## Impacts of paleoecology on the TEX<sub>86</sub> paleotemperature proxy

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The TEX<sub>86</sub> proxy, based on the distribution of isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) from planktonic Thaumarchaeota, is widely used to reconstruct sea surface temperature (SST). Recent observations of speciesspecific and regionally-dependent relationships between TEX<sub>86</sub> and SST in cultures and the modern ocean raise the question of whether ecological factors such as community composition may impact TEX<sub>86</sub> in the paleorecord. Here we evaluate the effects of rapid and dramatic ecological changes on GDGT distributions using one Pliocene and two Pleistocene sapropels from the Mediterranean Sea. During sapropel events, the Mediterranean experienced transient eutrophic and stratified euxinic conditions before returning to an oligotrophic background state. We find that  $U_{37}^{K'}$ -derived SSTs exhibit stable or monotonically increasing trends, thus forming a baseline against which ecological effects on TEX<sub>86</sub> can be investigated. TEX<sub>86</sub>-derived SSTs deviate from  $U_{37}^{K'}$ derived SSTs before, during, and after each sapropel. TEX<sub>86</sub>derived SSTs are consistently warmer in the Pleistocene, while the Pliocene samples exhibit both warmer and cooler (by up to 10 °C) TEX<sub>86</sub>-derived SSTs. GDGT distributions from all three sapropels differ systematically from both globally-distributed and Mediterranean core top samples within the same temperature range. The major differences are increased abundances of GDGT-2 and crenarchaeol regioisomer. Compound-specific carbon isotope ( $\delta^{13}$ C) compositions and branched-over-isoprenoid indices indicate a single, thaumarchaeal source of GDGTs and no confounding influence on TEX<sub>86</sub> from exogenous sources, such as methanotrophs or terrestrial Thaumarchaeota. Therefore, we interpret the changing relationship between TEX<sub>86</sub> and SST through time to be a result of shifts between distinct thaumarchaeal communities. Through characteristic GDGT distributions, we identify three independent populations of Thaumarchaeota in the Pleistocene, Pliocene, and modern Mediterranean Sea, respectively. Importantly, these communities prevailed not only during sapropel events but also during oligotrophic conditions. Our findings imply that shifts in thaumarchaeal ecology may affect TEX<sub>86</sub> paleorecords even within short geologic timeframes. Characteristic GDGT distributions may be used to identify communities discordant with modern calibrations.