

Control and distribution of anammox bacteria and ladderane lipids

Jayne E. Rattray, Ellen C. Hopmans, Stefan Schouten and Jaap S. Sinninghe Damsté*

Anaerobic ammonium oxidizing bacteria (anammox) are newly discovered bacteria in the marine nitrogen cycle, with the unique ability to oxidize ammonium and nitrite under anoxic conditions. Samples from a 2005 cruise have provided us with evidence that anammox bacteria play a major role in oceanic nitrogen cycling in the Peruvian upwelling. Evidence for the presence of anammox bacterial cells was, in part, determined by analysing for their unique 'biomarker' ladderane lipids. In a separate project we further investigated the effect of temperature on the production of ladderane lipids in anammox bacteria, in different environments (enrichment cultures, particulate organic matter and surface sediments). This resulted in the calculation of the index of Ladderane lipids with 5 cyclobutane rings (NL₅). The NL₅ has application in determining the origins of anammox bacteria in surface sediments (i.e. if anammox bacteria originated from the upper warmer water column or the colder surface sediments). The NL₅ index could be of relevance for future paleo-environmental studies.

Introduction

Until recently it was understood that the most important process responsible for the removal of nitrogen from the marine environment was denitrification (Fig. 1). However, in 1995 the discovery of the anaerobic ammonium oxidation (anammox) process in a wastewater treatment reactor has changed the way we view the cycling of nitrogen under anoxic conditions. Anammox bacteria performing the anaerobic oxidation of ammonium were found to be members of the bacterial group, the planctomycetes. Like all other bacteria classified as planctomycetes, anammox bacteria contain special compartments in their cell (Fig. 2a). However, unlike their closest relatives, they contain a unique compartment called the anammoxosome, the postulated site of the anammox reaction. In addition, anammox bacteria have been found to contain

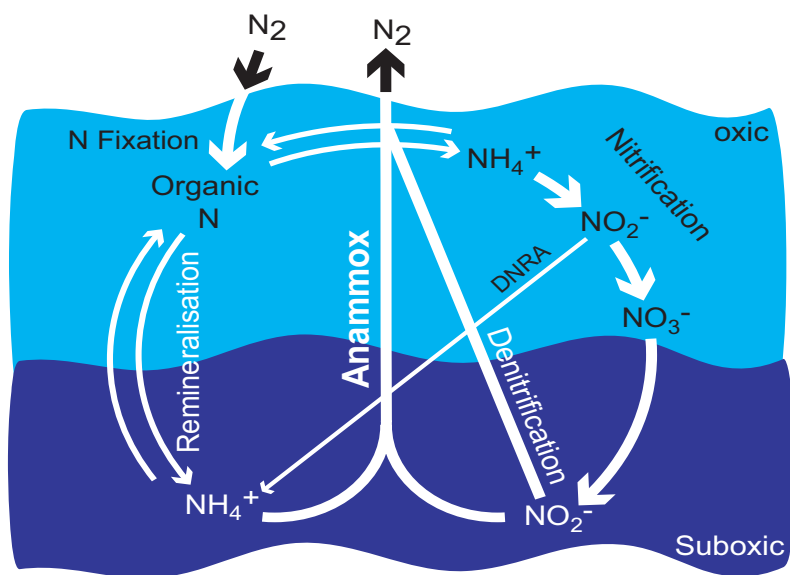


Fig. 1. Simplified version of the marine nitrogen cycle following the discovery of the anammox reaction. DNRA – disimilatory nitrite reduction to ammonium.

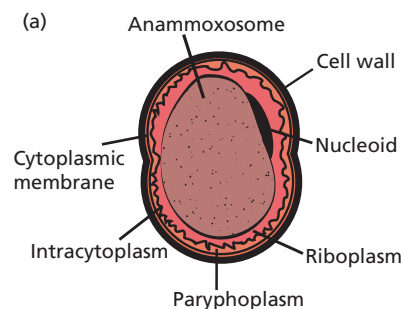


Fig. 2. The anammox cell (a) and ladderane fatty acid structures (b)

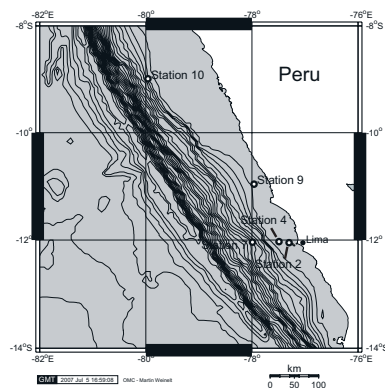


Fig. 3. Sampling sites and stations along the Peruvian oxygen minimum zone during the R/V Olaya 2005 cruise

*Corresponding author: damste@nioz.nl

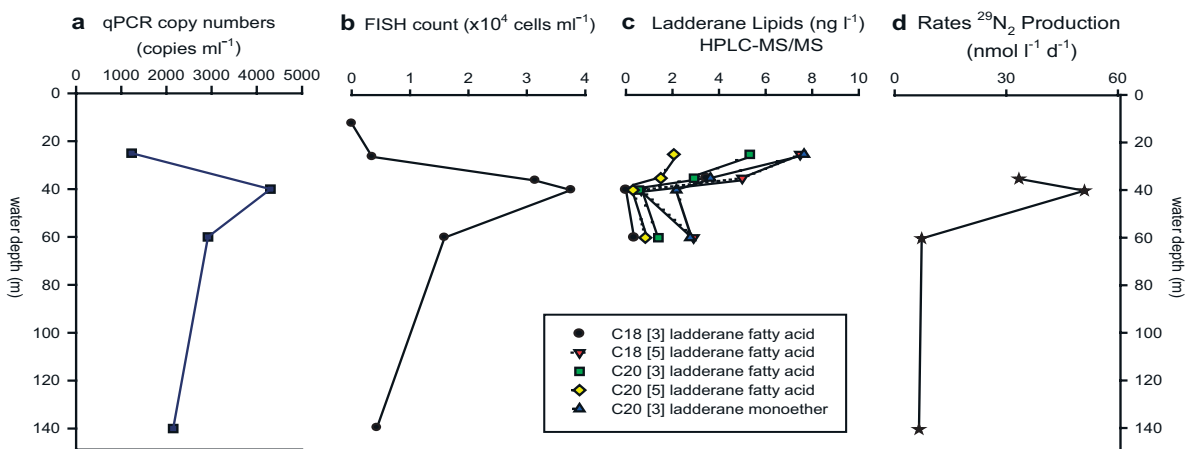


Fig. 4. Distribution of anammox cell counts, ladderane lipids and rates of N_2 production via the anammox process, at Peruvian upwelling station 7.

unusual membrane lipids with either three or five cyclobutane rings, named ladderane lipids (Fig. 2b). Since these lipids are unique to anammox they can be successfully used as ‘biomarkers’ or tracers for anammox cells and the anammox process.

Peruvian OMZ

The Peruvian oxygen minimum zone (OMZ) was chosen as a suitable site for investigating the occurrence of anammox bacteria, because it is an important area of global primary and secondary productivity (providing a large supply of

ammonium), and forms one of the largest oceanic masses of suboxic water.

Therefore, studying this upwelling system could give us a good indication to the magnitude that the anammox process plays in nitrogen cycling on a global scale. From samples taken at the stations (Fig. 3), the rates of the anammox reaction were measured using ^{15}N -labelling techniques, the anammox species using DNA, anammox cell numbers using FISH/qPCR, and the ladderane biomarker lipids using HPLC/APCI-MS/MS. Samples were taken on board using conductivity, temperature, depth (CTD) sampling



Anammox enrichment sequencing batch reactor, used during the temperature experiment.



Sampling using CTD equipment on board the RV Olaya

equipment, or *in situ* pumps (not shown). Typical depth profiles of the anammox rates, cell counts and ladderane lipids are shown in figure 4. In general, results showed that only the anammox process (and not denitrification) was responsible for the removal of nitrogen at all stations sampled. Thus, providing more evidence that the anammox process is a major sink for oceanic nitrogen, and thus constitutes an important process in the global biogeochemical cycling of nitrogen.

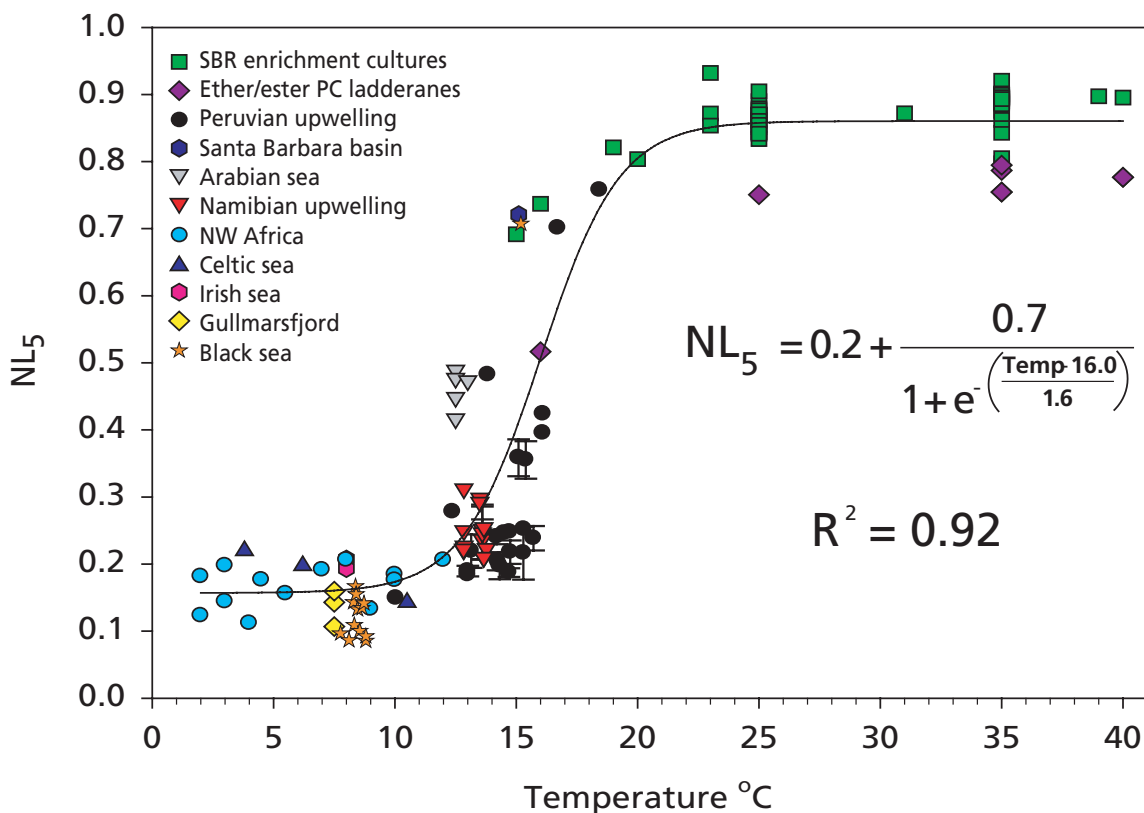


Fig. 5. Graph of the NL₅ index plotted in relation to temperature.

Temperature index

Ladderane lipids are excellent biomarkers for the occurrence of anammox bacteria however, nothing was known about what controls the distribution of these unusual membrane lipids in anammox bacteria. To investigate the influence of temperature on ladderane lipid production in anammox bacteria, an inoculum of the species "*Candidatus Brocadia fulgida*" was cultured at 35°C and then split between three different sequencing batch reactors set at 16°C, 25°C and 35°C. The biomass was cultured, harvested and the lipids extracted. Results showed lad-

derane lipids with a shorter acyl chain (C₁₈) were more predominant at lower temperatures while ladderanes with a longer acyl chain (C₂₀) were more abundant at higher temperatures. According to this relationship we were able to calculate the NL₅ index, which describes the relationship of the two different ladderane lipid chain lengths (i.e. 20 or 18 carbon atoms, where [5] is the number of cyclobutane rings) in response to temperature:

$$NL_5 = \frac{C_{20} [5] \text{fatty acid}}{(C_{18} [5] \text{fatty acid} + C_{20} [5] \text{fatty acid})}$$

We subsequently discovered that the NL₅ relationship holds true for all ladderane lipid samples analysed, including anammox bacteria from enrichment cultures, water column particulate matter and sediments (Fig. 5). The index can be used to determine the origin of the anammox cells producing ladderane lipids in sediments (if they are water column or surface sediment derived) and therefore, the NL₅ relationship could be used in future paleo studies as an indicator to reveal the origin of fossil ladderanes.