

AMINO ACID SYSTEMATICS WITH LIPIDS: INSIGHT FROM $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ AND $\Delta^{14}\text{C}$ IN THE DEEP-SEA ARCHAEOAL METHANOTROPHS AT THE BLACK SEA

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Introduction. In the deep-sea sediment, biological methane consumption, especially for anaerobic oxidation of methane (AOM) is a still enigmatic process from the scope of microbial ecology and biogeochemistry. Extremely ^{13}C -depleted lipids (*ca.* -110‰ vs. VPDB) mediated by modern ANME in deep-sea sediments (*e.g.*, Hinrichs et al., 1999; Elvert et al., 1999) have been recognized as modern AOM processes. Due to the critical reason that laboratory-based pure culture and isolation of ANME microbes are currently difficult, the fate of ^{13}C -depleted methane and biochemical mechanisms controlling AOM process are still largely unknown, especially on their biosynthetic pathways to produce ^{13}C -depleted lipids. We have developed the high resolution method for the relevant organic molecules of archaeal methanotrophy including amino acids, lipids, and coenzyme factor 430 (Kaneko et al., 2014) to trace the carbon and nitrogen in the AOM process.

Scientific objectives. To better define the biochemistry of AOM, we verified carbon isotopic composition of protein amino acids, fundamental building blocks of protein, by using ANME-1 and ANME-2 dominated mats collected from the northwestern Black Sea (44°46.46'N, 31°59.50'E, depth 235 m). The ANME-1 and ANME-2 can be observed in tall reef-like chimney structures (< 5 m height) composed of carbonate and dense microbial biomass ($>10^{10}$ cells cm^{-3} ; Blumenberg et al., 2004; Knittel and Boetius, 2009) where methane seep rises vertically through their porous calcified interior (Fig. 1). After suitable pre-treatment procedures, we conducted bulk and compound-specific stable carbon and nitrogen isotope analysis ($^{13}\text{C}/^{12}\text{C}$ & $^{15}\text{N}/^{14}\text{N}$) of 10 individual protein amino acids as *N*-pivaloyl isopropyl ester derivatives (Chikaraishi et al., 2010; Takano et al., 2010), together with radiocarbon analysis ($^{14}\text{C}/^{12}\text{C}$) by the accelerator mass spectrometry (Yokoyama et al., 2010).

Results and Discussion. The $\Delta^{14}\text{C}$ values of ANME-1 (-815.3 ± 1.4 ‰), ANME-2 (-770.4 ± 1.8 ‰) and carbonate (-855.9 ± 1.6 ‰) are lower than that of ambient seawater (Fig. 1A). The cross-plot of $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ clearly indicates that methane-derived relict carbon has been utilized directly into the ANME biomass at the seep chimney. We observed a clear stepwise ^{13}C -depletion trend with increasing carbon numbers of protein amino acids: for examples, C_2 -glycine and pyruvate family amino acids for C_3 -alanine, C_5 -valine, C_6 -isoleucine, and C_6 -leucine. Fig. 1B represents the comparison with the carbon isotopic composition of amino acids in photoautotrophs of phytoplankton and estuary diatom (Keil and Fogel, 2001). Valine is synthesized by the addition of ^{13}C -depleted acetyl group to the alanine carbon skeleton (Hayes, 2001), resulting in a ^{13}C -depleted stream would be further progressed to leucine eventually. Assuming the $\delta^{13}\text{C}$ of methane in the Black Sea seep environment (-65 to -50‰), the isotopic fractionation from initial methane to the end products of branched-chain amino acids (*i.e.*, isoleucine and leucine) and archaeal ether lipids (*i.e.*, C_{20} - and C_{40} -isoprenoids) were estimated up to *ca.* 50‰ during the entire archaeal methanotrophy. We further suggest that protein amino acids and the most ^{13}C -depleted signature of leucine, having a specific branched-chain structure, are potentially propagated as precursor molecules into archaeal biosynthesis of soft tissues, resulting in the extremely ^{13}C - and ^{14}C -depleted nature of ANME cells in the deep microbial oasis.

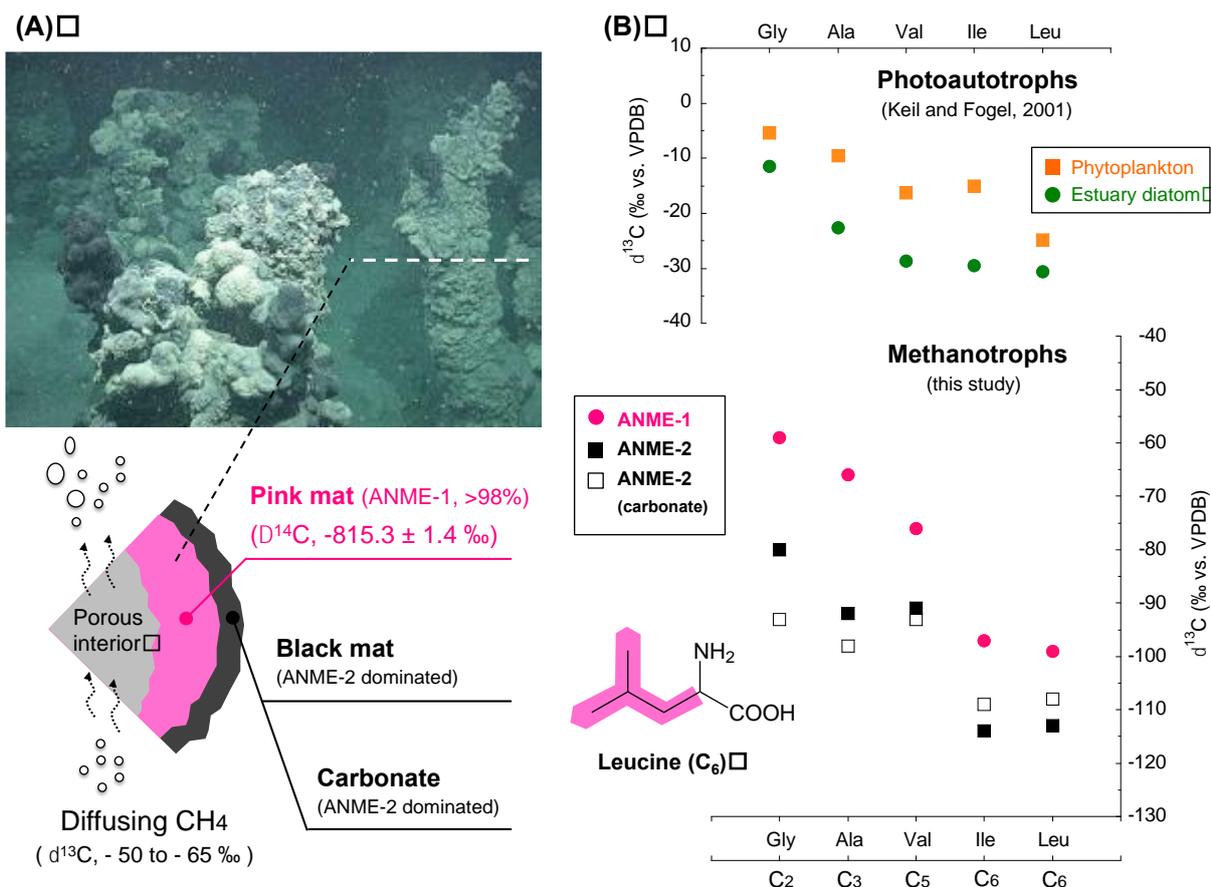


Figure 1 (A) Cross section of the ANME habitat in the seep chimney structure showing interior and exterior sections for pink (ANME-1), black (ANME-2 dominated) and carbonate (ANME-2 dominated) at the Black Sea. (B) Neutral amino acids (C_2 -glycine and pyruvate family amino acids for C_3 -alanine, C_5 -valine, C_6 -isoleucine, and C_6 -leucine) from photoautotrophs (Keil and Fogel, 2001) and methanotrophs (this study) with the corresponding carbon isotopic composition. Based on the lipid analysis of ANME-1, abundance of archaeal lipids was more predominant (>98%) than that of bacterial lipids.

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