

BIOMARKER EVIDENCE FOR ANAMMOX IN THE WAQUOIT BAY SUBTERRANEAN ESTUARY

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The recent discovery of a novel **anaerobic ammonium oxidation** (anammox) microbial pathway has generated a need to reevaluate the mechanisms of nitrogen cycling in aquatic environments, especially coastal settings where nitrogen cycling has strong implications for societally relevant phenomena such as eutrophication and the emergence of harmful algal blooms. In this study we reveal the presence of anammox organisms via their unique ladderane lipids in a coastal aquifer in northeast America. This is the first time that ladderanes have been observed in permeable sediments, and represents the first evidence for anammox in a subterranean estuary.

Mixing of seawater with fresh groundwater within coastal aquifers can be an important mechanism for chemical exchange between terrestrial and marine environments. In the past decade geochemical studies have demonstrated that strong redox gradients exist and active chemical cycling occurs within such environments, termed “subterranean estuaries.” The coexistence of high concentrations of ammonium and nitrate, along with the oxygen limiting conditions in some subterranean estuaries suggests the potential for removal of fixed nitrogen through the formation of dinitrogen gas (N₂) via anammox and this is presently unaccounted for in mass balance estimates of coastal nutrient cycling.

In this study we address two main questions: 1) is anammox occurring in the subterranean estuary of Waquoit Bay, Massachusetts, and 2) how are anammox organisms distributed throughout this environment? We have begun to address these questions through the detection of ladderanes — lipid biomarkers specific to anammox organisms. Our results demonstrate the presence of the ladderanes in one core (PZ10) from the subterranean estuary between 2.5 and 5.0 meters below the surface, and in the intertidal zone between 0 and 20 cm below the surface. The concentrations of the three ladderane fatty acid methyl esters (FAMES) that we have detected exhibit comparable trends downcore and all three FAMES occur at roughly similar abundance within each sample. We had predicted that anammox would be most prevalent at redox boundaries where ammonium and nitrate/nitrite co-exist in roughly equal

proportions. However, the maximum ladderane FAME abundance at PZ10 appear to coincide with a maximum ammonium concentration at 3.0 m and a maximum nitrate/nitrite concentration at 5.2 m. We also predicted that anammox would be restricted to depths with the lowest pore water dissolved oxygen concentrations. In culture, anammox is inhibited by oxygen levels exceeding roughly 1.3 mg/L (0.5% air saturation) and yet, in our samples, maximum ladderane abundances occur at depths with pore water dissolved oxygen concentrations ranging from about 1 to 3 mg/L. In fact, we observed the highest ladderane FAME abundances in the intertidal beach sediments. This suggests that anammox in this environment may not be restricted to redox boundaries and that anammox may exist in microenvironments that are not represented by measurements of bulk pore water chemistry.

To better understand the distribution of anammox within the subterranean estuary we are analyzing additional samples from three sediment cores taken from the subterranean estuary. In addition to measuring the abundance of ladderane core lipids, we are analyzing the intact polar lipids as evidence for the presence of active anammox communities. From this preliminary evidence we conclude that: 1) Ladderanes are present in coarse-grained, permeable sediments in sufficient abundance for detection with HPLC/APCI MS-MS; 2) Anammox is present deep within the subterranean estuary and in shallow sediments from the intertidal zone. This suggests that anammox may be a ubiquitous component of the coastal marine nitrogen cycle; and 3) anammox can exist in aerobic systems, possibly by taking advantage of anoxic micro-environments. To the best of our knowledge, this is the first observation of anammox existing in aerobic conditions in a natural system.