Characterization of methane cycling and redox coupling in Lake Kinneret using carbon isotopes
Introduction

It is known that in stratified lakes developing an anoxic hypolimnion, Methane (CH$_4$) production (methanogenesis) and sulfate reduction are key terminal processes in anaerobic carbon mineralization (Capone and Kiene 1988). It is assumed that in the presence of sulfate, bacterial sulfate reduction (BSR) can out-compete methanogenesis, restricting this process to the deeper sediments (e.g. Lovley and Klug, 1983). Because of methane oxidation (methanotrophy) in the aerobic waters of lakes (Cicerone and Oremland, 1988), at the oxic-anoxic chemocline in the water column of eutrophic stratified lakes (Casper et al., 2000) or at the same boundary near the sediment-water interface (Frenzel et al., 1990), Hessen and Nygaard (1992) reported that microbial cellular production by methanotrophs can be as important a carbon source to pelagic food webs as microbial secondary production. These organisms represent the “bacterial filter” that lowers methane emissions to the troposphere (by 60-90 %) from inundated terrestrial and aquatic surfaces (Galchenko et al. 1989).

It has been shown that anaerobic methane oxidation (AOM) by sulfate plays a major role in the methane consumption in marine sediments. The first geochemical evidence for AOM by BSR came from porewater profiles of inorganic rich marine sediments (Martens and Berner, 1974; Reeburgh, 1976; Barnes and Goldberg, 1976; Martens and Berner, 1977). When porewater SO$_4^{2-}$ is consumed by continuous organic matter oxidation its profile typically shows a concave down curve. In places where sulfate is consumed largely due to AOM, which can be tens of meters below the sediment-water interface (Sansone and Martens, 1981; Borowski et al., 1996), SO$_4^{2-}$ profiles exhibit linear diffusion to the zone of AOM (Niewöhner et al., 1998; Sivan et al., 2007). About ten years ago microbiologists showed that a consortium of archaea and bacteria is involved in AOM in some seep environments (Hoehler et al., 1996; Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001). About three groups of archaea preformed AOM, the ANME-1, ANME-2 and ANME-3, usually associated to SRB (Orcutt et al., 2008).

Theoretically, other electron acceptors can oxidize methane at a greater free energy yield. In anoxic sediments from a canal in the Netherlands, Raghoebarsing et al. (2006) discovered consortia of microorganisms capable of AOM coupled to denitrification.
Sivan et al. (2007) suggested a possible oxidation of CH$_4$ in deep marine sediments by Fe(III) reduction. Recently, Beal et al. (2009) showed the potential of such a process to occur in laboratory enrichment cultures from marine sediments. Studies in aquatic environments showed the potential of increased remineralization of organic matter by recycling of S via the microbially mediated redox coupling to iron (Straub and Schink, 2004a, 2004b; Turchyn et al., 2006). The authors suggested that S is used as an electron shuttling that increase the solubility of Fe(III) minerals. The last authors used the stable isotopes of sulfur and oxygen in sulfate ($\delta^{34}$S$_{SO_4}$, $\delta^{18}$O$_{SO_4}$) to quantify the recycling processes and suggested the future use of stable isotopes of iron ($\delta^{56}$Fe) for better understanding of the system.

Based on our dissolved CH$_4$ and measurements of the stable carbon isotope ($\delta^{13}$C) in the dissolved inorganic carbon (DIC) we have indirect evidence for AOM in LK sediments (Fig. 1) via iron reduction in the zone below the methanogenesis zone, and maybe via sulfate reduction in the deep water column.

In this research we intend to quantify the important redox couplings between CH$_4$, S and Fe in Lake Kinneret (LK) by combined geochemical and microbiological approach. We anticipate elucidating new pathways in the cycling of these environmentally important species in the lake, as they might change with decreasing of water levels and be of critical importance for the overall methane budget.

**Fig. 1:** Profiles and possible redox reactions of critical redox species at the sediment water interface and the upper sediments in Lake Kinneret based on profiles measured during the mixed period at the central station.
Results up to date

Field results

Prior to this study we conducted seasonal profiles in the water column and the sediments of Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, SO\textsubscript{4}\textsuperscript{2-}, S\textsuperscript{2-}, Fe(II), Fe(III), alkalinity, DIC, CH\textsubscript{4} and \(\delta^{13}\text{C}_{\text{DIC}}\) (Adler et al., 2011). In this grant we planned to sample for analyses of \(\delta^{13}\text{C}_{\text{CH}_4}\), \(^{14}\text{C}_{\text{DIC}}\), \(^{14}\text{C}_{\text{CH}_4}\), \(\delta^{34}\text{S}_{\text{SO}_4}\), \(\delta^{18}\text{O}_{\text{SO}_4}\) and Fe isotopes. Up to date we measured already \(\delta^{13}\text{C}_{\text{CH}_4}\), \(^{56}\text{Fe}\) and also the \(\delta^{13}\text{C}\) values of the total lipids extracted (TLE) in the porewater and \(\delta^{34}\text{S}_{\text{SO}_4}\) and \(\delta^{18}\text{O}_{\text{SO}_4}\) in the water column.

The results of the porewater profiles (Fig. 2) support the existence of iron driven AOM in LK below the methanogenesis zone. Most sedimentary methane profiles (Fig. 2A) show an increase from bottom water values of 100-200 \(\mu\)mol L\(^{-1}\) to maximum values of about 2 mmol L\(^{-1}\) (the saturation level at atmospheric pressure (after Yamamoto et al. 1976) around 5 to 12 cm depth, decreasing again at greater depths to less than 500 \(\mu\)mol L\(^{-1}\) by 27 cm. Such a decrease of methane in the deep sediments requires a sink for methane. Further evidence for AOM below the main methanogenesis zone comes from carbon stable isotope profiles. The \(\delta^{13}\text{C}_{\text{CH}_4}\) profile (Fig. 2B) shows a decrease from -60‰ at 1 cm depth to about -65‰ at 7 cm depth and then an increase to maximum value of -53.5‰ at 24 cm depth. Such an increase in \(\delta^{13}\text{C}_{\text{CH}_4}\) values can be explained by methanotrophy, in which the residual methane becomes isotopically heavier. The \(\delta^{13}\text{C}\) values of the total lipids extracted (TLE) from the sediments could have imprints of the AOM process, since at least some individual components will have very negative values of \(\delta^{13}\text{C}\) (Hinrichs et al. 1999). In LK sediments, a decrease in \(\delta^{13}\text{C}_{\text{TLE}}\) from -27‰ at the methanogenesis zone down to -31‰ at 27 cm depth is observed (Fig. 2C). As expected from the above, the values of \(\delta^{13}\text{C}_{\text{CH}_4}\) and \(\delta^{13}\text{C}_{\text{TLE}}\) show opposing patterns of \(^{13}\text{C}\).

Porewater sulfate disappears in these cores by 10 cm depth, and maximum methanogenesis rates occur between ~5 to 12 cm (Adler et al., 2011) Manganese oxides are too low throughout the sediment column of LK (0.04%) to support methane oxidation, as well as nitrate. Nitrate is found in LK only episodically in the water column, and it is consumed rapidly during a denitrification event at the beginning of seasonal stratification (Hadas and Pinkas, 1995). Hence, the most probable terminal electron
acceptors in these deep sediments are iron oxides. Indeed, porewater concentrations of Fe(II) (Fig. 2D) increase monotonically below a depth of 12 cm. Porewater iron isotopes (Fig. 2E) are also consistent with active Fe(III) reduction. At the zone where AOM appears to occur, $\delta^{56}$Fe values are isotopically negative (-1.66‰ to -2.33‰), similar to other sediments with active dissimilatory bacterial iron reduction (Severmann et al. 2006). In the upper sediments, positive values of $\delta^{56}$Fe likely are the result of fractionation during precipitation of FeS minerals.

Fig. 2: Profiles in Lake Kinneret sediments. (A) Dissolved methane in porewater (error bar is marked when duplicates were measured). (B) $\delta^{13}$C of dissolved methane in porewater. (C) $\delta^{13}$C of total lipids extracted from the sediment (TLE). (D) typical dissolved Fe(II) and Fe(tot) in the porewater. (E) $\delta^{56}$Fe in porewater.

Incubation experiments on top cores

The potential of highly reactive Fe(III) oxides to support AOM in LK was tested by mesocosm incubation studies with intact sediment cores from the central lake station (Figs. 3-4). Amorphous Fe(III) was added to sulfate-free overlying water of a core to which ca. 1 g of sediment sampled from the deep AOM horizon (~25 cm) was added. In the first incubation experiment, 0.2 g L$^{-1}$ Fe(III) oxide was added, and increases in Fe(II) and decreases in methane were measured simultaneously (Fig. 3). The ratio of the Fe(II) increase to methane decrease was about 2:1 over the first 210 hours, increasing to about 6:1 by the termination of the experiment (280 hours).
Fig. 3: Incubation experiment (#1) conducted on core from LK sediments. Time-separated amendments of 0.2 g·L\(^{-1}\) amorphic Fe(III) oxide demonstrated the potential of the mesocosm to generate Fe(II) by oxidation of methane. The error bar is smaller than the symbol, unless marked.

Fig. 4: Incubation experiment (#2) conducted on two cores from LK sediments. One untreated incubated control (grey), and one treatment core that amorphic Fe(III)-oxides were added to (black). The error bar of duplicates is smaller than the symbol, unless marked.

The first experiment demonstrated the potential of the mesocosm to generate Fe(II) by methane oxidation (Fig. 3), however the methane decrease was small. Therefore, in the second experiment, higher concentrations of amorphic Fe(III)-oxides (0.8 g·L\(^{-1}\)) were
added and a control was incubated in parallel to the treated core (Fig. 4). Indeed, a significant decrease in methane concentrations was observed, presumably due to addition of higher concentrations of iron. Methane in the control remained constant at around 70 μmol L⁻¹ until the end of the experiment (600 hours), while it decreased in the treated core to around 10-20 μmol L⁻¹ after 600 hours. The clear significant difference between the control and the treated core suggests an iron-dependent AOM process.

These results clearly show evidence for coupling between methane and iron in LK sediments. Because anaerobic Fe(III) respiration is thermodynamically more favorable than both sulfate-dependent methanotrophy and methanogenesis, its occurrence below the zone of CH₄ production supports the idea that reduction of sedimentary iron oxides is kinetically or biologically limited. Similar conditions are likely to prevail in other incompletely pyritized aquatic sediments, suggesting that AOM with Fe(III) is an important global sink for CH₄. This part of the study was summarized to a paper that has been recently accepted to L&O (Sivan et al., 2011).

**Slurry experiments results**

Slurry experiments are commonly used for direct rate measurements of processes in sediments. Slurry experiments are easier to manipulate than core experiments, where the entire sedimentary column is homogenized and involved.

The first slurry experiment was an experiment examining the different parameters that may influence the iron-driven AOM process (Fig. 5). Measurements of methane concentrations of homogenized sediments from the methanogenesis and methanotrophy zones, that are treated differently, can help us to quantify the processes involved. 15 cm of the upper sediment was discarded, while the rest was homogenized and divided into five triplicate treatments. 10 g of pore water was added to 10 g of sediment and 0.5 ml of amorphous iron (0.3 g FeCl₃·6H₂O salt in ~7 ml of DDW [0.159 M]) was added to the amorphous iron treatment, while a 1.5 ml sulfide solution (0.045 g NaS salt in 11 ml of pore water [0.017 M]) was added to the slurries with sulfide and sulfide with methane treatments. The samples were flushed with N₂ until the samples whose treatment did not contain methane had around 2 μM of methane, and the treatments with methane had around 10 μM of methane. At the end of the experiment, after 300 hours, the samples with the sulfide treatment showed a rapid increase in methane concentration to ~130 μM.
The control treatment also showed a rapid increase of its methane concentration to \( \sim 105 \, \mu\text{M} \), while the other three treatments increased almost at the same rate to \( \sim 60 \, \mu\text{M} \). Altogether, this experiment showed that some treatments (methane addition, sulfide and methane addition, and amorphic iron addition) can decrease the accumulation of methane, while others (sulfide addition) can increase the accumulation of methane.

The second experiment attempted to quantify the change in methane and Fe(II) concentrations in the methanogenesis and methanotrophic zones, using different additions of amorphic iron and methane. The slurries were made from two sections of the sediment. The upper 6 cm of the core was discarded, and the 8 cm underneath was collected to be representative of the methanogensis zone. The 5 cm below this was also discarded and the bottom 10 cm (depth of 20–30 cm) was collected to be representative of the AOM zone. 5 g of sediment was diluted using 5 g of pore water from the same zone. Amorphic iron (0.6 g FeCl\(_3\)-6H\(_2\)O salt in \( \sim 15 \) ml of pore water (0.15 M) was added in different volume quantities to the slurries (0.15, 0.3, 0.5, and 0.7 ml of amorphic iron) creating different concentrations (2.5, 5, 8, and 12 mM, respectively). After the slurries were flushed with \( \text{N}_2 \), different volumes of methane (0, 60, 135, and 180 \( \mu\text{l} \)) were injected into them. The dead natural slurries were treated with 0.1 g of HgCl\(_2\). Triplicates were made for the natural and the dead slurries, while the rest were in duplicates.

The addition of methane and amorphic iron to the slurries caused a decrease in methane production in all the slurries compared to the untreated ones. Also as expected, the dead natural slurries did not show any change in methane concentrations during the experiment, meaning that the processes involving methane are biological. The difference between the methane concentration in natural conditions (no additions) in the methanogenesis zone (230 \( \mu\text{M} \)) and in the suspected methanotrophic zone (1 \( \mu\text{M} \)) strengthens the existence of AOM (overlapping methanogenesis in this case).

In slurries with different amorphic iron addition, 135 \( \mu\text{l} \) of methane was added. Methane measurements showed an increase in all of the slurries within the methanogenesis zone (25 to 85 \( \mu\text{M} \) methane concentration change), while the slurries of the suspected AOM methane concentrations remained constant or even showed some decrease (0.5 to 9 \( \mu\text{M} \)) (Fig. 6). Fe(II) concentration change in this slurry experiment showed even more clearly the effect of amorphic iron additions on the AOM process.
Fe(II) concentrations (Fig. 7) showed that at different amorphic iron additions within the AOM suspected zone, Fe(II) increase of amoriphic Fe(III). In the dead slurry, there was no increase in Fe(II), and in the natural slurry (with no amorphic iron addition), Fe(II) increase was 9 µM. In the highest amorphic iron addition (12 mM), Fe(II) increased to 32 µM. In the methanogenesis zone, on the other hand, the addition of amorphic iron caused a much smaller increase in Fe(II). Comparing Fe(II) concentrations in the methanotrophic and methanogenesis zones indicate that indeed amorphic iron reduction is much greater in the deep sediments than in the methanogenesis zone, however, there is also a reduction process in the methanogenesis zone (overlapping between methanogenesis and AOM).

In slurries with different additions of methane volumes and constant amorphic iron (5 mM), methane measurements (Fig. 8) also showed an increase in all of the slurries within the methanogenesis zone (11 to 37 µM methane concentration change). In the slurries of the suspected AOM zone, only 185 µl of methane addition showed a substantial decrease in methane (36 µM decrease). The Fe(II) concentration change in different additions of methane volumes (Fig. 9) also showed that in the suspected AOM zone, there was a larger increase in Fe(II) concentration than in the methanogenesis zone. The largest increase (22 µM) in Fe(II) concentration change was in the slurries with the 135 µl addition of methane.

![Slurry experiment I](image)

*Fig. 5: Methane concentration VS time in slurry experiment I (different treatments).*
Fig. 6: Methane concentration change after two weeks in slurry with different amorphic iron additions.

Fig. 7: Ferrous concentration change after two weeks in slurries with different amorphic iron addition.
**Fig. 8:** Methane concentration change after two weeks in slurries with different methane additions experiment.

**Fig. 9:** Ferrous concentration change after two weeks in slurries with different methane additions.
We plan to continue and investigate this AOM process, and to quantify its rates and controlling parameters, by more sets of incubation experiments on slurries. The biological work of identifying the population is continuing as well.

References (* - funded partly by this grant)


