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Evidence for microbial methane oxidation at cold seeps along the main active fault in the Marmara Sea

Nicolas CHEVALIER (1), Daniel BIRGEL (2), Purificacion LOPEZ-GARCIA (3), Marie-Helene TAPHANEL (1), and Ioanna BOULOUBASSI (1)

(1) Laboratoire d'Océanographie et du Climat: Expérimentation et Approches Numériques (LOCEAN/IPSL), Université Pierre et Marie Curie, Paris, France (* corresponding author : nicolas.chevalier@locean-ipsl.upmc.fr), (2) MARUM, Zentrum für Marine Umweltwissenschaften, University of Bremen, Post Box 330 440, D-28334 Bremen,Germany , (3) Unité d'Ecologie, Systématique et Evolution - CNRS UMR8079 Université Paris-Sud 11, bâtiment 360, 91405 Orsay Cedex, France

The North Anatolian Fault in the Marmara Sea is a spectacular example of a seismically active fault where, in recent years, numerous sites of active fluid venting have been discovered and explored. During the MARNAUT cruise (2007), multidisciplinary sampling was carried out with the *Nautile* submersible in order to investigate biogeochemical and hydrogeological processes taking place at these newly discovered cold seeps.

We have studied short sediment cores (< 20 cm) and authigenic carbonate crusts retrieved with the *Nautile* submersible from sub-basins of the Marmara Sea, aiming at gaining insight into microbial processes and assemblages in this recently discovered methane-rich setting and at comparing it with previously studied cold seeps. To do so, we investigated diagnostic microbial lipids and their carbon isotope composition, and, in selected sediment samples, we carried out cloning and sequencing of 16S rRNA genes.

The sediment core retrieved from the southern slope of the Çinarcik Basin, in an area of black patches, bacterial mats and polychaetes, contained abundant and strongly ^{13}C-depleted archaeal and bacterial lipids. Archaeal lipids consisted mainly of archaeol, *sn*-2-hydroxyarchaeol, crocetane, and unsaturated PMIs, and showed δ^{13}C values as low as –125 per mille. Concurrently, bacterial lipids (e.g. cyclopropyl-$C_{17:0}$, $C_{16:1\omega5}$, i-/*ai*-$C_{15:0}$, and non-isoprenoidal glycerol monoethers), previously assigned to sulphate reducing bacteria (SRB), were identified with low δ^{13}C values (-55 to –115 per mille). The structural and isotopic features of microbial lipids provided compelling evidence for anaerobic oxidation of methane (AOM) taking place within the upper 17 cm of the sediment core, mediated by methanotrophic archaea (ANME) and sulphate reducing bacteria. No biomarker evidence for aerobic oxidation of methane was found. Depth profiles of microbial lipids revealed the vertical zonation of AOM and associated microbial biomass, and implied that AOM is highest at the 10-12 cm sediment horizon, in agreement with pore water profiles.

Lipid fingerprints suggested the occurrence of significantly diverse archaeal/bacterial assemblages. Among them, ANME-2 (likely ANME-2c) methanotrophic archaea and sulphate reducing bacteria from the *Desulfosarcina/Desulfococcus* (DSS) cluster appeared to dominate the ANME/SRB communities. Complementary evidence was provided by analyses of the archaeal and bacterial diversity based on the amplification, cloning and sequencing of 16S rRNA genes from the core surface and the 10-12 cm-deep layer. A large variety of archaeal and bacterial phylotypes was identified. In accordance with lipid profiles, sequences belonging to the groups ANME-2a and ANME-2c within the archaea and to δ-proteobacterial SRB within the bacteria were dominant in our gene libraries.

In contrast with the core from the south of Çinarcik Basin, cores retrieved from the north of Çinarcik Basin and the Central Basin did not contain lipids diagnostic for AOM. Evidence for aerobic methanotrophy was also absent. However, pore water profiles have implied that, in these cores, the methane/sulphate transition zone occurs in deeper sediment layers.

Diagnostic microbial lipids with very low δ^{13}C values were also very abundant in carbonate crust samples from the Marmara Sea pointing to their formation through AOM. Alike in sediments, lipid fingerprints indicated the prevalence of ANME-2/DSS assemblages. Yet, significant differences in the composition of bacterial lipids were observed.

Overall, our data provide first evidence for AOM taking place in the newly explored active fluid venting sites along the main active fault in the Marmara Sea, expand on existing observations and knowledge of AOM at cold seeps, and highlight similarities/differences with cold seeps in the Mediterranean/Black Sea realm.