

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.

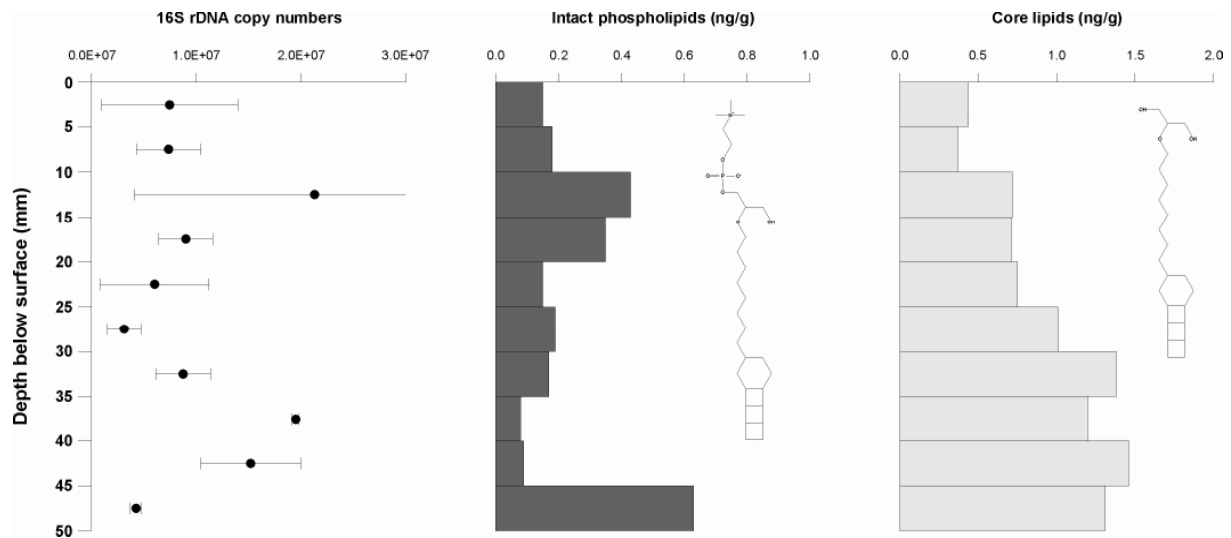


Figure 1. Depth profiles of (*left*) anammox bacterial 16S rDNA copy numbers, (*centre*) C_{20} -[3]-ladderane monoalkyl-phosphocholine and (*right*) C_{20} -[3]-ladderane monoalkyl-glyceride in sediment cores from the Gullmar Fjord.

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