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## Abstract

Iron-coupled anaerobic oxidation of methane (AOM) was evident in the methanic sediments of lake Kinneret (LK, Sea of Galilee, Israel) (Fig.1 and 7), however, the mechanism has been only partly understood. In an attempt to enrich the AOM activity in LK sediments prior to setting up experiments, 1:1 (sediment to pore-water (PW) ratio) long-term slurry incubations (Fig. 3) with the addition of <sup>13</sup>CH<sub>4</sub> were set up. After AOM was observed, several months later, 1:3 slurry experiments (Fig. 4) were set up with and without the addition of iron oxide (hematite) expecting to see more intensive AOM with this addition. Here we present the results of the geochemical analysis of four 1:3 long-term slurry experiments (Fig. 6) compared to the results of a fresh sediment experiment (Fig. 5, Bar-Or et al., 2017). Together with specific lipid isotopes and metagenomic analyses (not shown), our results suggest that the AOM process is not similar in the two experiment types. This indicates an evolution of the AOM process during the incubation period.

## Methods

- ❖ 40 cm sediment cores and sediment stock (for PW extraction) were collected from station A in LK (Fig. 2).
- ❖ Extraction of PW from sediment stock.
- ❖ Setting up 1:1 slurry incubation (Fig. 3) with sediment and PW from below 25 cm depth with PW exchange every 3 months, to “enrich” the AOM activity.
- ❖ Setting a 1:3 slurry experiments from the incubations after observing AOM (Fig. 4).

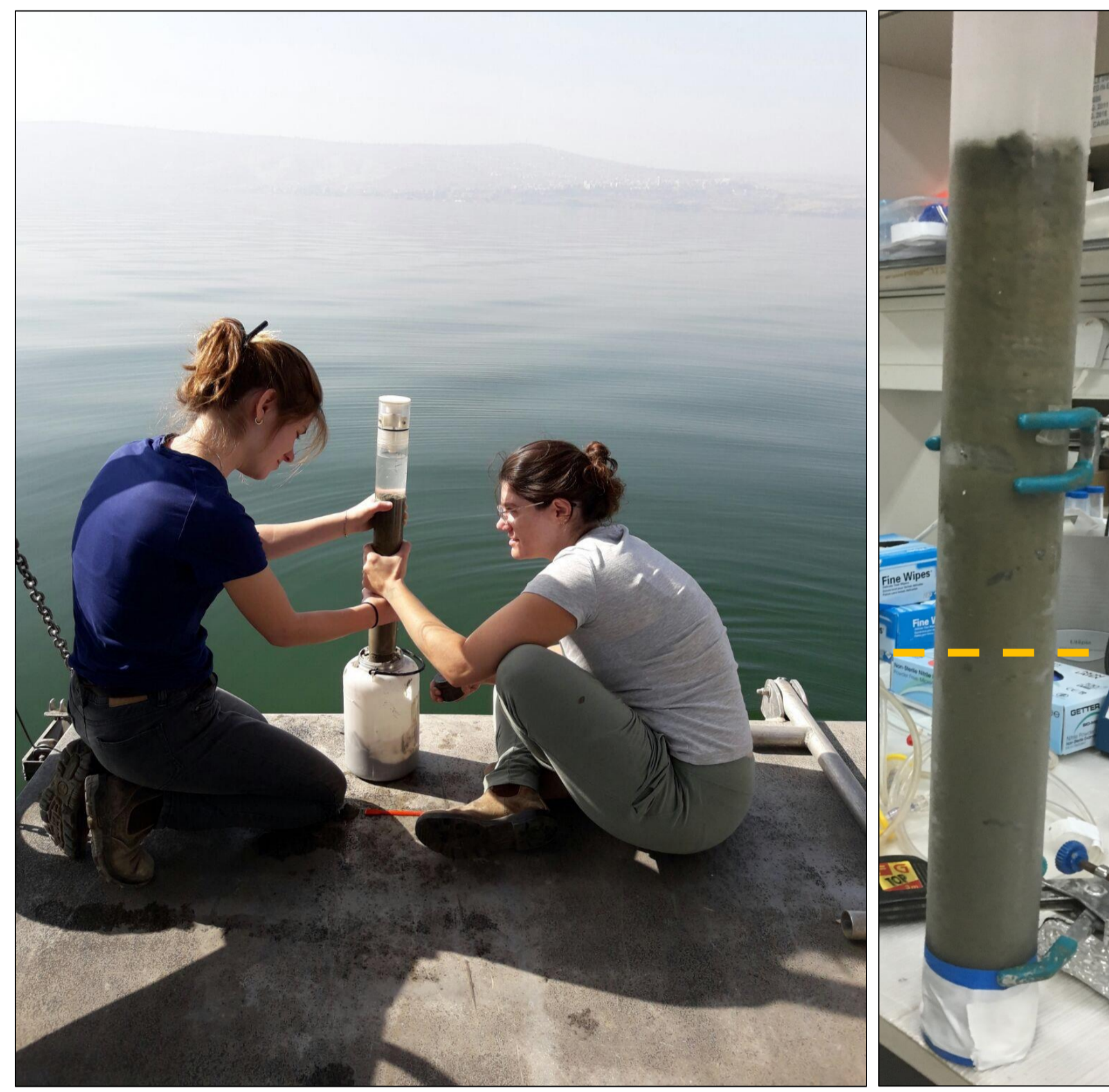


Figure 2: Collecting cores and sediment stock

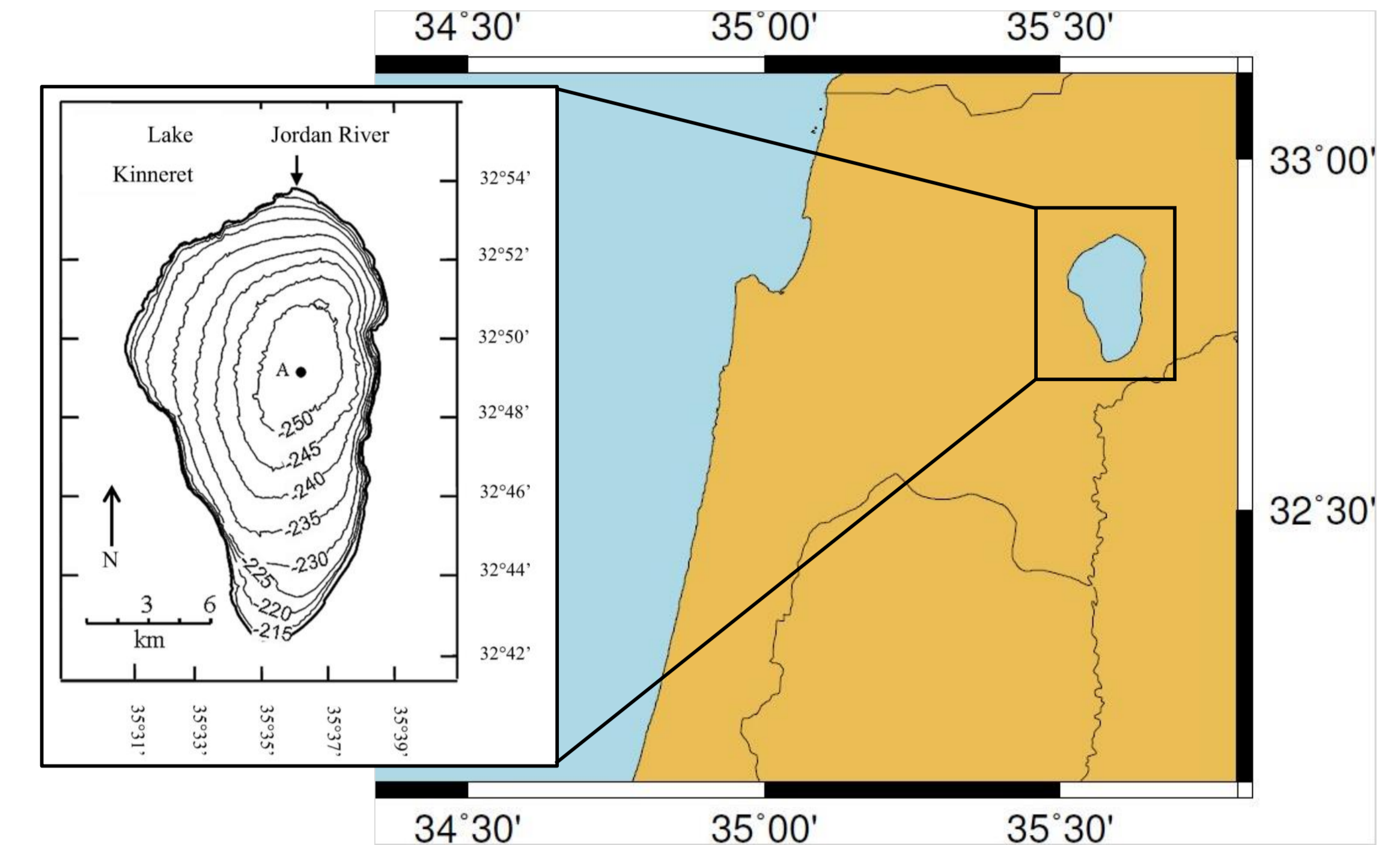


Figure 1: Lake Kinneret (Sea of Galilee) (Map on the right hand side is after Adler et al. (2011))



Figure 3: 1:1 long-term incubation



Figure 4: 1:3 slurry experiment

## Results

### Fresh sediment experiment

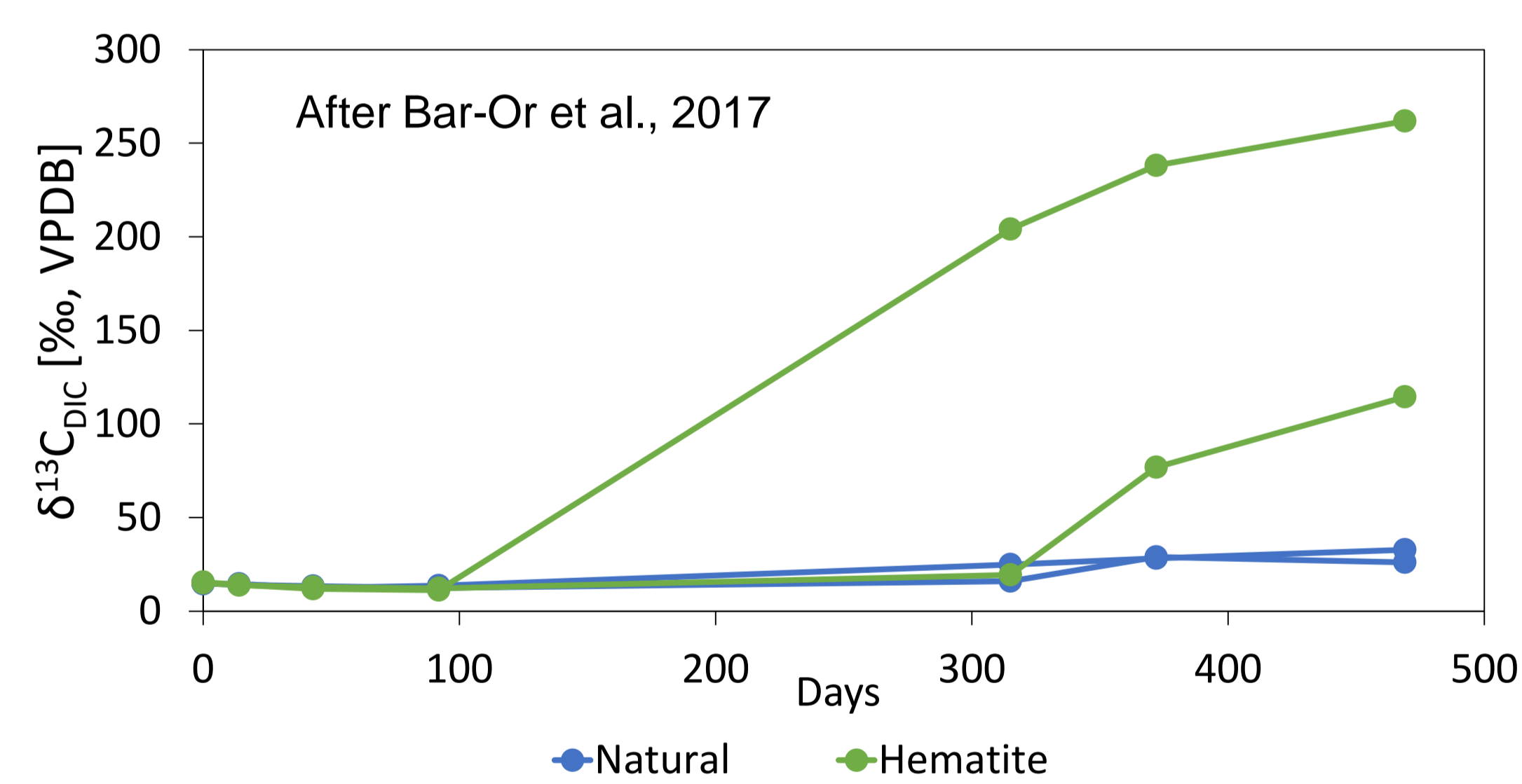


Figure 5:  $\delta^{13}\text{C}_{\text{DIC}}$  results of fresh sediment slurry experiment.

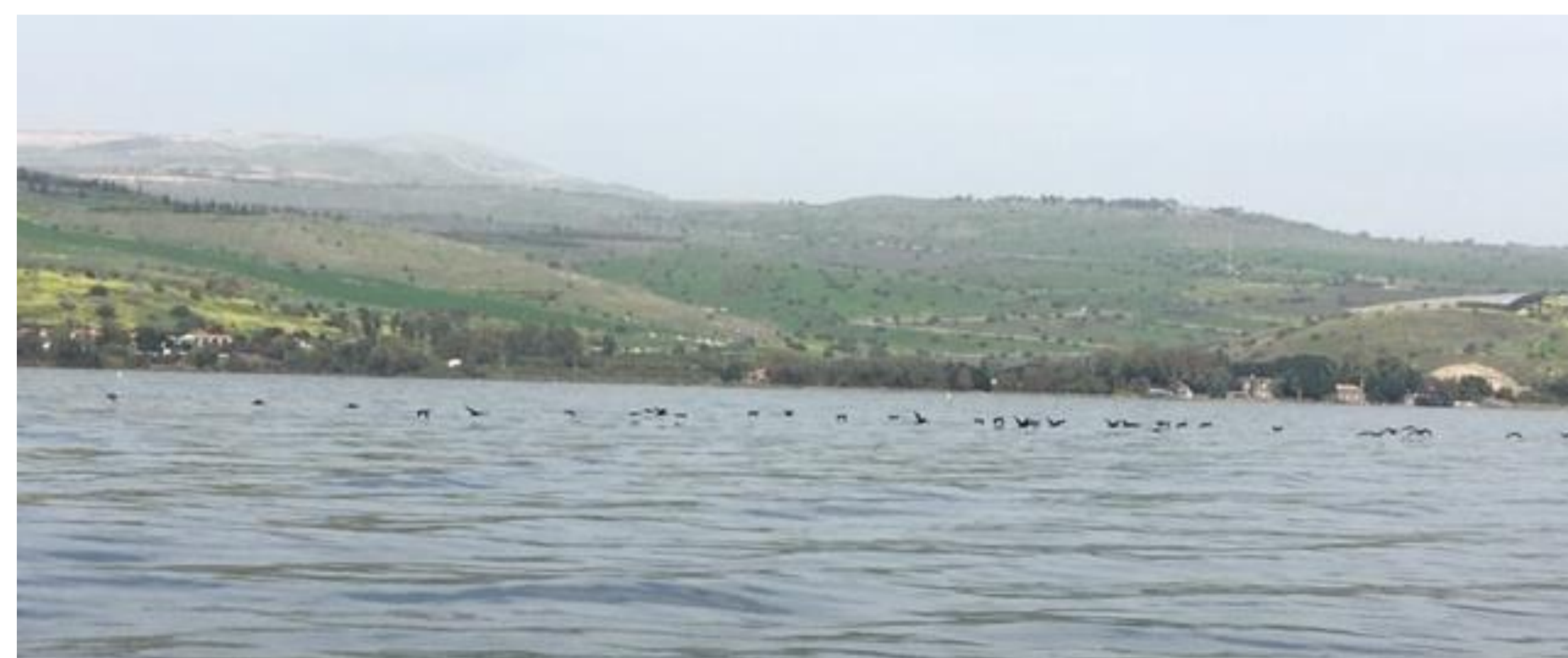


Figure 7: Lake Kinneret (Sea of Galilee)

VS.

### Incubated sediment experiment

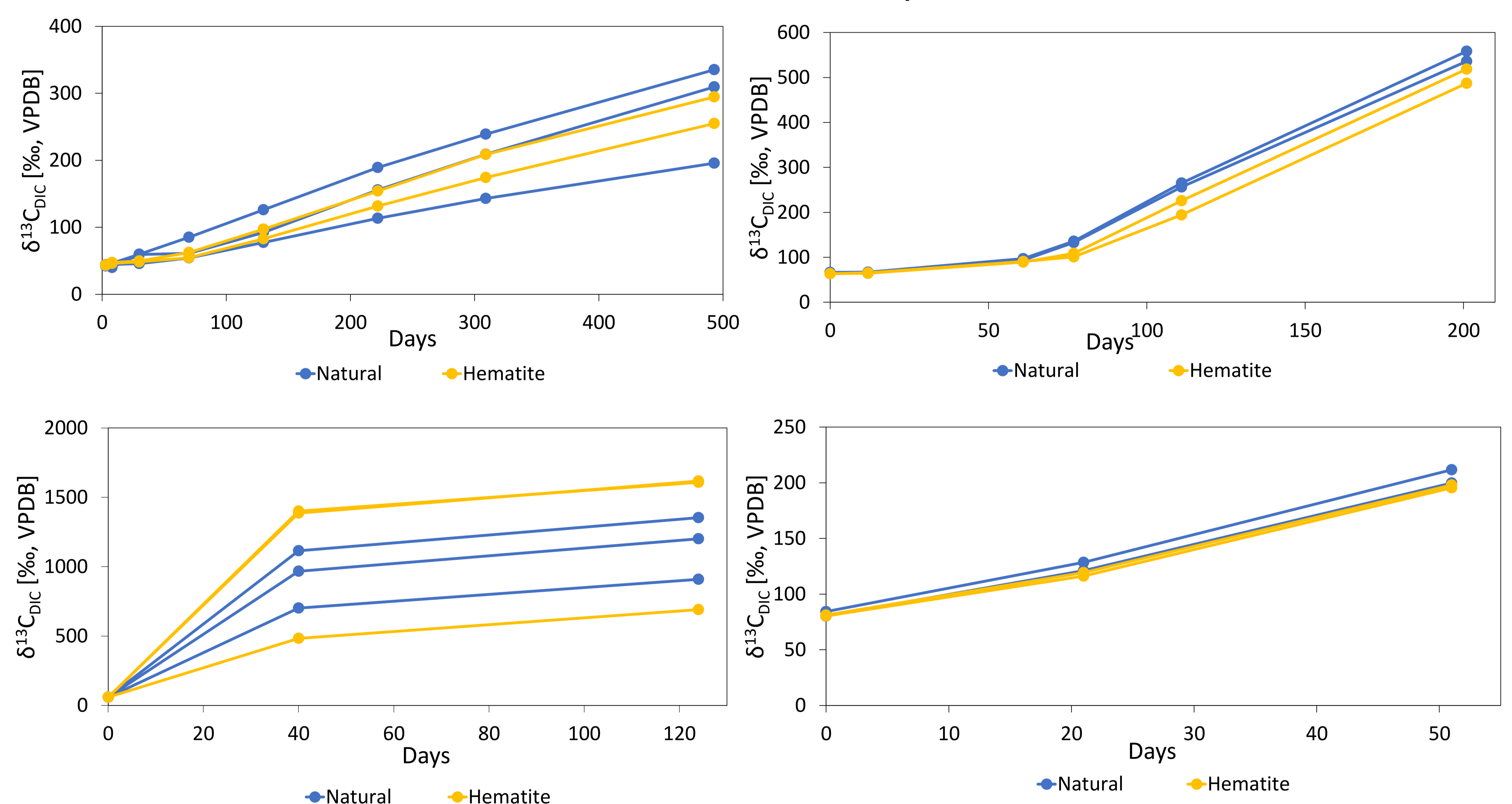


Figure 6:  $\delta^{13}\text{C}_{\text{DIC}}$  results of four long term incubated sediment slurry experiments.

## Conclusions

- The  $\delta^{13}\text{C}_{\text{DIC}}$  values of the long-term experiments suggest that either iron oxide does not act as the electron acceptor (as opposed to the fresh sediments experiment) or is not limiting.
- Metagenome analysis and  $\delta^{13}\text{C}$  values of the specific lipids indicate that in the fresh sediment experiment aerobic methanotrophs and methanogens are both involved in the AOM, and in the long-term incubations only methanogens are involved.
- It seems that during the incubation period the bacterial methanotrophs collapse, leaving methanogens as the only active microorganism involved in the AOM process.