3.1 Geochemical evidence for iron coupled AOM in Lake Kinneret iron-rich methanic sediments

We explore here slurries amended with Lake Kinneret sediments from the deep methanic zone (26-41 cm). In this potentially ferruginous zone, sedimentary profiles show that the concentration of methane decreases from its maximum values of above 2mM at around 10 cm depth to 500 μM at 40 cm depth, and that of dissolved ferrous iron increases (from 1-6μM at the first 10 cm depth to ~60-100μM, depending on sampling season). This, combined with an increase of $\delta^{13}C$ of methane (from -65‰ at 7 cm depth to -53.5‰ at 24 cm depth) and a decrease of $\delta^{13}C$ of total lipid compounds (from 27‰ at 23 cm depth to -31‰ at 27 cm depth), suggests AOM in the deep sediment coupled to iron reduction (Adler et al. 2011; Sivan et al. 2011). This was supported by rate modeling and by microbial profiles (Adler et al. 2011; Sivan et al. 2011; Bar-Or et al. 2015, 2017). Alternative electron acceptors are scarce: dissolved manganese oxides concentrations are ~ 0.04% and nitrate and sulfate are below the detection limit (Sivan et al. 2011).

The slurries investigated microbially here were amended with isotopically labeled $^{13}CH_4$, $^{13}CH_4$ + hematite and $^{13}CH_4$ + amorphous iron + molybdate for 470 days. In these incubations, we observed a marked enrichment of labeled carbon after ten months of incubation (up to 250‰ enrichment in the treatment with hematite addition, up to 80‰ enrichment in the natural treatment and up to 450‰ in the treatment with amorphous iron + molybdate Fig. S1 in the Supplement). Ferrous iron concentrations increased by ~20–50 μM following iron oxide amendments (with and without molybdate addition), indicating that iron was reduced. The BES amendments resulted in the highest increase in ferrous iron concentrations (~50-110 μM), most likely due to the abiotic reaction of BES with iron minerals. The evidence for iron reduction, together with the fact that $\delta^{13}C_{DIC}$ values increased by 250-450‰ in the different iron amended treatments, but not in methane-only additions (only up to 80‰, Fig. S1 in the Supplement), indicate iron coupled AOM. Sulfate did not play a role in the AOM, as the addition of molybdate, sulfate reduction and disproportionation antagonist, did not inhibit methane turnover (Fig. S1 in the Supplement). The addition BES to specific slurries inhibited the production of $\delta^{13}C_{DIC}$, indicating the essential role of methanogens in the AOM activity (Fig. S1 in the Supplement).