The sample tube was then attached to the PreCon device where the isotopic composition of N_2O and CH_4 were determined[8,9]. For those samples where we did not need N_2O data, a wet extraction technique was used to liberate the occluded air. Ice samples weighing 500-700 g were placed into a stainless steel extraction cylinder and sealed with a copper gasket. After evacuation, the ice was allowed to melt for 40 min in 50 °C water before being placed into a liquid nitrogen Dewar for 40 min to refreeze the meltwater. The headspace was then flushed with He (40 ml/min) through a H_2O trap (-110 °C) with the CH_4

ultimately trapped on a Hayesep D trap at -130 °C. After 40 min of flushing, the Hayesep D trap was isolated and attached to the PreCon for CH_4 isotopic analyses[10]. Both extraction systems were routinely checked for contamination/fractionation using standard air samples of varying sizes to mimic the amount of CH_4 we extract from the ice core samples (1-3 nmol of CH_4). The air standards were introduced over the residual crushed ice or degassed refrozen meltwater and processed as if they were a real ice sample. For the dry extraction runs, the average of eight separate standard runs was -47.30 ± 0.31 ‰ (1σ). The average value is 0.17 ‰ lower than the assigned $\delta^{13}CH_4$ value for the air tank (-47.13 ‰ VPDB) but within the uncertainty associated with the method itself. For the wet extraction system, we processed 15 standard air samples through degassed water samples. The average $\delta^{13}CH_4$ value for these runs was -47.10 ± 0.34 ‰. These results are close to the assigned value for the standard (-47.13 ‰) and 0.2 ‰ higher than the results from the dry extraction system. To account for the $\delta^{13}CH_4$ difference between the two extraction systems, we add 0.2 ‰ to all the dry extraction data to be consistent with the wet extraction data and the assigned value for the air standard. We estimate the overall uncertainty based on the replicate analyses of the standard air samples to be 0.3 ‰ (1σ).

2.3 Correction of $\delta^{13}CH_4$ data due to Kr interference during IRMS measurements

The chromatographic separation of CH_4 and the noble gas krypton (Kr) imposes special demands on a setup used to separate air components, owing to the very similar physico-chemical properties of these compounds. Kr has previously not been accounted for in $\delta^{13}CH_4$ studies, as none of the multiple stable isotopes of Kr are close to the mass to charge ratio (*m/z*) 44, 45 and 46 considered in CO_2 based $\delta^{13}CH_4$ measurements. Recently Schmitt et al. (2013) demonstrated[11], that the doubly charged isotope $^{86}Kr^{2+}$ does in fact interfere with the $\delta^{13}C$ measurement of CH_4, if Kr enters the ion source of the IRMS.

After a thorough review of the raw data for the ice core $\delta^{13}CH_4$ time series, we were able to identify irregularities in the raw chromatograms at the peak flanks of the CH_4-derived CO_2 for the measurements performed with the AWI instrument. The Kr peak causes anomalies in the ratio of *m/z* = 45 to *m/z* = 44 and the *m/z* = 46 to *m/z* = 44 ratio which generates higher $\delta^{13}CH_4$ values the more Kr contributes to the total peak areas (hereafter referred to as Kr effect). While the symptoms are distinct due to instrumental differences of the two setups, we also found the PSU system to be affected by the Kr interference.

Because polar ice core samples are not easily replaced, we were unable to repeat the measurements of the EDML and Vostok $\delta^{13}CH_4$ time series with enhanced instrumental setups. Instead we corrected the $\delta^{13}CH_4$ values for this Kr effect as described in detail in Schmitt et al. (2013)[11], which we briefly summarize below.

A posteriori correction of the Kr effect

We applied the following strategies to correct PSU and AWI $\delta^{13}CH_4$ measurements for the Kr effect and account for relative laboratory offsets with respect to the VPDB scale. AWI EDML measurements are corrected for Kr individually by a method applied to the respective raw data chromatograms. The method uses the visible anomalies seen in the *m/z* ratios 45/44 and *m/z* 46/44 and subtracts the derived interference from the raw data time series[11]. Afterwards, the chromatograms are reprocessed and the isotopic composition of the CH_4 peak calculated. We refer to these values as the Kr-corrected $\delta^{13}CH_4$ values. In contrast, $\delta^{13}CH_4$ values obtained without the subtraction algorithm are referred to as original $\delta^{13}CH_4$ values. The difference between original and corrected values are termed Kr correction values or $\Delta\delta^{13}C_{Kr}$. For the EDML time series $\Delta\delta^{13}C_{Kr}$ range between 0.4 ‰ for interstadial (medium CH_4 mixing ratios) to 0.8 ‰ during glacial (MIS 2) and stadial conditions (lower CH_4 levels) (compare also Supplementary Figure S1). The results are further calibrated internally and are tied to the VPDB scale as outlined in section 1.1.

Unfortunately, an analogous direct approach could not be applied to the Vostok ice core $\delta^{13}CH_4$ acquisitions performed at the PSU, as the raw chromatograms were not stored after processing the $\delta^{13}C$ data. Instead, we had to choose an indirect way to correct for the Kr effect in the Vostok

$\delta^{13}CH_4$ time series.

For the purpose of this correction the atmospheric krypton mixing ratio can safely be considered constant over time[11,12]. If instrumental conditions are uniform, the Kr effect scales only with the atmospheric concentration of methane, it is thus directly proportional to the Kr/CH_4 ratio[11]. We inferred the linear relationship between Kr effect and 1/CH_4 from a series of air samples with differing methane mixing ratios but similar Kr levels. The three ambient air samples were retrieved at Niwot Ridge preserve, Colorado, US in 2007, and were part of the „2007 - IPY International Ice Core Gas Intercalibration Exercise“ launched by Todd Sowers (Penn State University) and Ed Brook (Oregon State University). The samples of recent ambient air were diluted with ultra pure air (free of CH_4) in order to simulate the full spectrum of atmospheric variability from present day, pre-industrial to glacial conditions. Note, that the dilution did not affect the noble gas concentration in the cylinders.

The raw data chromatograms of “IPY” air samples performed with the PSU instrument were treated in the same manner as the EDML ice core samples, with the routine adapted to the specific characteristics of the PSU setup. Based on these PSU measurements we obtained Kr correction values of 0.12 ‰, 0.26 ‰ and 0.66 ‰ for cylinder “CA03560”, ”CC71560” and “CA01179”, respectively. To a first approximation, $\Delta\delta^{13}C_{Kr}$ scales with the inverse of the CH_4 mixing ratios of 1852 ppb, 906 ppb and 365 ppb (illustrated in Figure S2), yielding a dependence of $\Delta\delta^{13}C_{Kr}$ to CH_4 for the PSU measurements. Supplementary table 1 provides a detailed compilation of results for the “IPY” air samples for both the PSU and the AWI instruments.

When the linear $\Delta\delta^{13}C_{Kr}$ - 1/CH_4 relationship was applied to the CH_4 mixing ratios[13] of the Vostok ice core, we derived $\Delta\delta^{13}C_{Kr}$ values in the range of 0.4 ‰ for interglacial conditions (e.g. MIS 5.5) to 0.7 ‰ and 0.8 ‰ for the glacial stages MIS 2 and MIS 6, respectively (see Figure S1). Note that while these corrections on the PSU and AWI data of several tenth of a permille are significant, they are still small compared to the atmospheric changes of several permille observed in our ice core data on glacial/interglacial time scales, and do not influence our interpretations.

2.4 ***AWI and PSU laboratory inter-calibration***

In order to minimize offsets between the absolute standardization of both laboratory setups at the AWI and the PSU, we performed inter-calibration measurements on three IPY air samples and on ice core samples from the WAIS Divide ice core WDC05 A (79°27.70S 112°7.510W; 1.759 masl.). We applied the CH_4 dependence of $\Delta\delta^{13}C_{Kr}$ to account for the Kr effect encountered in the ice core and air sample measurements using the PSU instrument, and the raw data correction procedure described for the EDML ice samples in section 1.3 for the IPY air and WDC05 A measurements performed with the AWI setup. The results for both systems, presented in more detail in Supplementary table 1 and 2, lead to an AWI-PSU laboratory offset of 0.09 ‰ with respect to $\delta^{13}CH_4$. As a final adjustment, the Vostok $\delta^{13}CH_4$ time series is hence shifted by 0.09 ‰ towards lower values to account for the differences in the absolute standardization of both laboratories. Note that this inter-laboratory offset is significantly smaller than the measurement uncertainty of 0.3 ‰, showing that the AWI and PSU data sets are fully compatible after correction of the Kr effect.

2.5 Firn column corrections applied to the $\delta^{13}CH_4$ ice core records

During the enclosure of air bubbles in the Antarctic ice sheet, the methane molecules like other air components encounter diffusion processes in the open pore space of the firn column, which also affect their isotopic composition archived after bubble close-off. In the diffusive firn zone[14] methane is subject to gravitational settling, that enriches the heavier isotope at the bottom of the firn column[15]. In addition, strong concentration gradients caused by rapid atmospheric methane concentration changes, induce diffusive fluxes that lead to isotopic fractionation[16]. Finally, thermal diffusion corrections are required when large temperature gradients exist in the firn layer[17].

Temperature variations at Kohnen Station (EDML core site) and Vostok have been slow during the last glacial cycle. Hence, the firn column down to the close-off depth was essentially in thermal equilibrium and thermal diffusion effects are negligible for the datasets presented here.

In order to quantify the order of magnitude of the respective diffusion effects, we used a firn diffusion model[18] with a parameter set adapted to the glacial EDML core characteristics to calculate the combined effects of diffusive fractionation due to gravitation and concentration changes at the surface (see Figure S3). A methane pulse of 200 ppb with an initial rate of increase of 4 ppb/yr and a respective decline of 0.25 ppb/yr (grey line, subfigure **a**)) was prescribed to mimic the most vigorous natural methane rises throughout the glacial cycle (e.g. at the end of terminations or into DO 21). The $\delta^{13}CH_4$ signature of methane at the surface is prescribed at -45 ‰. Accordingly, all changes to this value recorded at the model bubble close-off on the bottom of the firn column (blue line, subfigure **b**)) are solely due to the two diffusion processes. To account for uncertainties concerning the two most important physical parameters of firnification, site temperature and accumulation rate, we illustrate the range of model results for the fractionation of $\delta^{13}CH_4$ with a minimal (-52.14 °C, 2.978 cm water equivalent per year (w.e./yr)), maximal (-46.64 °C, 5.075 cm w.e./yr) and a best guess scenario (-49.52 °C, 3.859 cm w.e./yr). This range of site temperatures and snow accumulation rates is a realistic representation of possible glacial conditions at the core site.

The initial $\delta^{13}CH_4$ value, after steady state is reached in the model, is -44.57 ‰. This offset of 0.43 ‰ relative to the atmospheric value is due to the gravitational settling that is established in the firn column. It is also on very good agreement with measured $\delta^{15}N_2$ for the EDML core for glacial conditions[19]. The consecutive prescribed methane rise at the surface causes an additional shift in $\delta^{13}CH_4$ after bubble close-off in the range of -0.73 ‰ for coldest temperatures and lowest snow accumulation and up to -1.05 ‰ for maximal temperature and accumulation rate, while the best guess scenario amounts to -0.85 ‰. However, the effect is short-lived and decreases to levels below our experimental uncertainty in less than 150 years after the initial methane increase. After about 500 years the $\delta^{13}CH_4$ value is essentially back at its starting value before the CH_4 increase. During the slower decline of methane concentration back to the base level of 350 ppb, the highest observed diffusional fractionation of -0.24 ‰ does not even exceed the measurement uncertainty. In conclusion, only those data points of our record may be affected that fall within the relatively short time window during major methane concentration increases. Accordingly, we did not correct our data for these diffusion effects, but have to keep in mind that individual samples that coincide with the rapid CH_4 changes may be biased by a few tenth of a permille towards lower (more negative) $\delta^{13}CH_4$ values.

Accordingly, all our $\delta^{13}CH_4$ data are solely corrected for gravitational fractionation. Vostok samples have been corrected with interpolated $\delta^{15}N_2$ data according to published procedures[14]. No $\delta^{15}N_2$ record covering the whole time interval of our $\delta^{13}CH_4$ data is available for the EDML ice core to date. However, $\delta^{15}N_2$ data over the last glacial/interglacial transition vary only between 0.4 to 0.45 ‰. Thus, we shifted EDML values by a constant offset of 0.41 ‰ to higher values. This 0.41 ‰ shift does reflect expected values for glacial conditions very well[19,20], and is also in line with model studies[21]. The error introduced by this constant correction is 0.05 ‰ at the most, and thus negligible compared to our overall analytical uncertainty of 0.3 ‰.

2.6 dD(CH_4) measurements performed at University of Bern

$\delta D(CH_4)$ measurements were performed using a purge and trap extraction coupled to a gas chromatography pyrolysis isotope ratio mass spectrometer (GC/P/IRMS) system as described in detail in Bock et al. (2010)[22] with some improvements which will be published elsewhere. External precision of the presented data is about 2.5 ‰ (1σ) based on standard air measurements of corresponding size. In Figure 3 of the main text the error bars represent the standard deviation of standard air measurements (1σ: 1.8 ‰ to 2.9 ‰) used to calibrate the corresponding samples. All $\delta D(CH_4)$ values are given with respect to the international Vienna Standard Mean Ocean Water (VSMOW) scale. No corrections (e.g. gravitational enrichment) have been applied, as these corrections would only be of minor importance regarding the analytical uncertainty, and do not affect our conclusions. Note, that no Kr interference occurs for our δD(CH_4) system.

2.7 *Age scales*

Ice core records

If not stated otherwise, all gas ages in this document are reported according to a chronology based on the new synchronization effort for EPICA and various other ice core chronologies by Lemieux-Dudon et al. (2010)[23], hereafter denoted as "Unified" age scale. Where applicable, a direct age calculation was performed by linear interpolation of the depth-age relationship provided by Lemieux-Dudon et al. (2010)[23]. Other records required additional conversion steps according to the procedures described below.

Vostok $\delta^{13}CH_4$ and CO_2 records

For Vostok we adopted the published Vostok depth to EDC3 gas age relationship of the Vostok CO_2 record and interpolated corresponding EDC3 ages for the Vostok $\delta^{13}CH_4$ data. However, we observed an evident misalignment of the fast methane concentration rises at Dansgaard/Oeschger (DO) event 24 compared to EDC CH_4 data[26]. As part of the focus of our work is directed at relative timings of $\delta^{13}CH_4$ and CH_4 rises, we performed a manual methane synchronization to account for this offset. Therefore, we picked five tie-points in between DO event 21 and the Termination 2 methane rise at peak flanks (Supplementary table 3) and interpolated the included data points linearly to the EDC3 age scale[27]. The EDC3 ages including the adjusted section between 83.6 kyr BP and 128.9 kyr BP were then converted to the target "Unified" age model. The adjustments were also applied to the Vostok CO_2 record used in this work[26,28]. Note that the applied adjustments do not affect the conclusions in this publication on the decoupling of CH_4 and $\delta^{13}CH_4$ or on the coupling of $\delta^{13}CH_4$ and atmospheric CO_2.

Byrd atmospheric CO_2

No official age conversion to the "Unified" age scale exists for the Byrd ice core. Hence, we performed another methane synchronization between the Byrd[29] and EDML[30] CH_4 records in order to obtain corresponding EDML depths. Those were then converted to "Unified" ages. We are aware of the limitations of the methane synchronization approach[31], especially if the resolution differences of the compared CH_4 datasets are large, or at times were CH_4 variations are low and, thus, tie-points are scarce. However, the very good temporal agreement of the Byrd[32] and the EDML CO_2 data[33] show that the relative CH_4 synchronization error is small and does not affect the conclusions in our paper. The list of manual tie-points applied in the synchronization are presented in Supplementary table 4.

Relative sea-level

No direct age conversion to the "Unified" target age scale could be applied to the relative sea-level (rsl.) data without invoking an ad-hoc phase relationship between the sea level and the ice core records. Therefore, we used the sea level record of Rohling et al. (2009)[24] on the speleothem synchronized age-scale provided by Grant et al. (2012)[25] without any modifications.

3. Complementary information on past $\delta^{13}CH_4$ changes

3.1 DO methane variability and its missing responses in $\delta^{13}CH_4$

Figure S4 illustrates the missing imprint of the rapid atmospheric methane concentration variability on the carbon isotopic signature of methane in conjunction with the six strongest DO warmings throughout the last glacial. Segments of the CH_4[30] and $\delta^{13}CH_4$ data sets, centered around the respective methane rise, have been aligned (Figure S4 **a**)) in order to study the phasing and individual timing of the concentration changes and its counterpart in $\delta^{13}CH_4$ (Figure S4 **b**)). Note that the data points in $\delta^{13}CH_4$ closest to the most vigorous methane increases are likely biased by diffusional fractionation in the firn column (see discussion above). This may lead to offsets of $\delta^{13}CH_4$ data points close to major CH_4 changes. For example, the two negative excursions in

$\delta^{13}CH_4$ of less than 1 ‰ at DO 8 and termination I are located so close to the corresponding methane increase, that they can be attributed to the diffusional fractionation effect. We mark the range of possibly affected values in Figure S4 with a box of the width of 150 yr (gray bar), according to the maximum duration in our firnification model exercise, where the effect of the diffusional fractionation exceeded our experimental uncertainty range of 0.3 ‰. We also show the two major methane increases of termination I and II (right pair of panels in Figure S4). There is no apparent imprint of rapid methane variability on its carbon isotopic signature over the respective DO events and deglaciations.

3.2 Proxy evidence for C4 plant expansion

Methane production under natural conditions involves the decomposition of organic precursor material that has previously been accumulated by plants through photosynthetic sequestration of CO_2. Owing to fundamental physiological differences of the two major photosynthetic pathways, which characteristically discriminate the heavy isotope ^{13}C during carbon assimilation, the typical ranges of isotopic signatures imposed on the plant material differ considerably[34,35]. 85–90 % of terrestrial plant species today, covering the whole spectrum of vegetation from grasses and herbs to shrubs and trees, follow the C3 photosynthetic pathway[36]. C3 plant biomass is characterized by a depleted $\delta^{13}C$ signature (-32 ‰ - -22 ‰), caused by the lower reactivity of $^{13}CO_2$ with the primary carboxylating enzyme RUBISCO[37]. C4 vegetation on the other hand, mostly grasses and sedges, are able to pre-concentrate CO_2 internally at the cost of reduced quantum yield[38]. As a consequence, C4 plant carbon fixation fractionates less against ^{13}C (~-16 ‰ - -9 ‰). The isotopic composition of the terrestrial biosphere, i.a. the pre-cursor biomass for methanogenesis, is controlled by the primary productivity of an assemblage of plants under local growth conditions, the individual adaptation of its members to this conditions, as well as its tolerance against limitation factors. Plants of both photosynthetic pathways are unequally tolerant to limitations in CO_2, light intensity, local temperature, and to moisture and nutrient availability[35,39,40]. Seasonality of precipitation has an equally significant impact on the local balance between C3 and C4 vegetation[40]. However, the relative importance of any of the limitations, especially in terms of a competitive advantage of plant families against others in the struggle for habitats, remains an unresolved and vitally discussed question[36,41-45].

Accordingly, it is neither physiologically well constrained how strong a C3 to C4 plant shift might have been in tropical regions under generally colder, drier conditions, and low CO_2 levels that were characteristic for the glacial period, nor is it extensively documented by the scarce terrestrial proxy evidence from these areas. Such a shift, however, is one of the relevant processes of our hypothesis to explain the observed, very pronounced $\delta^{13}CH_4$ changes. Therefore, we will now discuss some of the ecosystem evidence that is available and relevant for our hypothesis.

In temperate regions in Northern- and Meso-America[46] and the Chinese loess plateau[47-49], growing season temperature and the local climatic constellation seems to out-compete the physiological effect of low CO_2 level as predominant control upon the C3/C4 ratio. With warm growing seasons in the tropics, however, water insufficiency and low CO_2 possess an increased influence as plant-growth limitation factors and pose high adaptive pressure on prevalent ecosystems[50-53].

Recent vegetation model experiments indicate high vegetation sensitivity to low atmospheric CO_2 levels during glacial periods[44,45,54,55]. Globally, simulations for glacial climate conditions and typical CO_2 concentrations lead to significant retractions of closed canopy forest habitats in favor of open vegetation types[43,56,57]. Tropical rainforests seem especially affected by the combined effects of increased aridity and low CO_2, and large arboreal areas are replaced by open savanna- and shrub-like vegetation.

Analogue findings are well documented by terrestrial proxy data from tropical regions in Africa[58-61], Meso- and South America[61-64]. These studies also report large proportions of C4 vegetation contributing to the widespread grasslands in equatorial Africa and South America in the LGM.

Glacial-Interglacial differences in $\delta^{13}C$ of vascular plant waxes from sediment cores off the East Atlantic coast close to the river mouths of the Congo and Angola basin, indicate 3-4 ‰ shifts towards higher $\delta^{13}C$ values and thus, relative increases of C4 abundance in the range of 20-40 %[59]. Increased C4 contribution has also been inferred from another marine core retrieved at the Guinea Plateau margin recording Sahara/Sahel vegetation[60]. It also indicates raised aridity, falling temperatures and exceptionally high C4 predominance for the period between 71 and 65 kyr BP, i.e. the MIS5/4 transition, which is also characterized by a strong increase (+4 ‰) in our $\delta^{13}CH_4$ record.

A comparable study from the Cariaco Basin in the tropical west Atlantic, reported a 4-5 ‰ $\delta^{13}C$ decrease in leaf waxes from the LGM to the Preboreal Holocene[62]. This probably documents a reoccupation of forest vegetation in the peripheral Amazonian lowlands, that potentially retracted under glacial conditions[65-69]. Moreover, huge land masses the size of Europe from South Thailand to Sumatra, Borneo and Java became exposed in South-East Asia when sea-level fell in glacial periods. This territory, known as Sundaland, was also vastly covered by savanna type vegetation with considerable C4 contribution[70,71].

A global shift of C3 to C4 plants may not be representative for the conditions encountered in permanent (tropical) wetlands. Intuitionally, one may expect that the missing water limitation in such a wetland would reduce the adaptive pressure on plants in that ecosystem and hence level competitive advantages of one species against another. C3 plants, for example, are not forced into the natural trade-off between necessary stomatal opening for carbon sequestration and excessive water loss. In this light it is yet not fully understood, why large modern wetland ecosystems in tropical East Africa[72], South Africa[73,74] or areas of the Amazon floodplain[75] exhibit clear C4 plant predominance (mostly papyrus). Moreover, there is evidence that C4 dominance in east-equatorial Africa (at least near lake Challa) persisted during both wet and dry phases under glacial conditions[76]. For the $\delta^{13}CH_4$ change observed in our record we speculate that seasonally inundated wetlands played an increasing role for CH_4 emissions during cold climate conditions. These tropical non-permanent wetlands should foster the shift to open grasslands with high C4 contribution, as the higher water use efficiency and productivity of the C4 plants under low CO_2 levels in glacial periods should proof advantageous in this environment of seasonally contrasting very dry to very wet conditions.

3.3 Impact of location and habitat on the isotopic signature of C3 plants

Environmental factors as humidity, light availability, CO_2 concentration and use of recycled CO_2 differ largely between C3 plant habitats like rainforest and savanna[77]. Dense closed canopy rainforest habitats provide a higher degree of natural protection from wind movement and hence reduced water loss and air mass exchange, but on the other hand increase the competition for light intensity. The low photon flux caused by the light deficit for example diminishes carbon fixation rates, especially in the undergrowth vegetation. Lower vegetation layers furthermore assimilate more respired CO_2 that already underwent fractionation[78,79]. Rainforest habitats have much higher water supply and air moisture levels compared to open vegetation types. Reduced evapo-transpirative water loss in closed canopies allows longer periods of opened leaf stomata and, thus, increased CO_2 levels in the leafs. Light deficit and high intracellular CO_2 levels enhance the discrimination of the heavier ^{13}C isotope during photosynthesis[80]. In contrast, open shrub, herb and grassy C3 vegetation is, especially in low latitudes, exposed to high levels of direct sunlight and high leaf temperatures. To avoid extensive water loss, the stomatal conductance of these plants is usually highly restricted[37], and carbon dioxide limitation in the leaf cells reduces the relative discrimination of the heavier isotope by the enzymes involved in photosynthesis. As a consequence of these effects, C3 rainforest plant material is found to be 3-4 ‰ more depleted than C3 plant material from open savanna[77].

3.4 Role of water table changes

Hydrological changes in the past have direct implications on water table heights in wetlands. The soil oxidative layer thickness directly affects the net/gross primary productivity and thus CH_4 emission strength of a given wetland system. But an increased/decreased oxidative layer thickness would also induce an enrichment/depletion in the mean $\delta^{13}CH_4$ signature of emitted methane as a higher/lower proportion of methane is oxidized on its way to the surface. This parameter is highly dependent on the local hydrology, topography and soil characteristics and poorly constrained spatially and over time. Our record suggests that it is of minor importance for the millenial scale variability of methane over the DO cycles as there is no remarkable imprint on $\delta^{13}CH_4$. A general contribution to the glacial-interglacial difference in $\delta^{13}CH_4$ due to enhanced global aridity in the course of the glaciation, however, cannot be ruled out.

References

1. Behrens, M.; Schmitt, J.; Richter, K.-U.; Bock, M.; Richter, U. C.; Levin, I. & Fischer, H. A gas chromatography/combustion/isotope ratio mass spectrometry system for high-precision $\delta^{13}C$ measurements of atmospheric methane extracted from ice core samples. *Rapid Commun Mass Spectrom*, **22**, 3261-3269 (2008).
2. Fischer, H.; Behrens, M.; Bock, M.; Richter, U.; Schmitt, J.; Loulergue, L.; Chappellaz, J.; Spahni, R.; Blunier, T.; Leuenberger, M. & Stocker, T. F. Changing boreal methane sources and constant biomass burning during the last termination. *Nature*, **452**, 864-867 (2008).
3. Poß, C. *Analysis of the variability of the atmospheric methane budget in high polar regions with a regional trajectory model and using measurements of stable isotopes*. PhD thesis, University of Heidelberg, Germany, (2003).
4. Calibration of the Heidelberg $\delta^{13}CH_4$ measurements was performed using pure CO_2 IAEA Standard Reference Materials (RM 8562: $\delta^{13}C$ = -3.72 ‰, RM 8564: $\delta^{13}C$ = -10.45 ‰, RM 8563: $\delta^{13}C$ = -41.59 ‰[5]). The absolute agreement of the Heidelberg $\delta^{13}CH_4$ measurements on the VPDB CO_2 scale is estimated to be better than ±0.1 ‰. An intercomparison with three air samples from the southern hemisphere between Heidelberg and NIWA yielded a difference NIWA-Heidelberg $\delta^{13}CH_4 = 0.04 \pm 0.04$ ‰[3].
5. Sowers, T. & Jubenville, J. A modified extraction technique for liberating occluded gases from ice cores. *J. Geophys. Res.*, **105**, 29155-29164 (2000).
6. Sowers, T.; Alley, R. B. & Jubenville, J. Ice Core Records of Atmospheric N_2O Covering the Last 106,000 Years. *Science*, **301**, 945-948 (2003).
7. Miteva, V.; Sowers, T. & Brenchley, J. Production of N_2O by Ammonia Oxidizing Bacteria at Subfreezing Temperatures as a Model for Assessing the N_2O Anomalies in the Vostok Ice Core. *Geomicrobiology Journal*, **24**, 451-459 (2007).
8. Sowers, T.; Rodebaugh, A.; Yoshida, N. & Toyoda, S. Extending records of the isotopic composition of atmospheric N_2O back to 1800 A.D. from air trapped in snow at the South Pole and the Greenland Ice Sheet Project II ice core. *Global Biogeochem. Cycles*, **16**, 1129- (2002).
9. Sowers, T.; Bernard, S.; Aballain, O.; Chappellaz, J.; Barnola, J.-M. & Marik, T. Records of the $\delta^{13}C$ of atmospheric CH_4 over the last 2 centuries as recorded in Antarctic snow and ice. *Global Biogeochem. Cycles*, **19**, (2005).
10. Sowers, T. Atmospheric methane isotope records covering the Holocene period. *Quaternary*

Science Reviews, **29**, 213-221 (2010).

11. Schmitt, J.; Seth, B.; Bock, M.; Fischer, H.; Möller, L.; Sapart, C.; Prokopiou, M.; Röckmann, T. & Sowers, T. On the interference of Kr during carbon isotope analysis of methane using continuous-flow combustion–isotope ratio mass spectrometry. *Atmos. Meas. Tech.*, **6**, 1425-1445 (2013).
12. Headly, M. A. & Severinghaus, J. P. A method to measure Kr/N_2 ratios in air bubbles trapped in ice cores and its application in reconstructing past mean ocean temperature. *J. Geophys. Res.*, **112**, D19105- (2007).
13. Petit, J. R.; Basile, I.; Leruyuet, A.; Raynaud, D.; Lorius, C.; Jouzel, J.; Stievenard, M.; Lipenkov, V. Y.; Barkov, N. I.; Kudryashov, B. B.; Davis, M.; Saltzman, E. & Kotlyakov, V. Four climate cycles in Vostok ice core. *Nature*, **387**, 359-360 (1997).
14. Sowers, T.; Bender, M.; Raynaud, D. & Korotkevich, Y. S. $\delta^{15}N$ of N_2 in Air Trapped in Polar Ice: a Tracer of Gas Transport in the Firn and a Possible Constraint on Ice Age-Gas Age Differences. *J. Geophys. Res.*, **97**, 15683-15697 (1992).
15. Schwander, J.; Stauffer, B. & Sigg, A. Air mixing in firn and the age of the air at pore close-off. *Annals of Glaciology*, **10**, 141-145 (1988).
16. Trudinger, C. M.; Enting, L. G.; Etheridge, D. M.; Francey, R. J.; Levchenko, V. A.; Steele, L. P.; Raynaud, D. & Arnaud, L. Modeling air movement and bubble trapping in firn. *J. Geophys. Res.*, **102**, (1997).
17. Severinghaus, J. P.; Grachev, A. & Battle, M. Thermal fractionation of air in polar firn by seasonal temperature gradients. *Geochem. Geophys. Geosyst.*, **2**, (2001).
18. Schwander, J.; Sowers, T.; Barnola, J.-M.; Blunier, T.; Fuchs, A. & Malaize, B. Age scale of the air in the summit ice: Implication for glacial-interglacial temperature change. *J. Geophys. Res.*, **102**, 19483–19493 (1997).
19. Landais, A.; Barnola, J.; Kawamura, K.; Caillon, N.; Delmotte, M.; Van Ommen, T.; Dreyfus, G.; Jouzel, J.; Masson-Delmotte, V.; Minster, B.; Freitag, J.; Leuenberger, M.; Schwander, J.; Huber, C.; Etheridge, D. & Morgan, V. Firn-air $\delta^{15}N$ in modern polar sites and glacial-interglacial ice: a model-data mismatch during glacial periods in Antarctica?. *Quaternary Science Reviews*, **25**, 49-62 (2006).
20. Landais, A.; Dreyfus, G.; Capron, E.; Masson-Delmotte, V.; Sanchez-Goñi, M.; Desprat, S.; Hoffmann, G.; Jouzel, J.; Leuenberger, M. & Johnsen, S. What drives the millennial and orbital variations of $\delta^{18}O_{atm}$? *Quaternary Science Reviews*, **29**, 235-246 (2010).
21. EPICA community members, One-to-one coupling of glacial climate variability in Greenland and Antarctica. *Nature*, **444**, 195-198 (2006).
22. Bock, M.; Schmitt, J.; Behrens, M.; Möller, L.; Schneider, R.; Sapart, C. & Fischer, H. A

gas chromatography/pyrolysis/isotope ratio mass spectrometry system for high-precision δD measurements of atmospheric methane extracted from ice cores. *Rapid Commun. Mass Spectrom.*, **24**, 621-633 (2010).

23. Lemieux-Dudon, B.; Blayo, E.; Petit, J.-R.; Waelbroeck, C.; Svensson, A.; Ritz, C.; Barnola, J.-M.; Narcisi, B. M. & Parrenin, F. Consistent dating for Antarctic and Greenland ice cores. *Quaternary Science Reviews*, **29**, 8-20 (2010).
24. Rohling, E. J.; Grant, K.; Bolshaw, M.; Roberts, A. P.; Siddall, M.; Hemleben, C. & Kucera, M. Antarctic temperature and global sea level closely coupled over the past five glacial cycles. *Nature Geosci*, **2**, 500-504 (2009).
25. Grant, K. M.; Rohling, E. J.; Bar-Matthews, M.; Ayalon, A.; Medina-Elizalde, M.; Ramsey, C. B.; Satow, C. & Roberts, A. P. Rapid coupling between ice volume and polar temperature over the past 150,000 years. *Nature*, **491**, 744-747 (2012).
26. Loulergue, L.; Schilt, A.; Spahni, R.; Masson-Delmotte, V.; Blunier, T.; Lemieux, B.; Barnola, J.-M.; Raynaud, D.; Stocker, T. F. & Chappellaz, J. Orbital and millennial-scale features of atmospheric CH_4 over the past 800,000 years.. *Nature*, **453**, 383-386 (2008).
27. Parrenin, F.; Barnola, J.-M.; Beer, J.; Blunier, T.; Castellano, E.; Chappellaz, J.; Dreyfus, G.; Fischer, H.; Fujita, S.; Jouzel, J.; Kawamura, K.; Lemieux-Dudon, B.; Loulergue, L.; Masson-Delmotte, V.; Narcisi, B.; Petit, J.-R.; Raisbeck, G.; Raynaud, D.; Ruth, U.; Schwander, J.; Severi, M.; Spahni, R.; Steffensen, J. P.; Svensson, A.; Udisti, R.; Waelbroeck, C. & Wolff, E. The EDC3 chronology for the EPICA Dome C ice core. *Clim. Past*, **3**, 485-497 (2007).
28. Lüthi, D.; Floch, M. L.; Bereiter, B.; Blunier, T.; Barnola, J.-M.; Siegenthaler, U.; Raynaud, D.; Jouzel, J.; Fischer, H.; Kawamura, K. & Stocker, T. F. High-resolution carbon dioxide concentration record 650,000-800,000 years before present.. *Nature*, **453**, 379-382 (2008).
29. Blunier, T. & Brook, E. J. Timing of Millennial-Scale Climate Change in Antarctica and Greenland During the Last Glacial Period. *Science*, **291**, 109-112 (2001).
30. Schilt, A.; Baumgartner, M.; Schwander, J.; Buiron, D.; Capron, E.; Chappellaz, J.; Loulergue, L.; Schüpbach, S.; Spahni, R.; Fischer, H. & Stocker, T. F. Atmospheric nitrous oxide during the last 140,000 years. *Earth and Planetary Science Letters*, **300**, 33-43 (2010).
31. Blunier, T.; Spahni, R.; Barnola, J.-M.; Chappellaz, J.; Loulergue, L. & Schwander, J. Synchronization of ice core records via atmospheric gases. *Clim. Past*, **3**, 325-330 (2007).
32. Ahn, J. & Brook, E. J. Atmospheric CO_2 and Climate on Millennial Time Scales During the Last Glacial Period. *Science*, **322**, 83-85 (2008).
33. Bereiter, B.; Lüthi, D.; Siegrist, M.; Schüpbach, S.; Stocker, T. F. & Fischer, H. Mode change of millennial CO_2 variability during the last glacial cycle associated with a bipolar

marine carbon seesaw. *Proceedings of the National Academy of Sciences*, **109**, 9755-9760 (2012).

34. O'Leary, M. H. Carbon Isotope Fractionation In Plants. *Phytochemistry*, **20**, 553-567 (1981).
35. Farquhar, G. D.; Ehleringer, J. R. & Hubick, K. T. Carbon Isotope Discrimination and Photosynthesis Rid A-3722-2008. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503-537 (1989).
36. Gerhart, L. M. & Ward, J. K. Plant responses to low [CO_2] of the past. *New Phytologist*, **188**, 674-695 (2010).
37. Farquhar, G. D.; Oleary, M. H. & Berry, J. A. On the Relationship Between Carbon Isotope Discrimination and the Inter-cellular Carbon-dioxide Concentration In Leaves Rid A-3722-2008 Rid B-8211-2009. *Australian Journal of Plant Physiology*, **9**, 121-137 (1982).
38. Ehleringer, J. R. Implications of quantum yield differences on the distributions of C3 and C4 grasses. *Oecologia*, **31**, 255-267 (1978).
39. Cowling, S. A. & Sykes, M. T. Physiological Significance of Low Atmospheric CO_2 for Plant/Climate Interactions. *Quaternary Research*, **52**, 237-242 (1999).
40. Ehleringer, J. (2005). *The Influence of Atmospheric CO_2, Temperature, and Water on the Abundance of C3/C4 Taxa*. In: Baldwin, I.; Caldwell, M.; Heldmaier, G.; Jackson, R.; Lange, O.; Mooney, H.; Schulze, E.-D.; Sommer, U.; Ehleringer, J.; Denise Dearing, M. & Cerling, T. (Ed.), *Ecological Studies*, Springer New York.
41. Sankaran, M.; Hanan, N. P.; Scholes, R. J.; Ratnam, J.; Augustine, D. J.; Cade, B. S.; Gignoux, J.; Higgins, S. I.; Le Roux, X.; Ludwig, F.; Ardo, J.; Banyikwa, F.; Bronn, A.; Bucini, G.; Caylor, K. K.; Coughenour, M. B.; Diouf, A.; Ekaya, W.; Feral, C. J.; February, E. C.; Frost, P. G. H.; Hiernaux, P.; Hrabar, H.; Metzger, K. L.; Prins, H. H. T.; Ringrose, S.; Sea, W.; Tews, J.; Worden, J. & Zambatis, N. Determinants of woody cover in African savannas. *Nature*, **438**, 846-849 (2005).
42. Edwards, E. J.; Osborne, C. P.; Strömberg, C. A. E.; Smith, S. A. & Consortium, C. 4. G. The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science. *Science*, **328**, 587-591 (2010).
43. Prentice, I. C.; Harrison, S. P. & Bartlein, P. J. Global vegetation and terrestrial carbon cycle changes after the last ice age. *New Phytologist*, **189**, 988-998 (2011).
44. Bragg, F. J.; Prentice, I. C.; Harrison, S. P.; Eglinton, G.; Foster, P. N.; Rommerskirchen, F. & Rullkötter, J. Stable isotope and modelling evidence for CO_2 as a driver of glacial–interglacial vegetation shifts in southern Africa. *Biogeosciences*, **10**, 2001-2010 (2013).
45. Claussen, M.; Selent, K.; Brovkin, V.; Raddatz, T. & Gayler, V. Impact of CO_2 and climate on Last Glacial Maximum vegetation - a factor separation. *Biogeosciences Discuss.*, **9**, 15823-

15852 (2012).

46. Huang, Y.; Street-Perrott, F. A.; Metcalfe, S. E.; Brenner, M.; Moreland, M. & Freeman, K. H. Climate Change as the Dominant Control on Glacial-Interglacial Variations in C3 and C4 Plant Abundance. *Science*, **293**, 1647-1651 (2001).
47. Zhang, Z.; Zhao, M.; Lu, H. & Faiia, A. M. Lower temperature as the main cause of C4 plant declines during the glacial periods on the Chinese Loess Plateau. *Earth and Planetary Science Letters*, **214**, 467-481 (2003).
48. Vidic, N. J. & Montañez, I. P. Climatically driven glacial-interglacial variations in C3 and C4 plant proportions on the Chinese Loess Plateau. *Geology*, **32**, 337-340 (2004).
49. Wang, G.; Feng, X.; Han, J.; Zhou, L.; Tan, W. & Su, F. Paleovegetation reconstruction using δ13C of Soil Organic Matter. *Biogeosciences*, **5**, 1325-1337 (2008).
50. Ehleringer, J. R.; Cerling, T. E. & Helliker, B. R. C4 photosynthesis, atmospheric CO_2, and climate. *Oecologia*, **112**, 285-299 (1997).
51. Street-Perrott, F. A.; Huang, Y.; Perrott, R. A.; Eglinton, G.; Barker, P.; Khelifa, L. B.; Harkness, D. D. & Olago, D. O. Impact of Lower Atmospheric Carbon Dioxide on Tropical Mountain Ecosystems. *Science*, **278**, 1422-1426 (1997).
52. Sage, R. F.; Wedin, D. A. & Li, M. (1999). *10 - The Biogeography of C4 Photosynthesis: Patterns and Controlling Factors*. In: Sage, R. F. & Monson, R. K. (Ed.), *C4 Plant Biology*, Academic Press.
53. Sage, R. F. The evolution of C4 photosynthesis. *New Phytologist*, **161**, 341-370 (2004).
54. Harrison, S. P. & Prentice, C. I. Climate and CO_2 controls on global vegetation distribution at the last glacial maximum: analysis based on palaeovegetation data, biome modelling and palaeoclimate simulations. *Global Change Biology*, **9**, 983-1004 (2003).
55. Prentice, I. C. & Harrison, S. P. Ecosystem effects of CO_2 concentration: evidence from past climates. *Clim. Past*, **5**, 297-307 (2009).
56. Jolly, D. & Haxeltine, A. Effect of Low Glacial Atmospheric CO_2 on Tropical African Montane Vegetation. *Science*, **276**, 786-788 (1997).
57. Woillez, M.-N.; Kageyama, M.; Krinner, G.; de Noblet-Ducoudré, N.; Viovy, N. & Mancip, M. Impact of CO_2 and climate on the Last Glacial Maximum vegetation: results from the ORCHIDEE/IPSL models. *Clim. Past*, **7**, 557-577 (2011).
58. Wooller, M. J.; Swain, D. L.; Ficken, K. J.; Agnew, A. D. Q.; Street-Perrott, F. A. & Eglinton, G. Late Quaternary vegetation changes around Lake Rutundu, Mount Kenya, East Africa: evidence from grass cuticles, pollen and stable carbon isotopes. *J. Quaternary Sci.*, **18**, 3-15 (2003).
59. Rommerskirchen, F.; Eglinton, G.; Dupont, L. & Rullkötter, J. Glacial/interglacial changes

in southern Africa: Compound-specific δ ^{13}C land plant biomarker and pollen records from southeast Atlantic continental margin sediments. *Geochem. Geophys. Geosyst.*, **7**, (2006).

60. Castañeda, I. S.; Mulitza, S.; Schefuß, E.; Lopes dos Santos, R. A.; Sinninghe Damsté, J. S. & Schouten, S. Wet phases in the Sahara/Sahel region and human migration patterns in North Africa. *Proceedings of the National Academy of Sciences*, **106**, 20159-20163 (2009).
61. Hessler, I.; Dupont, L.; Bonnefille, R.; Behling, H.; González, C.; Helmens, K. F.; Hooghiemstra, H.; Lebamba, J.; Ledru, M.-P.; Lézine, A.-M.; Maley, J.; Marret, F. & Vincens, A. Millennial-scale changes in vegetation records from tropical Africa and South America during the last glacial. *Quaternary Science Reviews*, **29**, 2882-2899 (2010).
62. Hughen, K. A.; Eglinton, T. I.; Xu, L. & Makou, M. Abrupt Tropical Vegetation Response to Rapid Climate Changes. *Science*, **304**, 1955-1959 (2004).
63. Punyasena, S. W.; Mayle, F. E. & McElwain, J. C. Quantitative estimates of glacial and Holocene temperature and precipitation change in lowland Amazonian Bolivia. *Geology*, **36**, 667-670 (2008).
64. Lane, C.; Horn, S.; Mora, C.; Orvis, K. & Finkelstein, D. Sedimentary stable carbon isotope evidence of late Quaternary vegetation and climate change in highland Costa Rica. *Journal of Paleolimnology*, **45**, 323-338 (2011).
65. Mayle, F. E.; Burbridge, R. & Killeen, T. J. Millennial-scale dynamics of southern Amazonian rain forests. *Science*, **290**, 2291-2294 (2000).
66. Turcq, B.; Cordeiro, R. C.; Sifeddine, A.; Simões Filho, F. F.; Albuquerque, A. L. S. & Abrão, J. J. Carbon storage in Amazonia during the Last Glacial Maximum: secondary data and uncertainties. *Chemosphere*, **49**, 821-835 (2002).
67. Mayle, F. E. & Beerling, D. J. Late Quaternary changes in Amazonian ecosystems and their implications for global carbon cycling. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **214**, 11-25 (2004).
68. Mayle, F. E.; Beerling, D. J.; Gosling, W. D. & Bush, M. B. Responses of Amazonian ecosystems to climatic and atmospheric carbon dioxide changes since the last glacial maximum. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, **359**, 499-514 (2004).
69. Anhuf, D.; Ledru, M.-P.; Behling, H.; Da Cruz Jr., F.; Cordeiro, R.; Van der Hammen, T.; Karmann, I.; Marengo, J.; De Oliveira, P.; Pessenda, L.; Siffedine, A.; Albuquerque, A. & Da Silva Dias, P. Paleo-environmental change in Amazonian and African rainforest during the LGM. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **239**, 510-527 (2006).
70. Bird, M. I.; Taylor, D. & Hunt, C. Palaeoenvironments of insular Southeast Asia during the Last Glacial Period: a savanna corridor in Sundaland? *Quaternary Science Reviews*, **24**, 2228-

2242 (2005).

71. Wurster, C. M.; Bird, M. I.; Bull, I. D.; Creed, F.; Bryant, C.; Dungait, J. A. J. & Paz, V. Forest contraction in north equatorial Southeast Asia during the Last Glacial Period. *Proceedings of the National Academy of Sciences*, **107**, 15508-15511 (2010).

72. Jones, M. B. & Muthuri, F. M. Standing biomass and carbon distribution in a papyrus (*Cyperus papyrus* L.) swamp on Lake Naivasha, Kenya. *Journal of Tropical Ecology*, **13**, 347-356 (1997).

73. Kotze, D. C. & O'Connor, T. G. Vegetation variation within and among palustrine wetlands along an altitudinal gradient in KwaZulu-Natal, South Africa. *Plant Ecology*, **146**, 77-96 (2000).

74. Saunders, M.; Jones, M. & Kansiime, F. Carbon and water cycles in tropical papyrus wetlands. *Wetlands Ecology and Management*, **15**, 489-498 (2007).

75. Piedade, M. T. F.; Junk, W. J. & Long, S. P. The Productivity of the C4 Grass Echinochloa-polystachya On the Amazon Floodplain Rid A-2488-2008. *Ecology*, **72**, 1456-1463 (1991).

76. Sinninghe Damsté, J. S.; Verschuren, D.; Ossebaar, J.; Blokker, J.; van Houten, R.; van der Meer, M. T.; Plessen, B. & Schouten, S. A 25,000-year record of climate-induced changes in lowland vegetation of eastern equatorial Africa revealed by the stable carbon-isotopic composition of fossil plant leaf waxes. *Earth and Planetary Science Letters*, **302**, 236-246 (2011).

77. Vogts, A.; Moossen, H.; Rommerskirchen, F. & Rullkötter, J. Distribution patterns and stable carbon isotopic composition of alkanes and alkan-1-ols from plant waxes of African rain forest and savanna C3 species. *Organic Geochemistry*, **40**, 1037-1054 (2009).

78. Medina, E. & Minchin, P. Stratification of $\delta^{13}C$ values of leaves in Amazonian rain forests. *Oecologia*, **45**, 377-378 (1980).

79. Buchmann, N.; Guehl, J.-M.; Barigah, T. S. & Ehleringer, J. R. Interseasonal comparison of CO_2 concentrations, isotopic composition, and carbon dynamics in an Amazonian rainforest (French Guiana). *Oecologia*, **110**, 120-131 (1997).

80. Ehleringer, J. R.; Field, C. B.; Lin, Z.-F. & Kuo, C.-Y. Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. *Oecologia*, **70**, 520-526 (1986).

81. Mitchell, L. E.; Brook, E. J.; Sowers, T.; McConnell, J. R. & Taylor, K. Multidecadal variability of atmospheric methane, 1000-1800 C.E.. *J. Geophys. Res.*, **116**, (2011).

82. Mischler, J. A.; Sowers, T. A.; Alley, R. B.; Battle, M.; McConnell, J. R.; Mitchell, L.; Popp, T.; Sofen, E. & Spencer, M. K. Carbon and hydrogen isotopic composition of methane over the last 1000 years. *Global Biogeochem. Cycles*, **23**, (2009).

83. Chappellaz, J.; Barnola, J. M.; Raynaud, D.; Korotkevich, Y. S. & Lorius, C. Ice-core record

of atmospheric methane over the past 160,000 years. *Nature*, **345**, 127-131 (1990).

Figure legends

Figure S1 | Magnitude of the Kr effect $\Delta\delta^{13}C_{Kr}$ derived from two distinct methods for the AWI and PSU instrument

a) the new $\delta^{13}CH_4$ time series of Vostok (light blue) and EDML (red), the Holocene GISP II record[11] (dark blue, on its original time scale), and the published EDML record[2] over termination I, that was processed anew according to the procedures in this publication (purple). The corrected time series are illustrated with thicker lines and large circle markers. The original records before the correction are shown as thin lines with small white markers.

b) The range of $\Delta\delta^{13}C_{Kr}$ values applied as individual correction to the respective datasets to account for the Kr-bias on $\delta^{13}CH_4$. The color coding is the same as in a). Note that $\Delta\delta^{13}C_{Kr}$ of both, Vostok and GISP II data measured at the PSU, were inferred indirectly from CH_4 mixing ratios, while for the two EDML records the correction is based on the Kr-induced anomaly derived from the ion-current ratios (see section 1.3 for a detailed description of both approaches).

Figure S2 | Linear scaling of the size of interference on carbon isotope measurements caused by Kr ($\Delta\delta^{13}C_{Kr}$) relative to variations in CH_4 mixing ratios for the PSU instrument

Atmospheric krypton mixing ratios are considered constant over time[13].The size of the interference (Kr effect or $\Delta\delta^{13}C_{Kr}$) thus scales with CH_4 concentrations. Measurements of three ambient air samples were used to infer a linear relationship between $\Delta\delta^{13}C_{Kr}$ and the inverted CH_4 mixing ratio for the PSU instrument, that is used to account for the Kr effect of ice core (Vostok, WDC05) and laboratory inter-calibration measurements (see section 1.3 for further details). Results for the IPY cylinder with a mixing ratio representative for glacial conditions ("glacial", 365 ppb) is shown as a light blue diamond, the one with "preindustrial" values (906 ppb) in orange, and the one representative for modern concentrations ("present-day", 1852 ppb) in dark red, all illustrated with its corresponding 1σ error range. The linear relationship in red allowed a first order estimate of

$\Delta\delta^{13}C_{Kr}$ for ice core samples measured at the PSU lab, based on interpolated CH_4 mixing ratios of the respective samples.

Figure S3 | Model results to quantify fractionation processes in the firn column due to atmospheric CH_4 concentration changes

a) Artificial atmospheric methane pulse at the model firn "surface" (grey line), and its corresponding concentration after the "bubble close-off" at the bottom of the firn column at three different accumulation rate and temperature regimens ("maximum" temperature: purple line; "minimum" temperature: red line and "best guess" temperature: light blue line). b) Shifts of the carbon isotope signature of methane caused by the gravitational and diffusional fractionation in the firn column from the constant value at the "surface" (gray dashed line) to the signals enclosed in the model "bubbles" (colored lines) at the firn bottom, according to their respective scenario (color coding similar to a))

Figure S4 | Representation of major Dansgaard-Oeschger methane rises during the last glacial period, and the respective imprint on its carbon isotope signature

a) Six strong methane rises (color coding in the figure legends) coeval to major Dansgaard/Oeschger (DO) events (left and middle figure column) and the two ultimate terminations (right column) in a 6,000 year time window aligned and centered around the major methane rise (illustrated by the vertical gray line). All CH_4 data from the EDML ice core[30] b) Corresponding time windows of the respective $\delta^{13}CH_4$ data sections from Vostok and EDML (this study, color coding and alignment according to a)). The shaded bar represents the time window, in which data points might be biased by diffusional fractionation beyond our measurement uncertainty.

Tables

Supplementary Table 1 | Comparison of $\delta^{13}CH_4$ results for the measurements of air samples performed at the AWI and PSU laboratories, that were part of the „2007 - IPY International Ice Core Gas Intercalibration Exercise".

Ambient air was diluted to resemble atmospheric methane mixing ratios typical for present day (1852 ppb), pre-industrial (906 ppb) and glacial (365 ppb) conditions. $\delta^{13}CH_4$ values are reported with respect to VPDB. The first two columns report original measurements, the following two columns the respective values after the correction for Kr interference. Deviations in the carbon isotopic signature caused by the ionized Kr ($\Delta\delta^{13}C_{Kr}$) and its dependency on CH_4 levels are summarized in the final column. The results are further used to infer the absolute standardization offset between both laboratories.

Sample ID | epoch	$\delta^{13}CH_4$ (‰)	1σ (‰)	$\delta^{13}CH_4$ (‰)	1σ (‰)	$\Delta\delta^{13}C_{Kr}$ (‰)
	Original		Corrected for Kr		
AWI analyses (5/2010)					
CA03560 | present day	-47.14	0.05	-47.33	0.06	0.19
CC71560 | preindustrial	-47.07	0.09	-47.40	0.12	0.33
CA01179 | glacial	-46.25	0.11	-46.97	0.13	0.72
PSU analyses (7/2007)					
CA03560 | present day	-47.08	0.16	-47.20	0.16	0.12
CC71560 | preindustrial	-47.15	0.10	-47.41	0.10	0.26
CA01179 | glacial	-46.86	0.06	-47.52	0.06	0.66
Difference (AWI - PSU)					
CA03560 | present day	-0.06		-0.13		
CC71560 | preindustrial	0.08		0.01		
CA01179 | glacial	0.61		0.55		
Avg. lab offset	**0.21**		**0.14**		
St.dev. (1σ)	**0.35**		**0.36**		

Supplementary Table 2 | $\delta^{13}CH_4$ values of WDC05 A ice core material measured with the AWI and PSU instruments to further test the alignment of both systems.

No adjustments have been applied to correct for gravitational settling. Values denoted as "original" are inferred according to the standard routines in the respective laboratories, before the correction for Krypton interference. Acquisitions with the AWI instrument were treated similar to EDML ice and IPY air samples correcting the chromatograms for the Kr interference, PSU measurements using the CH_4 dependent $\Delta\delta^{13}C_{Kr}$ to account for Kr. WDC05A [CH_4] values and approximate age of the samples (black diamond) were interpolated from data of Mitchell et al.,2011[81]. Note, that the two PSU measurements (marked by asterisks) are not from the same depth as the AWI sample, but $\delta^{13}CH_4$ variability (1σ) is less than 0.3 ‰ over the depth interval 161.5 m (1593 AD) to 173.4 m (1540 AD) and thus in the the order of our analytical uncertainty[82].

Sample ID	Lab	Depth (m)	Age◆ (AD)	$\delta^{13}CH_4$ (‰)	1σ (‰)	$\delta^{13}CH_4$ (‰)	1σ (‰)	$\Delta\delta^{13}C_{Kr}$ (‰)	n
				Original		Corrected for Kr			
WDC05A*	PSU	164.96	1571	-47.94	-	-48.28	-	0.34	-
WDC05A	AWI	166.78	1564	-47.53	0.03	-47.81	0.04	0.28	2
WDC05A*	PSU	169.80	1551	-47.53	-	-47.87	-	0.34	-

Supplementary Table 3 | Manual CH_4 tie-points used for the adjustment of the misaligned Vostok section in between 129-84 kyr BP[28].

The synchronization was performed with EDC[26] and Vostok[83] atmospheric methane records. The EDC3 ages of the five listed Vostok sample depths were adjusted according to the corresponding EDC tie points. All Vostok records presented in this study are dated according to this adjustments.

tie-point	EDC depth (m)	EDC3 age (years BP)	EDC CH_4 (ppb)	Vostok depth (m)	Vostok CH_4 (ppb)
1	1241.67	83627	500.7	1266.83	500.1
2	1367.89	95866	470.2	1440.34	470.0
3	1473.40	106781	510.1	1536.00	510.8
4	1543.59	115081	480.2	1635.97	480.4
5	1723.46	128871	559.9	1881.99	560.1

Supplementary Table 4 | Methane synchronization tie-points used for dating the Byrd ice core

records shown in this work. The age conversion to the “Unified” age scale[23] is based on EDML[30] and Byrd[29] atmospheric methane records.

tie-point	EDML depth (m)	“Unified” age (years BP)	EDML CH_4 (ppb)	Byrd depth (m)	Byrd CH_4 (ppb)
1	717.02	11599	609.35	1071.76	574.28
2	829.64	14543	529.66	1195.84	543.30
3	1070.95	23231	383.60	1446.29	361.95
4	1154.20	27748	392.50	1498.37	392.42
5	1173.61	28810	419.31	1526.10	417.05
6	1233.17	32339	435.59	1595.76	443.86
7	1260.48	33728	456.60	1617.35	417.67
8	1286.47	35417	467.63	1654.37	449.47
9	1337.80	38258	488.11	1716.45	490.03
10	1365.07	39433	423.15	1744.35	415.49
11	1403.97	41378	443.35	1780.49	422.27
12	1436.97	43074	452.35	1806.94	441.63
13	1489.88	46719	469.20	1863.57	461.97
14	1601.65	53264	491.10	1960.44	488.11
15	1627.36	54732	493.91	1973.59	491.04
16	1666.48	57333	532.10	2000.30	523.93
17	1680.64	58176	538.28	2011.48	533.62
18	1688.09	58586	497.84	2017.44	463.90
19	1760.37	63448	468.08	2062.90	476.49
20	1860.22	71706	470.32	2082.65	460.38
21	1914.24	75872	460.21	2100.52	455.18
22	1949.26	78706	480.43	2111.69	494.55
23	2023.03	85207	550.63	2133.58	546.72
24	2065.88	89241	493.35	2139.75	489.08