

Climate evolution in Anatolia across the late Eocene and Eocene–Oligocene Transition: isotopic evidence for warming, cooling and drying

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Supplementary Text 1: Clumped isotope analytical protocol at CEREGE

Carbonate samples are powdered with a mortar or a microdrill; they are then treated overnight with 3-8% NaOCl to remove nitrate contamination (Fiebig et al., 2024), rinsed and centrifuged 4 times and dried with a freeze-drier. Carbonate standards and sample material corresponding to ca. 80-120 µg of carbonate are then loaded into glass vials and placed in a dry oven at 50°C for at least 24h. A typical carousel of 46 vials generally includes 20 replicates of unknown samples, 20 primary standards (ETH1, ETH2, ETH3 and ETH4, see Bernasconi et al., 2018) and 6 vials of secondary standards: IAEA-603 (calcite certified reference material from IAEA, from the same marble slab as calcite standard IAEA-C1; Assonov et al., 2020), BDH (calcium carbonate laboratory standard from BDH Industries Ltd), POL2 (laboratory standard consisting of a low temperature carbonate deposited in cave pond in a Siberian cave, courtesy by S. Breitenbach from Northumbria University), and VIA1 and VIA2 (laboratory internal standard obtained from two blocks of the same travertine from a quarry in Southwestern France). Each sequence of measurements starts with 9 primary standards followed by blocks of 3 (or 4) sample aliquots interspersed with primary and secondary standards.

The powders are analyzed on a Thermo 253+ mass spectrometer attached to a Thermo Kiel IV device, piloted by Qtegra™ software. Vials are placed on the carousel in the Kiel oven held at 70°C. Each vial is in turn evacuated and held under vacuum, then acidified with 2 to 3 drops of 105% Phosphoric acid, with a reaction time of 300s. The resulting gas, released from the carbonate by acidification, is transferred to the mass spectrometer through a system of differential trapping on 2 cold traps (-190°C/-110°C /-80°C) as well as an extra Porapak-filled trap in between, which is held at -20°C by a Peltier system for the purification of the gas for clumped isotopes measurements. The transfer time is 500 seconds.

The resulting purified CO₂ gas is transferred to the 253+ mass spectrometer, where extra faraday cups will allow the measurement of clumped isotopes (m/z 47, 48, 49) on top of carbon and oxygen isotopes (m/z 44, 45, 46) and pressure baseline corrections (m/z 47.5 and 48.5). Each sample is integrated for 90 cycles.

Sessions of 46 vials (a full carousel) are acquired in Bracketed Long Integration Dual Inlet mode (LIDI bracketed, also known as LIDI2), where individual vials and reference gas are measured consecutively rather than alternately (change-over mode), with a drift correction by standardization of the sample data with the two bracketing reference data sets. The use of the LIDI2 approach in our laboratory, where daily temperature fluctuations can reach 4°C, significantly improves the analytical precision and reduces daily drifts observed in primary standard data.

To further reduce the impact of daily temperature fluctuations, the MAT253+ detector box has been customised by being wrapped with a water-circulation tubing system connected to a

chiller, with a temperature set to 21 °C. The system is then enclosed in a 3D-printed housing insulated with cork panels (designed by Technovalo Aix Plateform, Institute of Movement Sciences, Aix-Marseille University). This setup has proved to reduce daily temperature variations near the detectors to less than 1 °C and significantly improves data reproducibility within each session. The last 1/4 of the sessions were run with the system in place.

Session d45, d46, d47, d48 and d49 data are automatically calculated by Q-Tegra applying session-specific pressure baseline corrections. The beam intensity on $m/z=47$ is corrected by using the baseline signal and session-specific k-factor, the coefficient describing the proportionality between the baseline at mass 47.5 and the actual signal at mass 47, which is manually calculated before each run.

Baseline-corrected d data are screened with an in-house Matlab script for statistical outliers (based on a peirce criterion on d data, Zaruur et al., 2013), as well as for vials run with unusual acquisition parameters. These unusual acquisition parameters include: high leak rates (above 500 microbar/min), high residual pressure and non-condensable pressure (above 150 microbar), sample pressure that is too low (<600 microbar), bad bellow adjustments (pressure adjust target intensity between individual vials and the reference gas outside within +/-10%; bellow pressure position below 10% or over 90%) and poor temperature control during parts of the run. The results for the first vial of every session are also systematically off and therefore never included in the data processing. Outliers with unusual acquisition parameters are either tagged as “outliers”, or alternately as “rejected” if their d values are too off to be translatable into realistic D47 values (between 0 and 1). Note that samples with unusual acquisition parameters that are not statistical outliers are tagged but kept in the calculation of average D47 values for standards and unknowns. Screened and outlier d data are then translated into $d^{18}O$, $d^{13}C$ and D47 projected to the Intercarb-Carbon Dioxide Equilibrium Scale (I-CDES90; Bernasconi et al., 2021) using D47crunch (Daëron, 2021), pooling over data from all sessions of the dataset.

Paleotemperatures are calculated using the unified D47-T calibration of Anderson et al. (2021). Uncertainties around temperature estimates are given at 1s, with and without propagating intersession-related uncertainty (see the supplementary data). Raw data for unknown samples and standards are provided together with interpreted data, to allow the future reprocessing of D47 values and paleo-temperature estimates.

References:

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