Influence of ammonia oxidation rate on thaumarchaeal lipid composition and the TEX$_{86}$ temperature proxy

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Archaeal membrane lipids known as glycerol dibiphytanyl glycerol tetraethers (GDGTs) are the basis of the TEX$_{86}$ paleotemperature proxy. Because GDGTs preserved in marine sediments are thought to originate mainly from planktonic, ammonia-oxidizing Thaumarchaeota, the basis of the correlation between TEX$_{86}$ and sea surface temperature (SST) remains unresolved: How does TEX$_{86}$ predict surface temperatures, when maximum thaumarchaeal activity occurs below the surface mixed layer and TEX$_{86}$ does not covary with in situ growth temperatures? Here we used isothermal studies of the model thaumarchaeon Nitrospumilus maritimus SCM1 to investigate how GDGT composition changes in response to ammonia oxidation rate. We used continuous culture methods to avoid potential confounding variables that can be associated with experiments in batch cultures. The results show that the ring index scales inversely ($R^2 = 0.82$) with ammonia oxidation rate ($\phi$), indicating that GDGT cyclization depends on available reducing power. Correspondingly, the TEX$_{86}$ ratio decreases by an equivalent of 5.4 °C of calculated temperature over a 5.5 fmol cell$^{-1}$ d$^{-1}$ increase in $\phi$. This finding reconciles other recent experiments that have identified growth stage and oxygen availability as variables affecting TEX$_{86}$. Depth profiles from the marine water column show minimum TEX$_{86}$ values at the depth of maximum nitrification rates, consistent with our chemostat results. Our findings suggest that the TEX$_{86}$ signal exported from the water column is influenced by the dynamics of ammonia oxidation. Thus, the global TEX$_{86}$-SST calibration potentially represents a composite of regional correlations based on nutrient dynamics and global correlations based on archaeal community composition and temperature.

Thaumarchaeota | TEX$_{86}$ | GDGT | continuous culture | nitrification

The glycerol dibiphytanyl glycerol tetraether (GDGT) membrane lipids of Archaea are abundant in marine water columns and sediments. The major source of GDGTs to ocean sediments is thought to be planktonic, ammonia-oxidizing Archaea (AOA) affiliated with the phylum Thaumarchaeota (formerly Marine Group I Crenarchaeota) (1, 2). Thaumarchaeota play a primary role in the nitrogen cycle, performing the first and rate-limiting step of nitrification—namely, the oxidation of ammonia to nitrite (3–5). Accordingly, Thaumarchaeota are most abundant at the base of, or below, the euphotic zone (6–9). Based on the phylogeny of their ammonia monooxygenase gene, the planktonic Thaumarchaeota are divided into two distinct clusters, the Water Column Cluster A that is most abundant in the epi- and upper mesopelagic (above ~200 to ~500 m, depending on location) and the Water Column Cluster B that dominates thaumarchaeal assemblages in the deeper mesopelagic and bathypelagic (7–10). These clusters putatively represent thaumarchaeal ecotypes adapted to high and low ammonium flux, respectively (11, 12).

Thaumarchaeota produce GDGTs containing from zero to four cyclopentane rings (GDGT-0 to GDGT-4) or four cyclo-pentane rings and one additional cyclohexane ring (e.g., in crenarchaeal, see SI Appendix, Fig. S1). The ratio between a specific subset of these GDGTs is the basis of the TEX$_{86}$ paleothermometer, which assumes that planktonic Archaea adapt to higher growth temperatures by increasing cyclization in their GDGT membrane lipids (see SI Appendix, Eq. S3 (13)). This proxy is calibrated to either annual mean sea surface or subsurface temperature using modern surface sediments (e.g., 0–200 m; see SI Appendix, Eq. S4) (14, 15) and has been used for paleoclimate reconstructions over the past 100 Ma (16). Trends in reconstructed TEX$_{86}$ temperatures often agree well with other proxy records but can diverge, particularly during greenhouse climates (17, 18).

Despite its wide application, the mechanistic basis of the TEX$_{86}$ proxy remains unresolved. Culture studies have shown that the average number of cyclopentyl moieties in GDGTs can increase with growth temperature in marine Archaea (19–22); however, TEX$_{86}$ values of suspended particulate material (SPM) through the water column do not reflect in situ temperatures either in trend or in magnitude (23–29). TEX$_{86}$-calculated temperatures in sinking particles and core-top sediments are often colder than mean annual average, especially in highly productive regions such as upwelling systems (30–33). TEX$_{86}$-calculated temperatures also often reach their maximum values below 200 m, and at these depths, TEX$_{86}$ can yield temperatures that are greater than local sea surface temperatures (23, 26, 28, 34). These patterns indicate that variables other than surface or shallow subsurface temperatures are important in determining environmental TEX$_{86}$ signals.

A clue to explaining these observations comes from batch cultures of marine Thaumarchaeota. TEX$_{86}$ values increase in later growth phases (35) and at lower O$_2$ concentrations (22). The commonality between these experiments is that both experienced a variable rate of energy supply. Because energy limitation has been

**Significance**

The membrane lipids of marine Archaea form the basis of the temperature proxy called TEX$_{86}$, which is used for paleoclimate reconstructions from the Jurassic to the present. To date there remains no satisfactory explanation for how planktonic Archaea are able to record water column temperatures, because TEX$_{86}$ does not correlate well with in situ growth temperatures in the modern ocean. Here we show that the TEX$_{86}$ lipid ratio changes in response to cellular growth rate, which is controlled by the ammonia oxidation rate. This implies that variation in the TEX$_{86}$ ratio with water depth is influenced by the metabolic activity of Thaumarchaeota in the water column.

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suggested to be a defining feature of the domain Archaea (36) and GDGTs effectively reduce proton permeability and hence promote energy conservation (37, 38), we hypothesized that availability of adequate reducing equivalents, generated via an energy-dependent reverse electron flow from ammonia, could have a direct effect on GDGT distributions.

Here we performed a continuous culture (chemostat) experiment to isolate the influence of energy and e−-donor supply on the lipid composition of the marine AOA *Nitrosopumilus maritimus* SCM1. Chemostat approaches have been valuable in characterizing the response of marine taxa to changing CO2 concentrations (39) and to nutrient limitation (40), each with the objective of isolating a component of physiological response. In our experiments with *N. maritimus*, the chemostat not only controls the growth and corresponding ammonia oxidation rate but also maintains the thermal and chemical stability of the growth conditions. This approach is fundamentally different from all previous batch culture (21, 22, 35) and mesocosm (19, 20) studies of GDGT-producing planktonic Archaea, in which several potentially competing variables such as metabolic activity, temperature, community assemblage, and chemical composition of the medium simultaneously could have influenced the lipid distributions.

**Results**

**Lipid Response to Controlled Energy Supply.** We cultivated an isothermal (28 °C) continuous culture of *N. maritimus* (see SI Appendix, Fig. S2) to steady state at each of three dilution rates, corresponding to doubling times (T_d) of 71 h (slow growth rate), 30 h (intermediate growth rate), and 22 h (fast growth rate), thereby maintaining the system in continuous culture for 6 mo over the course of the experiments. Nearly identical cell concentrations (1.4–2.0 10^17 cells·mL^{-1}; see SI Appendix, Fig. S3 and Table S1) were achieved at the different growth rates by limited provision of ammonia (inflow ca. 150 μM). Cell-specific ammonia oxidation rates, as measured by the production of NO_2^−, were 1.6, 3.9, and 7.1 fmol cell^{-1}·d^{-1}, respectively. The concentration of NO_2^− in the chemostat varied by a maximum of 7% across all stages (mean value, 132.5 μM), and the pH remained within 0.07 units of the mean value (7.64). Biomass harvested during each growth rate was directly hydrolyzed to remove polar head groups and obtain total GDGTs, whereas nonhydrolyzed biomass was extracted to measure respiratory quinones and intact polar GDGTs (for structures, see SI Appendix, Fig. S1).

The relative abundance of respiratory menaquinones (MK; see SI Appendix, Fig. S1)—isoprenoid lipids that act as intramembrane electron and proton carriers within the thauamarchaeal respiratory chain (41)—decreased at lower ammonia oxidation rates (ϕ), representing between 1.0% and 2.5% of total lipids (Fig. L4). This is consistent with a down-regulated electron transport chain in more slowly metabolizing cells.

The cyclization of total GDGTs, or the relative number of cycloalkyl moieties known as the ring index (RI), decreased at higher ammonia oxidation rates (Fig. 1 B and C). This reflects an underlying decrease in the cyclization of individual intact polar GDGTs and core GDGTs (see SI Appendix, Fig. S4). However, the relative abundance of different intact polar GDGT classes did not change systematically with ammonia oxidation rate (see SI Appendix, Fig. S4). Therefore, the cyclization trend in total GDGTs represents the individual response of the most abundant intact polar GDGT classes. The cumulative effect in total GDGTs was a decrease in the most abundant GDGT structures, crenarchaeol and GDGT-2, at the benefit of GDGT-0 and GDGT-1, with increasing ammonia oxidation rates. GDGTs of minor abundance (GDGT-3 and the crenarchaeal regiosommer) did not show a systematic variation with ammonia oxidation rate. Accordingly, the GDGT RI (see SI Appendix, Eq. S5) decreased with higher ammonia oxidation rates (Fig. 1 B), and the RI calculated only from GDGTs with zero to four cyclopentane rings (GDGT-0 to GDGT-4; see SI Appendix, Eq. S6) had a similar response to ϕ (Fig. 1 C).

**Implications for Previous Batch Culture Experiments.** Batch cultures of the same thauamarchaeal strain (*N. maritimus* SCM1) grown at different metabolic energy levels provide the closest experimental analogs to our chemostat results. Elling et al. (35) showed that the apparent temperature calculated from TEX86 values increases over the course of a batch culture. Harvesting batch cultures during successive growth phases (i.e., early growth, late growth, and stationary phase) presumably samples decreasing ammonia oxidation rates in a system that is becoming more energy-limited as the ammonia supply is used up (35). We estimated the ammonia oxidation rates for the different growth phases from the reported TEX86 temperatures using Eq. 1. This resulted in estimated cell-specific ammonia oxidation rates that decrease by ca. 12 fmol cell^{-1}·d^{-1} from the early growth phase, when there is excess substrate, to the stationary phase, when the substrate is depleted (Fig. 24). We then used the published cell densities and time-dependent NO_2^− concentrations from ref. 35 to independently calculate the experimental ammonia oxidation...
rates and compare them to the TEX86-derived estimates. The experimental ammonia oxidation rates similarly decreased from early growth phase to stationary phase, however the magnitude of change was somewhat greater than the estimated rates from the TEX86 data and Eq. 1.

Qin et al. (22) showed that the apparent temperature calculated from TEX86 values increases in batch cultures incubated with lower headspace O2 concentrations. Varying the O2 concentration should similarly control the ammonia oxidation rate by limiting the availability of e−-acceptor, instead of (or collimiting with) the e−-donor. The cell-specific ammonia oxidation rates estimated from the reported TEX86 values and Eq. 1 decrease by ca. 5 fmol cell−1 d−1 under oxygen-limiting conditions (Fig. 2B). These estimated rates agree well with the experimental ammonia oxidation rates determined from ref. 22 using the reported cell densities and NO2− concentrations.

In all three culture systems—chemostat, high-nutrient batch (35), and O2-limiting batch (22)—the trends in TEX86-estimated ammonia oxidation rates are consistent with experimental ammonia oxidation rates. These experiments with N. maritimus point to a common TEX86 response, in which TEX86-derived temperatures decrease as ammonia oxidation rate increases.

**Applicability to Marine Water Columns.** Cell-specific ammonia oxidation rates in the chemostat (1.6–7.1 fmol cell−1 d−1), as well as those estimated for previous batch cultures (22, 35), fall well within activities of natural assemblages in pelagic and coastal settings (0.2–15 and 10 fmol cell−1 d−1, respectively) (8, 10).

The slow ammonia oxidation rate additionally resembled the pelagic AOA enrichment. Candidatus Nitrosopelagicus brevis (2 fmol NO2−·cell−1·d−1; Td, 98 h) (42, 43), as well as North Sea enrichments (2–4 fmol NO2−·cell−1·d−1) (44).

To examine the relationship between TEX86 ratios and ammonia oxidation rates in the environment, we measured GDGTs, NO2− concentrations, in situ temperatures, and nitrification rates for the upper water column of two locations in the South Atlantic Ocean (Fig. 3) (45). We calculated a mass-weighted integration of TEX86 values to approximate the export signal from the upper water column (see SI Appendix) and compared it to the nearest sedimentary value from the global calibration dataset (46). At station 15, the integrated and sedimentary TEX86 values (22.1 °C and 23.8 °C; Fig. 3, black and red triangles, respectively) are colder than measured SST (28.9 °C; Fig. 3, column 4), potentially due to productivity differences associated with equatorial upwelling (see SI Appendix, Fig. S5). At station 7, in the oligotrophic gyre, the integrated TEX86 temperature and the nearest sedimentary TEX86 value (29.8 °C and 26.5 °C, respectively) agree with measured SST within calibration error (27.4 °C; Fig. 3, column 4). GDGT abundances and TEX86 profiles from these stations showed depth-dependent features consistent with nitrification rates and NO2− concentrations. The abundance of GDGTs was low in surface waters and highest just below the depth of maximum NO2− concentrations and maximum measured nitrification rates (Fig. 3, columns 1–3, blue shading). TEX86 values decreased between surface waters and the zone of maximum nitrification rates. Minimum TEX86-calculated temperatures were observed coincident with maximum NO2− concentrations. Below this depth, TEX86-calculated temperatures increased by ~7–12 °C through at least 250 m, consistent with decreasing nitrification rates. Over the same depth range, however, in situ temperatures decreased by 5 °C. Similar observations of a TEX86 minimum and an inflection of the TEX86 profile near the NO2− concentration maximum have been made in other water columns sampled at high resolution (e.g., refs. 28, 34), suggesting that this is a ubiquitous phenomenon.

**Discussion**

The RI describes the relative numbers of cycloalkyl moieties of GDGTs. Because thermophilic Archaea use cyclization as an adaptation to growth temperature (47, 48), the similarly formulated TEX86 ratio was assumed to depend primarily on growth temperature in marine Archaea (13). Although it is now acknowledged that other factors such as community dynamics (e.g., refs. 29, 34) may affect TEX86, and that in situ temperatures and TEX86 are decoupled in the global subsurface ocean (23–28), these arguments remain centered around a temperature-driven response.

The temperature response argument was initially supported by the relationship of TEX86 with in situ temperatures in the surface ocean (0–100 m), as this strong correlation (R2 = 0.75) had a linear slope similar to the global core top calibration (24). However, no such correlation was found for water depths below 100 m, and updating this calibration to include all published SPM data shows that the relationship between 0–100 m is weaker than the early data indicated (see SI Appendix, Fig. S6). The cumulative data show that the TEX86 ratio of intact polar GDGTs is not correlated to temperature and that the coefficient of determination values for core GDGTs—irrespective of the chosen depth interval (0–100 m or 0–200 m)—are much lower (R2 = 0.56) than either the initial SPM or core-top calibrations (13–15, 24). Therefore factors other than growth temperature likely exert significant influence on GDGT distributions and the TEX86 core-top calibration, especially when considering that the sedimentary record includes additional confounding factors such as production of GDGTs throughout the water column, changes in community composition, and selective export mechanisms (e.g., refs. 18, 25, 29, 34).

Energy limitation has been suggested to be a defining feature of Archaea, and maintaining low proton permeability of their membranes is a common need of both thermophilic Archaea and of mesophilic Thaumarchaeota (36). Remarkably, mesophilic Thaumarchaeota and thermophilic Archaea seem to use the same mechanism, GDGT cyclization, to regulate membrane fluidity and proton permeability (21, 22, 37, 38, 47). At the depth of maximum thaumarcheal activity, between 50 and 200 m (e.g., 7, 10), most ocean waters have temperatures between 5 and 15 °C (with the exception of polar latitudes). These temperatures are much colder and represent a smaller dynamic range than experiments performed with thermophiles, yet GDGTs of marine Archaea consistently have higher RI values than thermophiles (22, 35, 47). The high RI of marine mesophilic Thaumarchaeota as well as increasing TEX86 values in later growth phases (35) and at lower O2 concentrations (22) suggest that energy conservation may be an important factor influencing GDGT composition in these Archaea.
Cellular Energy Balance and the Synthesis of GDGTs: The Reducing Power-Limitation Hypothesis. The in situ energetic stress imposed by low ammonia and/or O₂ availability, as well as the intrinsically low energetic yield of ammonia oxidation, may qualify the Thaumarchaeota as energetic extremophiles (36). This idea is consistent with studies that show a low half-saturation constant (K_{m,\text{NH}_4} + \text{NH}_4^+ = 98–132 nM for ammonia oxidation by N. maritimus (49, 50), and it suggests adaptation to growth under conditions of constantly low energy flux, as typically found in open ocean waters. N. maritimus (as well as all recently characterized Thaumarchaeota) fixes inorganic carbon using an energy-efficient hydroxypropionate/hydroxybutyrate (HP/HB) pathway that minimizes input of chemical energy from ATP and yields the products acetate (HP/HB full cycle) and succinate (HP half cycle) (43, 51). The results of our chemostat experiments are consistent with this view of broad metabolic adaptation to energy stress in Thaumarchaeota. How, then, does this observation explain GDGT biosynthesis? In the context of ammonia limitation? In addition to promoting energy conservation through decreased proton permeability, we argue that there may also be a biosynthetic explanation.

Most chemolithotrophs conserve only a small fraction of the energy obtained from the respiration of inorganic substrates—that is, most of the energy flux is dissipatory. From the conserved fraction, they must generate reducing power for biosynthetic processes, and in most cases, including AOA, this requires energy-dependent reverse electron transport. For example, the electrochemical potential of the NH₄⁺/NO₂⁻ redox couple is insufficient to reduce NAD(P)⁺ to NAD(P)H₂. However, the synthesis of GDGTs is a possible mechanism of energy conservation through decreased proton permeability, and energy as well as in the more traditional sense of maintaining low proton permeability (37, 48). In this way, adaptation to e⁻-donor limitation in Thaumarchaeota and to higher growth temperatures in both Thaumarchaeota and archaeal thermophiles operates via the same mechanism: It reduces proton permeability by increasing the packing density of membrane lipids (38). If there is an insufficient rate of FADH₂ regeneration under energy-limited conditions, DGGGP may form a cycloalkyl ring rather than saturating the double bond, thereby saving 2 e⁻ (Fig. 4F).

Although speculative, this hypothesis offers a direct, mechanistic explanation for greater numbers of cyclopentyl rings at lower ammonia oxidation rates. The idea may be testable through measurements of natural hydrogen isotope fractionation as an indicator of cellular hydrogenase activity (56). Our chemostat data, as well as previous batch culture studies from which oxidation rates may be inferred (Fig. 2) (22, 35) also point to the plausibility of this hypothesis, as the RI scales directly with respiratory activity as expressed in the abundance of the electron carrier MK (Fig. 4A). Further, RIs calculated with and without crenarchaeol yield the same slopes, suggesting that the cyclohexyl-bearing crenarchaeol may serve a similar physiological role as cyclopentyl-containing GDGTs (Fig. 1B and C).

![Fig. 4. Proposed synthesis of GDGT-0 from two molecules of DGGGP includes a saturation step (A) mediated by GGR and requiring the cofactors FADH₂ and NAD(P)H₂. Electron limitation (B) may slow the regeneration of one or more of these cofactors, causing internal cyclization rather than saturation.](https://example.com/fig4)

![Fig. 3. South Atlantic Ocean water column properties support the relationship between TEX₈₆ and ammonia oxidation rate (22.5°S 33.0°W: station 7; 2.7°S 28.5°W: station 13). Column 1: NO₂⁻ concentrations. Column 2: Measured nitrification rates (referto SI Appendix for methods). Column 3: GDGT abundance in small (0.3–0.7 μm, dotted line) and large (0.7–5.3 μm, solid line) suspended particulate size classes. Column 4: TEX₈₆ values from small and large size classes compared with in situ measured temperatures (blue line). The zone of maximum nitrification rates, NO₂⁻ concentrations, and TEX₈₆ minima is highlighted by the light blue horizontal shading. Red triangles represent the nearest sediment TEX₈₆ values compiled from ref. 46, whereas black triangles represent the integrated TEX₈₆ value from 0 to 500 m.](https://example.com/fig3)
Environmental Implications. The relationship between GDGT cyclization and energy limitation observed in N. maritimus provides a long sought-after mechanism to explain the directionality of water column TEX$_{86}$ patterns. While water column profiles typically show inflections in apparent TEX$_{86}$ temperatures, first falling toward the NO$_3^-$ maximum, while temperature is also decreasing, and then rising below this point, while temperature continues to decrease (Fig. 3) (23, 28, 34). Invoking a temperature-only control is not sufficient to explain patterns of water column TEX$_{86}$. In contrast, ammonia oxidation rate can uniquely explain the directionality of the observed TEX$_{86}$ changes with depth. Although it is possible that the ammonia oxidation rate itself depends on temperature—essentially rendering oxidation and temperature inseparable variables—the kinetic response of ammonia oxidation rate to increasing temperature appears to be insignificant in natural marine communities (50). The TEX$_{86}$–temperature relationship inferred from core-top sediments may represent the interplay of several variables (e.g., ammonia oxidation rate, temperature, export depth) or the covariation of temperature and TEX$_{86}$ with other, latitude-dependent, oceanographic and ecological parameters.

Our results suggest that the process of GDGT cyclization in the marine water column is not directly controlled by growth temperature, while simultaneously explaining how an apparent link to temperature may arise. Net primary production varies in response to density stratification and its effect on nutrient exchange through vertical mixing (57). This explains why environments with lower rates of primary production and sub-mesotrophic zone nutrient regeneration—that is, lower ammonia oxidation rates (8, 58)—have warm TEX$_{86}$-calibrated SSTs. Similarly, TEX$_{86}$ warming observed within oxygen minimum zones is likely caused by energy stress and a corresponding decrease in ammonia oxidation rates; that is, it is not directly caused by physiological response to low O$_2$ as suggested by Qin et al. (22). This explains why the subsurface TEX$_{86}$ warming trend also is observed in well-oxygenated water columns and not limited only to oxygen minimum zones (23, 27). In contrast, cold TEX$_{86}$ temperatures are observed in surface sediments and SPM at high-nutrient/high-productivity sites such as upwelling systems (e.g., refs. 30–33).

Further experiments with a broader range of thaumarchaeal ecotypes are necessary to understand the global relationship between TEX$_{86}$ ammonia oxidation rates, and temperature. N. maritimus was isolated from a tropical fish tank in the Seattle aquarium (3) and displays a narrow growth range (~20–30 °C) and a high temperature optimum (28 °C), which is particularly applicable to the low latitude environment studied here. However, the global TEX$_{86}$ calibration encompasses temperate and polar eco- and phylotypes (e.g., ref. 59) that may exhibit different magnitudes for the relationship between TEX$_{86}$ and ammonia oxidation rate (Eq. 1), as evidenced by latitude-dependent and ocean basin-dependent TEX$_{86}$ patterns (15, 18). Further, the range of ammonia oxidation rates observed in the ocean (8, 10) is much larger than in our culture system, indicating that the range of TEX$_{86}$ changes that might be attributable to metabolic activity is likely larger than shown here for N. maritimus.

Indeed, previous studies indicate that GDGT composition may significantly vary even between different strains of cultured planktonic Thaumarchaeota (21, 22). The transition from shallow-water to deep-water thaumarchaeal ecotypes has been invoked to explain different relative GDGT compositions through the water column (e.g., refs. 29, 34). However, the major shift to the deep-thaumarchaeal eco- and phylotype occurs well below the typical abundance and activity maxima and below the depth where TEX$_{86}$ is typically most variable (e.g., refs. 6–9, 11, 12). The fact that the inverse correlation between TEX$_{86}$ and ammonia oxidation rate determined for a single taxon, N. maritimus, agrees with the overall patterns of TEX$_{86}$ values produced by natural assemblages in the marine water column suggests that it may represent a universal mechanism. Thus, the TEX$_{86}$ inflection in the zone of maximum nitrification is likely not influenced, or only secondarily influenced, by variations in community composition.

The latitudinal distribution of thaumarchaeal ecotypes suggests that there are regionally distinct populations: Low-ammonia adapted ecotypes predominate in less productive, stratified regions such as subtropical gyres and deep water (ca. >200–500 m), whereas high-ammonia adapted ecotypes dominate more productive sites such as subarctic and equatorial upwelling regions (11, 12). Differences in the TEX$_{86}$-ammonia oxidation rate relationship inferred from core-top sediments may represent the calibration models of these ecotypes potentially offer an explanation for regional TEX$_{86}$–temperature correlations. In addition to focusing on depth distributions of ecotypes, future work also will need to focus on the influence these spatial trends in thaumarchaeal ecology have on GDGT distributions.

Currently, regional or spatially variable calibration models appear to be the most reasonable choice for paleotemperature reconstructions, as they account for modern variations in hydrographic and ecological effects. It may be challenging to accurately interpret TEX$_{86}$–temperature relationships from circulation-driven nutrient regeneration rates or total global nutrient budgets may have defined spatially and temporally distinct TEX$_{86}$ correlations. Thus, the development of calibration models based on analogous biogeochemical environments is highly warranted for assessing the complex ecological effects amalgamated into the TEX$_{86}$ ratio and for improving our ability to reconstruct past climate.

Materials and Methods

N. maritimus SCM1 was maintained at 28 °C and pH 7.6 in the dark in a 4.5-L-containing Synthetic Crenarchaeota Medium (150 μM NH$_4^+$; see SI Appendix, SI Materials and Methods and Fig. S2). Specific growth rate (d$^{-1}$) was controlled by dilution with fresh medium, and three steady-state experiments were performed (slow growth rate, 0.23 d$^{-1}$; 71 h $T_{	ext{max}}$ intermediate growth rate, 0.56 d$^{-1}$; 30 h $T_{	ext{max}}$ fast growth rate, 0.75 d$^{-1}$, 22 h $T_{	ext{max}}$). (SI Appendix, Fig. S3).

Depth profiles of (SPM) samples were retrieved from a latitudinal transect through the Atlantic Ocean (see SI Appendix, Fig. 35) using in situ pumps equipped with a sequence of filters (32 μm, 0.7 μm, 0.3 μm). Lipids were extracted from biomass harvested from chemostat effluent by cross-flow filtration or centrifugation. Intact polar lipids and quinones from biomass and SPM were analyzed using reversed-phase HPLC–MS. Core lipids extracted from hydrolyzed biomass were analyzed using normal-phase HPLC–MS. For details, see SI Appendix.

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