

REASSESSING INTACT POLAR MEMBRANE LIPIDS AS BIOMARKERS FOR LIVING MICROBIAL CELLS

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Polar membrane lipids, which include phospholipids, glycolipids and sphingolipids, constitute the bulk of cell membranes in most organisms and are widely used as biomarkers in microbial ecology and biogeochemistry. In particular, the presence of intact phospholipids, measured either directly by HPLC/ESI-MSⁿ or indirectly as core lipids derived from phospholipids, is viewed as indicative of living biomass (e.g., Sturt *et al.*, 2004). Based upon the observation that the bond between the phosphate-based headgroup and the glycerol backbone is rapidly hydrolyzed upon cell death (Harvey *et al.*, 1986), intact phospholipids are used to trace the presence of living microbial cells in a large variety of natural settings.

We applied this biomass proxy in an attempt to determine the *in situ* presence of living anaerobic ammonia oxidizing ('anammox') bacteria in marine sediments from the Swedish Gullmar Fjord. The ladderane lipids that make up the membranes of anammox bacteria have recently been identified as phospholipids, comprising either phosphocholine or phosphoethanolamine headgroups (Boumann *et al.*, 2006). Using purified ladderane lipid standards, we quantified the concentrations of C₂₀-[3]-ladderane monoalkyl-glyceride (core lipid) and of C₂₀-[3]-ladderane monoalkyl-phosphocholine (intact phospholipid) in a number of Gullmar Fjord sediment cores, by HPLC/ESI-MS² (Fig. 1). As expected, there are marked differences between the two lipid depth profiles. The amounts of intact phospholipid are consistently lower and more variable than the amounts of core lipid, implying that the total ladderane core lipid pool in the sediment is made up of ladderanes derived from both living and dead anammox bacteria. However, neither lipid profile correlates very well with anammox bacterial 16S rDNA copy numbers in the same sediments, as determined by quantitative real-time polymerase chain reaction (Q-PCR) (Fig. 1). In particular, the discrepancy between the amounts of intact phospholipid and the Q-PCR measurements is noteworthy, since 16S rDNA is considered a relatively good indicator for living cells. This discrepancy implies that there may be a fossil component to the total intact phospholipid and/or 16S rDNA pool as well.

While fossil DNA has indeed been found in some sedimentary records (e.g., Coolen and Overmann, 1998), the possibility of fossil phospholipids has, as yet, not been considered.

To address this possibility, we are using cultures of the marine diatom *Chaetoceros calcitrans* as a model system to study the phospholipid composition of both living and dead microbial cells. Degradation of the cell membranes is examined under a variety of conditions, such as oxic versus anoxic, and in the presence or absence of bacteria. The phospholipid data will be compared with *Chaetoceros* cell counts and quantitative 16S rDNA measurements. Thus, it is expected that these results will provide us with new insights into the various factors influencing the process and rate of phospholipid degradation and will elucidate the question if intact phospholipids can indeed be used as reliable tracers for living microbial cells.

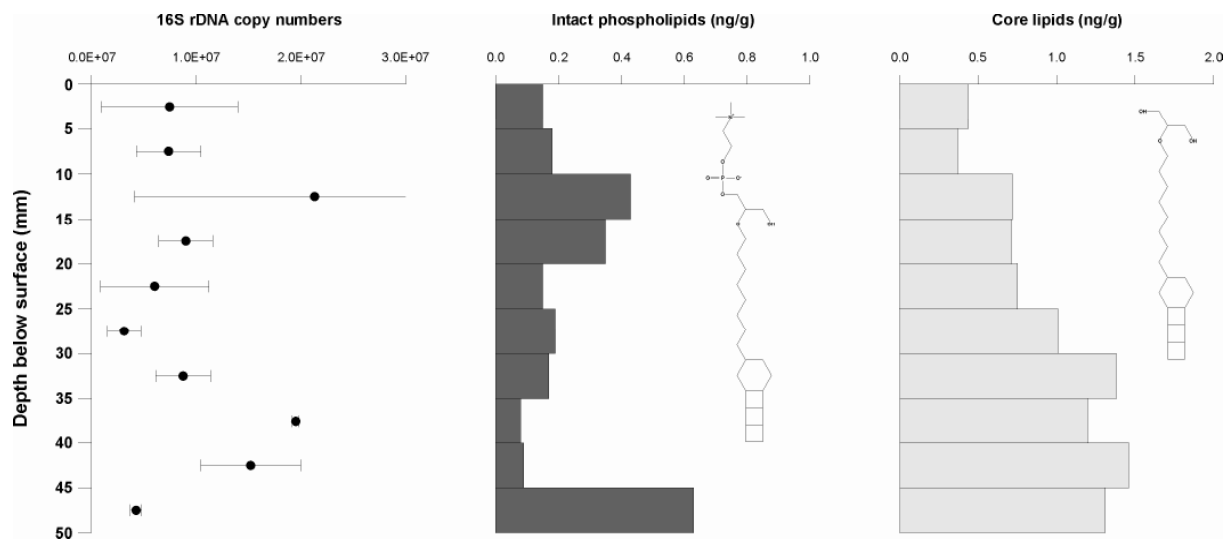


Figure 1. Depth profiles of (*left*) anammox bacterial 16S rDNA copy numbers, (*centre*) C₂₀-[3]-ladderane monoalkyl-phosphocholine and (*right*) C₂₀-[3]-ladderane monoalkyl-glyceride in sediment cores from the Gullmar Fjord.

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MARINE ARCHAEA LIPIDS: PATTERNS AND PROVENANCE IN THE WATER-COLUMN

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We measured archaeal lipids from globally distributed samples of freshwater, marine, and hypersaline suspended particulate matter. Cluster analysis of relative lipid distributions identified four environmentally distinct groups, including: 1) marine epipelagic waters, 2) marine mesopelagic/upwelling waters, 3) freshwater/estuarine waters, and 4) hypersaline waters. There is a distinct near-absence of ring-containing glycerol dialkyl glycerol tetraethers (GDGTs) at high salinity. Different archaeal communities populate marine (mesophilic Crenarchaeota and Euryarchaeota), and hypersaline environments (halophilic Euryarchaeota) and community shifts must regulate differences in lipid patterns between marine and hypersaline waters. We propose that community changes within mesophilic marine Archaea also regulate the lipid patterns distinguishing epipelagic and mesopelagic/upwelling zones. Changes in the relative amounts of crenarchaeol and caldarchaeol and low relative abundances of ringed structures in surface waters differentiate lipids from the epipelagic and mesopelagic/upwelling waters. Patterns of lipids in mesopelagic (and upwelling) waters are similar to those expected of the nitrifying Group I Crenarchaeota, with predominance of crenarchaeol and abundant cyclic GDGTs; non-metric multidimensional analysis (NMDS) shows this pattern is associated with high nitrate concentrations (likely tracking nitrite). In contrast, limited culture evidence indicates marine Group II Euryarchaeota produce mainly caldarchaeol and some, but not all, of the ringed GDGTs and we suggest that these organisms contribute significantly to lipids in epipelagic marine waters.

Calculated TEX₈₆ temperatures from particles in sediment traps in the northeastern Pacific and Arabian Sea are well-correlated to annual sea surface temperature, indicating that the TEX₈₆ signal reaching sediments is derived primarily from the surface waters (Wuchter et al. 2006). However, Calculated TEX₈₆ temperatures in mesopelagic samples (reported here and in Wuchter et al. 2004) are always much warmer than measured *in situ* temperatures. Furthermore, the residual temperature (calculated TEX₈₆ temperature-*in situ* temperature)

correlates with nitrate concentrations (Figure 1). Our cluster analysis, ordination, TEX_{86} results all suggest observed values of TEX_{86} are subject to changes in archaeal ecology as influenced by nutrient fluctuations or other perturbations which in turn can affect both surface water GDGT production and GDGT preservation and transport such as zooplankton grazing. Therefore, in ancient applications, reported extreme temperatures shifts (e.g. Zachos et al. 2006; Dumitrescu et al. 2006) may indicate the TEX_{86} lipids are not recording temperature alone, but equally interesting changes in nutrient concentrations, oceanographic conditions, and ecology.

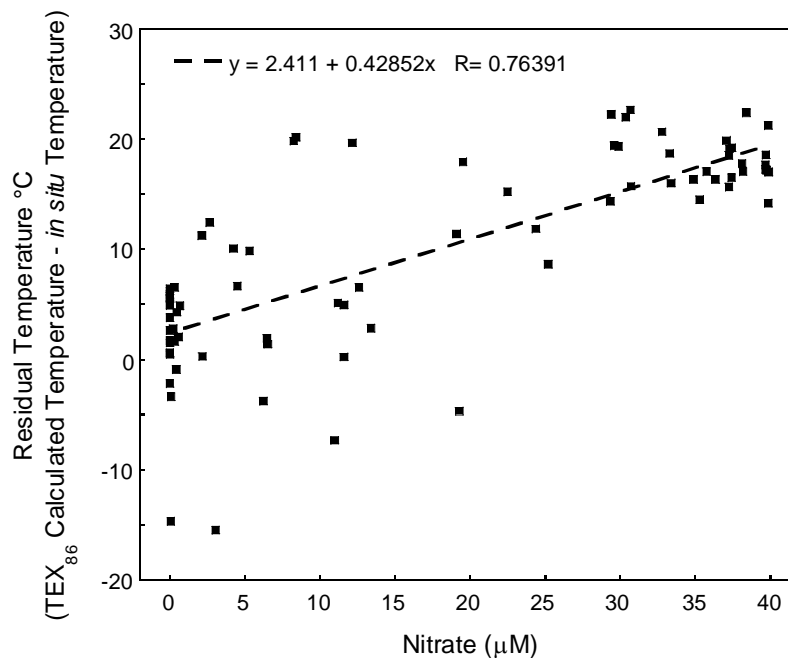


Figure 1. Temperature residuals (Calculated TEX_{86} temperatures – *in situ* temperature) from this study and from Wuchter et al. (2005) plotted against nitrate. Sites include the Arabian Sea, the Equatorial Pacific, and the Bermuda Time Series site and the Bermuda-Atlantic Time Series site.

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C₂₀-C₃₂ N-ALKAN-1-OL DISTRIBUTIONS AS MARKERS OF CONTRIBUTION FROM C₄ PLANTS IN MARINE SEDIMENTS

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Climate in the eastern tropical Indian Ocean is strongly influenced by the semiannually reversed wind regime of the Asian-Australian monsoon, the migration of the Intertropical Convergence Zone (ITCZ) and the land-sea distribution. Since Indonesia is enclosed between the thin Sunda and Sahul Shelf seas, changes in sea level during glacial-interglacial cycles have a pronounced effect on the climate, especially in relation to precipitation. As a result, this area is characterised by extreme climate conditions which are recorded in the land vegetation. Accordingly, the study of the sedimentary composition of higher plant markers, namely long-chain *n*-alkanes and *n*-alkan-1-ols, provides information on the climate conditions of the area. In this respect, contents, distribution patterns and molecular stable carbon isotope composition of these compounds have been studied as markers of continental vegetation over the last 320 kyr in a set of samples from the tropical Indian Ocean core MD98-2165 (9°38'96S, 118°20'31E, 2100 m).

The concentration changes of these compounds show a general pattern dominated by the well defined glacial-interglacial oscillation. Eolian inputs were much stronger during the glacial periods. The *n*-alkanes range between C₂₃ and C₃₃ and are characterised by high odd-over-even carbon number preference and predominance of the C₃₁ *n*-alkane, whereas the *n*-alkanols range between C₂₀ and C₃₂ and are dominated by even-over-odd carbon numbers with maxima at C₂₈ or C₃₂ *n*-alkanol. In this respect, warmer and wetter conditions appear to favour deposition and preservation of the C₃₀ homologue and colder and drier conditions favour the C₂₈ *n*-alkanol. However, the C₃₂ *n*-alkanol becomes an additional major homologue during the glacial times, suggesting an expansion of C₄ plants during these arid conditions as reported by Rommerskirchen *et al.* (2006). The stable carbon isotope weighted mean average of the *n*-alkanes (*n*-C₂₇ to *n*-C₃₃) fall in the range between -30.5 and -34.5‰, typical of leaf-wax *n*-alkanes biosynthesised by C₃ plants. The lower δ¹³C values are observed during warm and humid interglacial periods, when the estimated C₄ plants contribution decreased ~15 wt %. This is consistent with the negative relationship existent between δ¹³C of C₃ plants and water availability (Liu *et al.*, 2005; Miller *et al.*, 2001). The weighted mean average δ¹³C values of *n*-alkanols (C₂₀-C₃₂) fall in the range between -24.4 and -32.6‰, again with lower

$\delta^{13}\text{C}$ values during interglacials. Amazingly, the $\delta^{13}\text{C}$ of C_{32} n -alkanol reveals a clear C_4 plant signature during cold and dry conditions (Fig. 1). These results demonstrate that n -alkanes and n -alkanols, but most particularly the C_{32} n -alkanol, show a distinct pattern of contributions from C_4 plants to marine sediments during arid conditions and therefore, they can be used as indirect proxy of continental climate conditions in the tropics.

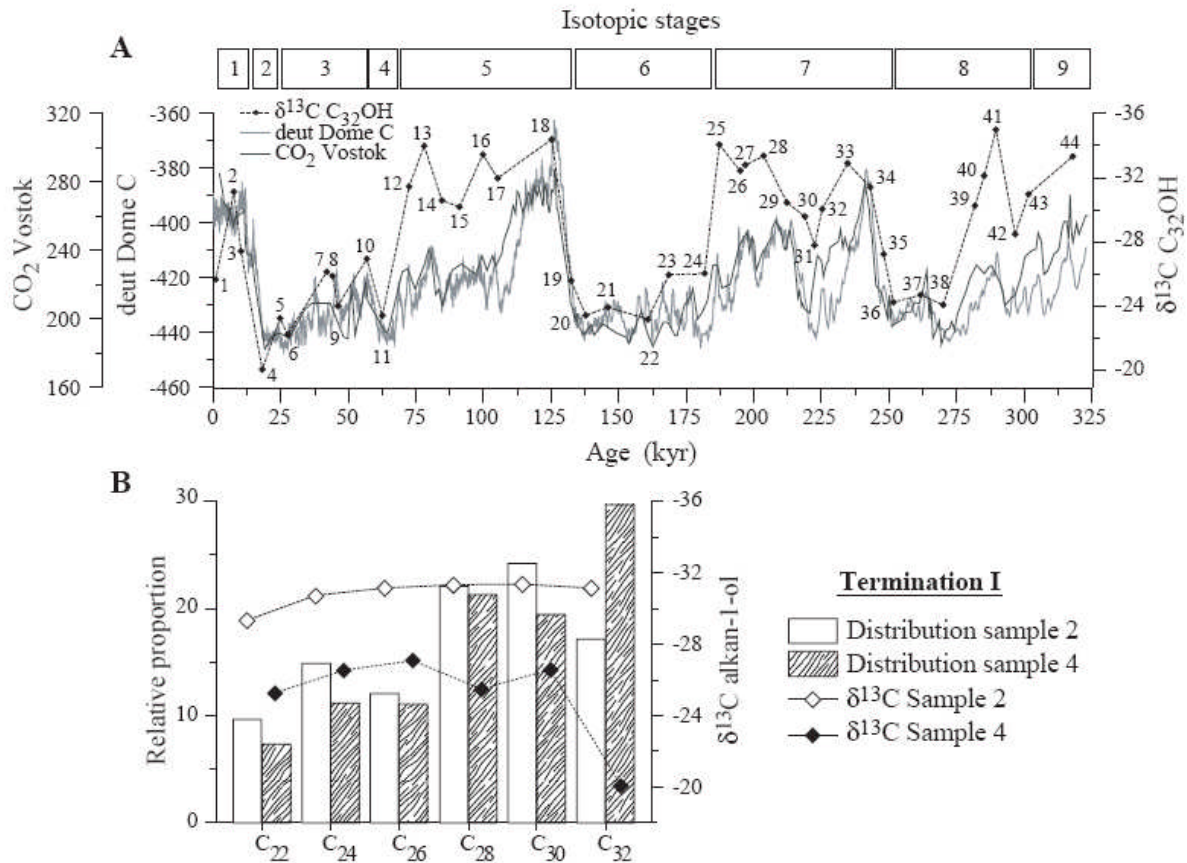


Figure 1. A. $\delta^{13}\text{C}$ variation of C_{32} n -alkanol along the last 320 kyr according to the CO_2 record from Vostok and the deuterium record from Dome C. B. Carbon number distributions and molecular carbon isotope shifts of long chain n -alkanols along the last deglaciation. The white and dark symbols and bars belong to interglacial and glacial samples, respectively.

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