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Geochemical evidence for iron-mediated anaerobic oxidation of methane

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Abstract

Anaerobic oxidation of methane (AOM) by sulfate has been recognized as a critical process to maintain this greenhouse gas stability by limiting methane flux to the atmosphere. We show geochemical evidence for AOM in deep lake sediments and demonstrate that AOM is likely driven by iron (Fe) reduction. Pore-water profiles from Lake Kinneret (Sea of Galilee, Israel) show that this sink for methane is located below the 20-cm depth in the sediment, which is well below the depths at which nitrate and sulfate are completely exhausted, as well as below the zone of methanogenesis. Iron-dependant AOM was verified by Fe(III)-amended mesocosm studies using intact sediment cores, and native iron oxides were detectable throughout the sediments. Because anaerobic Fe(III) respiration is thermodynamically more favorable than both sulfate-dependent methanotrophy and methanogenesis, its occurrence below the zone of methane 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, indicating that AOM with Fe(III) is an important global sink for methane.

During the remineralization of organic matter, microbes use the available electron acceptors in order of decreasing free energy yield, which under steady-state conditions results in a regular sequence of solute profiles in sedimentary pore waters (Berner 1980). The terminal inorganic oxidant in this series is sulfate, and below the depth of zero sulfate, traditionally the only presumed energy-yielding pathway is methanogenesis.

When the methane (CH_4) that is produced diffuses in contact with an available electron acceptor it can be consumed by microbial oxidation (methanotrophy). In marine sediments this methanotrophy occurs mainly through anaerobic oxidation of methane (AOM) coupled to sulfate reduction. The first evidence for this type of AOM derived from methane and sulfate profiles in anoxic organic-rich sediments (Martens and Berner 1974; Barnes and Goldberg 1976; Reeburgh 1976). AOM results in ^{13}C-depleted dissolved organic carbon (DIC) and slightly heavier $\delta^{13}C$ values of the residual CH_4 as a result of both a small fractionation (of 0‰ to 10‰) during methane oxidation and the initial $\delta^{13}C$ value of the methane itself (Alperin et al. 1988; Martens et al. 1999). Borowski et al. (2000) found very light values of $\delta^{13}C_{DIC}$ in marine sediments (−37.7‰) located at the sulfate–methane interface zone. Ussler and Paull (2008) found very low $\delta^{13}C_{DIC}$ values of −63.2‰ in sediments of the Gulf of Mexico, the most negative value reported so far; all are clear evidence for respiration of CH_4 to CO_2 by AOM.

This process was initially controversial among microbiologists because neither the responsible organism nor the mechanism had yet been identified. About 15 yr ago, field and laboratory studies showed evidence for coupling between methanogens and sulfate reducers (Hoehler et al. 1994). Later on microbiologists and geochemists showed that consortia of archaea and bacteria are involved in AOM in some deep environments (Hinrichs et al. 1999; Boetius et al. 2000; Orphan et al. 2001) and that at least three groups of anaerobic methanotroph (ANME) archaea (ANME-1, ANME-2, and ANME-3) may perform AOM, often associated with sulfate-reducing bacteria (SRB) (Boetius et al. 2000; Orphan et al. 2002; Niemann et al. 2006). Orphan et al. (2001) investigated the $\delta^{13}C$ values of a cell aggregate of ANME-2 and SRB. The archaea were characterized by extremely low $\delta^{13}C$ values (−96‰), best explained by assimilation of a highly depleted carbon source. The most likely source with highly ^{13}C-depleted values was methane.

Sulfate-driven AOM can result in a sharply detectable transition zone at the boundary of CH_4 with sulfate, with concomitant full consumption of upwardly diffusing methane (Niewöhner et al. 1998). Thus, in situ oxidation is critical to maintaining greenhouse gas stability by limiting methane flux to the atmosphere (Borowski et al. 1996; Valentine and Reeburgh 2000).

In contrast, sediments below the zone of methanogenesis are presumed to be largely inactive, because to date no specific microbial respiratory pathways have been demonstrated in these sediments, although recent evidence indicates that biological production of ethane and propane occurs by as yet–unknown processes (Hinrichs et al. 2006). This is despite the presence of oxidized mineral phases such as iron (Fe)-oxides throughout many deep sediments (Lovley and Phillips 1986; Wersin et al. 1991; Widerlund and Ingri 1995). These solid-phase oxides could be electron acceptors for microbiota, but it has remained difficult to establish the extent to which they are quantitatively

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important as electron acceptors in such systems. It seems that kinetic control on reduction of Fe-oxides may limit the rate and extent of Fe availability (Lovley and Phillips 1986; Canfield 1989; Postma 1993), even though there are bacterial mechanisms to reduce solid Fe(III) oxides (Kostka et al. 2002; Straub and Schink 2003).

Because of this possibility and the abundance of Fe(III) oxides, the apparent disappearance of methane in some deep marine sediments has led to a proposed additional AOM process mediated by reduction of mineral-bound Fe(III) (Sivan et al. 2007). Recently it was shown that AOM can be coupled to reduction of Fe(III)-oxides in laboratory enrichment cultures obtained from marine sediments (Beal et al. 2009). Similarly, incubation experiments using digested sewage showed that addition of manganese and iron increased the ratio of methanotrophy to methanogenesis (Zehnder and Brock 1980). Thus, some circumstantial evidence for coupling methane oxidation to Fe(III) reduction exists, but the geochemical importance of this process has not yet been demonstrated.

In this study we show geochemical evidence for AOM in deep Lake Kinneret (LK, Sea of Galilee, Israel) sediments and demonstrate that this AOM is likely driven by iron reduction. The results of this study fit our model results from this study site (Adler et al. 2011) and indicate that AOM coupled to iron reduction is an important global sink for methane.

Methods

Study site—LK is a subtropical, meso-eutrophic lake located in the northern part of the Afro-Syrian rift valley at 32°50′N latitude and 35°35′E longitude. The 37-m deep and 166-km^2 lake is thermally stratified between March and December. Detailed description of the study site is found in Adler et al. (2011).

Sampling—Water samples and 35-cm sediment cores were collected at the central deepest lake (Sta. A) from 2007 to 2010. The cores were immediately sectioned into slices of 1–2 cm in thickness under N_2 atmosphere to prevent oxidation. Half of each sediment slice was transferred into a gas-tight bottle for the head-space measurements of CH_4 and $\delta^{13}C_{CH4}$ (Adler et al. 2011). Pore water was extracted immediately from the other half through centrifuging under a nitrogen atmosphere at 4°C. The pore water was measured for major ions, dissolved organic carbon (DIC), CH_4, $\delta^{13}C_{DIC}$, Fe(II), and Fe(total), as described by Adler et al. (2011), and 1 mL was transferred into a bottle containing zinc acetate for S^{2-} determination. Cores for measurements of $\delta^{13}C_{CH4}$, $\delta^{13}C$ of the total lipids extracted from the sediments ($\delta^{13}C_{\text{Total lipids extracted [TLE]}}$), and $\delta^{56}Fe$ of the pore water were collected during 2009. Ammonium and phosphate in pore water were measured in May 2010. A core collected in September 2008 was measured at depths of 4–23 cm for highly reactive Fe(III) oxides.

Experiments—Three sets of incubation experiments were conducted in January and October 2009 and in February 2010 on cores taken (with a gravity corer) from Sta. A. The core liners are made of Perspex (60 cm long and with diameter of 5.5 cm). Each core was sealed immediately upon sampling and incubated in the dark at 15°C. Before the experiment, the overlying water in the core was siphoned off via N_2 overpressure and replaced with 280-mL of sulfate-free ($BaCl_2$ precipitation followed by filtration through 0.45 μm) surface water from the lake. Then ca. 1 g of sediment from approximately 25 cm in depth was added to the overlying water as an inoculum representative of the deeper sediment layers, and this sediment covered most of the surface of the core. Each core had a lid with an airtight O-ring and a built-in redox electrode (Mettler Toledo) set to measure the pε every 5 min. In the first experiments, total sulfide and nitrate concentrations were also monitored with built-in electrodes (Mettler Toledo) and showed constant very low values of 2.5 μmol L^{-1} and 0.01 μmol L^{-1} during the experiment, respectively (as expected by the removal of sulfate). The overlying water was circulated continuously via closed pump cycle. After dissolved methane concentrations reached steady concentrations (about 1 week), amorphous Fe(III) oxides ($Fe(OH)_{3(am)}$) were injected into the overlying water of one core (the controlled core was not treated). The latter was prepared by dissolving $FeCl_3 \cdot 6H_2O$ salt (molecular weight 240.2 g mol^{-1}) in ~ 3 mL of distilled water according to the desired concentration, adjusted to a pH of 7 with NaOH (1.5 mol L^{-1}). In the labeled methane experiment, 8 mL of treated water saturated with ^{13}C-enriched methane (99.9%) was injected to the overlying water of both cores. Samples were taken for CH_4, Fe(II) concentrations, DIC, and $\delta^{13}C_{DIC}$. Methane was measured via headspace analysis. To sample the water a syringe with a valve was connected to the tube that was placed close to the sediment–water interface. An open syringe with N_2-flushed water was connected to the other tube to prevent oxidized water from entering while sampling. The volume of the collected sample was about 1% of the water volume and therefore did not affect the concentrations in the tank.

Analytical methods—Sulfate concentrations were measured by inductively coupled plasma (ICP)–atomic emission (Perkin-Elmer optima 3300) with a precision of 2%. Methane concentrations in the headspace were measured by gas chromatograph–flame ionization detector, with a precision of 2 μmol L^{-1}. The error bar of duplicates was marked whenever duplicate sampling was enabled. Fe(II) was fixed immediately using Ferrozine solution, and its absorbance in 562 nm was measured on a spectrophotometer (Stookey 1970), with an error of less than 7 μmol L^{-1}. Fe(III) was reduced to Fe(II) using ascorbic acid and was also measured by the spectrophotometer. Pore-water sulfide concentration was measured by titration with 0.005 mol L^{-1} thiosulfate, with an estimated error of 0.02 meq L^{-1}. Total sulfide and nitrate concentrations in the experiments were measured by electrodes with built-in electrodes (Mettler Toledo) and an error of 0.01 μmol L^{-1}. Phosphate and ammonium in pore water were measured by a flow injection automated ion analyzer (Quikchem 8000 Instruments). The errors calculated by averaging duplicate samples were ± 0.03 μmol L^{-1} and ± 0.01 mmol L^{-1},

respectively. Values of $\delta^{13}C_{DIC}$ and $\delta^{13}C_{CH4}$ were measured using a conventional isotopic ratio mass spectrometer (IRMS, Thermo) equipped with PreCon interface, which enables one to perform methane isotopic ratio measurements.

The precisions of these measurements were ± 0.1‰ and ± 0.5‰, respectively, and are reported on the Vienna Pee Dee Belemnite (VPDB) scales. DIC concentrations were measured as well in the IRMS using the peak heights, with a precision of ± 0.05 mmol L^{-1}. Total lipids were extracted using the Bligh–Dyer procedure (Bligh and Dyer 1959), and $\delta^{13}C_{TLE}$ was measured on an elemental analyzer IRMS with a precision of 0.1‰. For iron isotope analysis, pore-water samples were acidified with 10% HCl for 1 week to dissolve any precipitated iron and purified by anion exchange chromatography (Borrok et al. 2007). δ^{56}Fe was measured on a Neptune multi-collector ICP–mass spectrometer in high-resolution mode according to standard methods, standardized against isotopic reference material (IRMM-014), with precision of 0.1‰ (John and Adkins 2010).

Measurements of highly reactive iron—For the most highly reactive Fe(III) oxide measurements, 50 mL of diluted ascorbic acid (0.01 mmol L^{-1}) was added to an 0.5-g sediment sample to reduce the Fe(III) to Fe(II), transferring it in this way to the highly soluble form. The tubes were shaken for 24 h and then centrifuged and the supernatant was measured for its Fe(II) concentrations. It is known that mild ascorbic acid extracts only the highly reducible Fe(III) oxides (Kostka and Luther 1994; Raiswell et al. 2010), and not Fe(II) compounds in the sediment, such as FeS and pyrite. However, in order to further verify it in the conditions of the extraction in LK sediments, a control experiment was conducted. Five centrifuge tubes were shaken for 24 h with the following solutions: (1) 0.1 g $Fe(OH)_{3(am)}$ in 40 mL of 0.01 mol L^{-1} ascorbic acid (final pH 3.8); (2) 0.1 g $Fe(OH)_{3(am)}$ + 0.1 g mixture of powdered FeS and pyrite in 40 cc of 0.01 mol L^{-1} ascorbic acid (final pH 3.9); (3) 0.1 g $Fe(OH)_{3(am)}$ in 40 mL of 0.01 mol L^{-1} ascorbic acid dissolved in ammonium acetate solution (final pH 5.4); (4) 0.1 g $Fe(OH)_{3(am)}$ + 0.1 g mixture of powdered FeS and pyrite in 40 mL of 0.01 mol L^{-1} ascorbic acid dissolved in ammonium acetate solution (final pH 5.5); and (5) 0.1 g mixture of FeS and pyrite in distilled water. The solutions were centrifuged and the supernatant was extracted and measured for its Fe(II) concentrations. Fe(II) concentrations in the first four tubes were very close to each other (1.73 ± 0.02 mmol L^{-1} for run 1, 1.43 ± 0.02 mmol L^{-1} for run 2, 1.32 ± 0.02 mmol L^{-1} for run 3, and 1.20 ± 0.02 mmol L^{-1} for run 4) and higher by two orders of magnitude than the control tube (0.04 ± 0.02 mmol L^{-1} for run 5). Therefore, FeS and pyrite are not dissolved at pH 3.9 and pH 5.5 and for this reason will be probably not be dissolved at less favorable conditions for dissolution at higher pH (Anderko et al. 1997), such as pH ~ 7 in LK sediments.

Results

The set of geochemical evidence for AOM coupled to iron reduction in deep LK sediments (at greater depths than 20 cm) comes from in situ sediment profiles (Fig. 1) and from incubation experiments (Figs. 2–4). Most sedimentary methane profiles (Fig. 1A) show an increase from bottom water values of 100–200 μmol 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 in depth, decreasing again at greater depths to less than 500 μmol L^{-1} by 27 cm. Such a decrease of methane in the deep sediments requires a sink for methane. This was shown in our reactive-transport Berner-type model with terms for diffusion, advection, sedimentation, and reaction (Adler et al. 2011). Further evidence for AOM below the main methanogenesis zone comes from carbon stable isotope profiles. The $\delta^{13}C_{CH4}$ profile (Fig. 1B) shows a decrease from −60‰ at 1 cm in depth to about −65‰ at 7 cm in depth and then an increase to maximum value of −53.5‰ at 24 cm in depth. Such an increase in $\delta^{13}C_{CH4}$ values can be explained by methanotrophy, in which the residual methane becomes isotopically heavier. The $\delta^{13}C$ values of the TLE from the sediments could bear the imprints of the AOM process, since at least some individual components will have very negative values of $\delta^{13}C$ (Hinrichs et al. 1999). In LK sediments, a decrease in $\delta^{13}C_{TLE}$ from −27‰ at the methanogenesis zone down to −31‰ at 27-cm depth is observed (Fig. 1C). As expected from the above, the values of $\delta^{13}C_{CH4}$ and $\delta^{13}C_{TLE}$ show opposing patterns of ^{13}C.

Pore-water sulfate disappears in these cores by 10 cm in depth, and maximum methanogenesis rates occur between ~ 5 and 12 cm in depth (Adler et al. 2011). Manganese oxide concentrations are very low (~ 0.04%) throughout the sediment column of LK (Serruya et al. 1974). 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, pore-water concentrations of Fe(II) (Fig. 1D) increase monotonically below a depth of 12 cm. Pore-water iron isotopes (Fig. 1E) are also consistent with active Fe(III) reduction. At the zone in which AOM appears to occur, δ^{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 δ^{56}Fe likely are the result of fractionation during precipitation of FeS minerals.

The increase in Fe(II) in the deep sediments is certainly favored by the low concentrations of sulfide. Sulfide, the product of microbial sulfate reduction (BSR), complements the sulfate concentration profiles with maxima of 0.3 meq L^{-1} at the sediment–water interface and a decrease to concentrations of less than 0.1 meq L^{-1} at depths greater than 12 cm (Fig. 1F) and in the overlying thermocline of the lake (not shown). Other species that may precipitate with Fe(II) and affect its profile can include phosphate (Fig. 1G).

Iron-dependent AOM requires accessible Fe(III). Indeed, Fe(III) minerals are abundant at all depths in LK sediments at concentrations of about 400 μmol g dry weight (dry wt)$^{-1}$ (~ 2%; Eckert 2000). Mild acid extraction

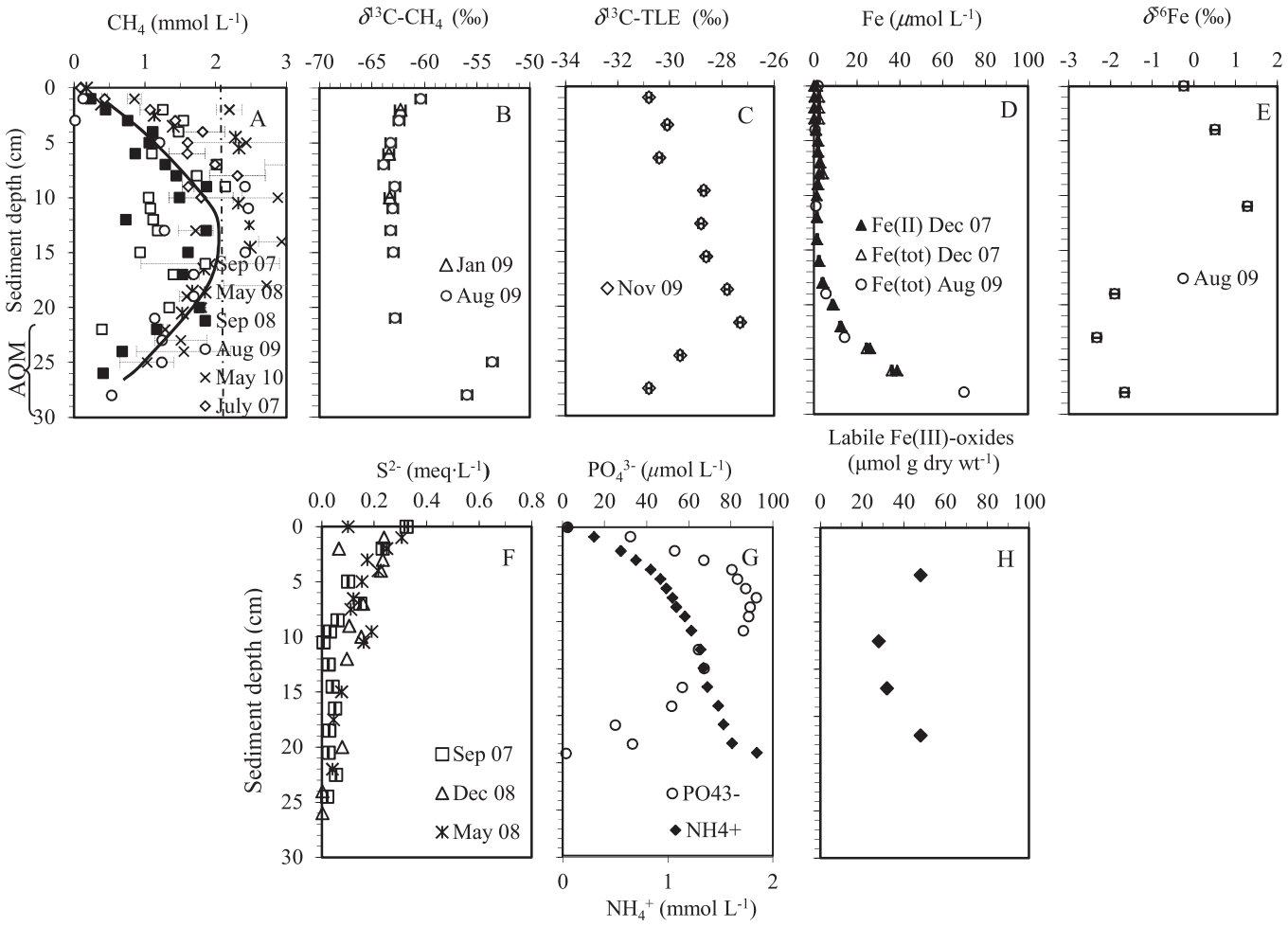

Fig. 1. Profiles in LK sediments. (A) Dissolved methane in pore water (error bar is marked when duplicates were measured). (B) $\delta^{13}C$ of dissolved methane in pore water. (C) $\delta^{13}C$ of TLE from the sediment. (D) Typical dissolved Fe(II) and Fe(total (tot)) in the pore water. (E) $\delta^{56}Fe$ in pore water. (F) Sulfide in pore water. (G) Phosphate and ammonium in pore water. (H) Highly accessible Fe(III)-oxides in LK sediments extracted by diluted ascorbic acid. The depth range of the sampling is 0.5–2 cm, and the error bar is smaller than the symbol, unless marked.

yielded about 40 μmol g dry wt^{-1} sediment of easily accessible Fe(III) throughout the sediments (Fig. 1H). This means that the reservoir of available Fe(III) for methane oxidation is on the order of 40–400 μmol g dry wt^{-1} (between the minimum estimation and the total Fe(III)).

The potential of highly reactive Fe(III) oxides to support AOM in LK was tested by three mesocosm incubation studies with intact sediment cores from the central lake station (Figs. 2–4). Amorphous Fe(III) was added to the sulfate-free overlying water containing ca. 1 g of sediment sampled from the deep AOM horizon (~ 25 cm). It was used in order to verify that the resident microbial population is able to utilize the available electron donor (the methane), as was done in previous experiments with amorphous iron (Lovley and Phillips 1986).

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. 2). The ratio of the Fe(II) increase to methane decrease was about 2 : 1 over the first 210 h, increasing to about 6 : 1 by the termination of the experiment (280 h).

The first experiment demonstrated the potential of the mesocosm to generate Fe(II) by methane oxidation (Fig. 2); however, the methane decrease was small. Therefore, in the second experiment, higher concentrations of amorphous Fe(III)-oxides (0.8 g L^{-1}) were added, and a control was incubated in parallel to the treated core (Fig. 3). 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 h), while it decreased in the treated core (to around 10–20 μmol L^{-1}) after 600 h. The clear significant difference between the control and the treated core indicates an iron-dependent AOM process.

In the third experiment (Fig. 4) methane labeled with ^{13}C was added to the overlying water of two cores. One core was kept untreated, and 0.4 g L^{-1} of amorphous Fe(III)-oxides was added to the other core. The significant increase with time

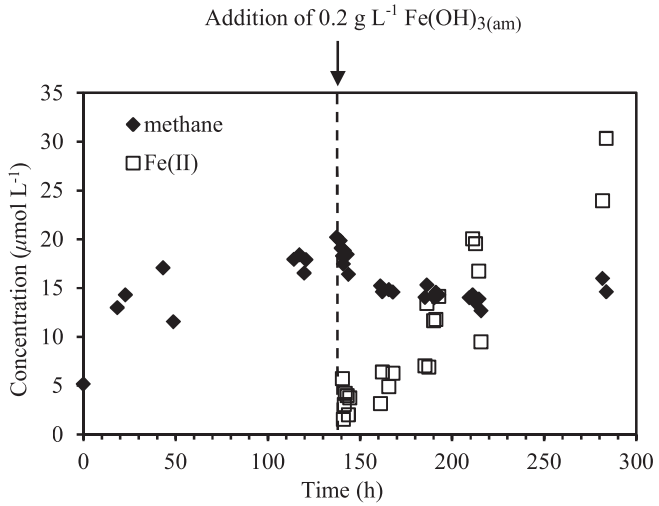

Fig. 2. Incubation Experiment 1 conducted on core from LK sediments. Time-separated amendments of 0.2 g L^{-1} amorphous Fe(III)-oxide demonstrated the potential of the mesocosm to generate Fe(II) by oxidation of methane. The error bar is smaller than the symbol, unless marked.

of $\delta^{13}C_{DIC}$ of the overlying water of the treated core (Fig. 4A) shows production of labeled DIC. In this experiment, a larger increase in Fe(II) was observed in the treated core compared to the control core (Fig. 4B), indicating a correlation between Fe(III) additions and Fe(II) increase.

Discussion

Concentration profiles indicate that although some sulfate is consumed in the hypolimnion of LK, the main zone of sulfate reduction is in the upper few centimeters of the sediment (Adler et al. 2011). There sulfate is exhausted following a classic concave profile, indicating continuous microbial sulfate reduction by organic matter (~ 2–3% dry

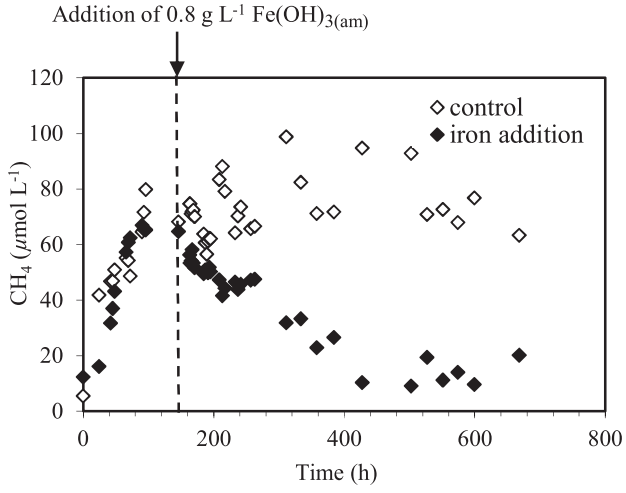

Fig. 3. Incubation Experiment 2 conducted on two cores from LK sediments: one untreated incubated control and one treatment core to which amorphous Fe(III)-oxides were added. The error bar of duplicates is smaller than the symbol, unless marked.

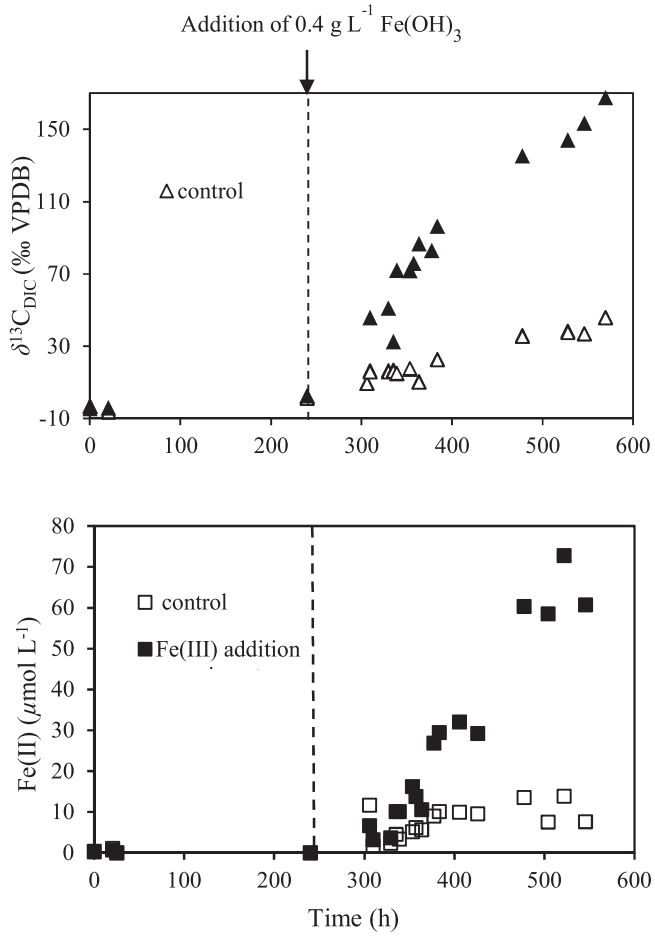

Fig. 4. Incubation Experiment 3 with labeled methane conducted on two cores from LK sediments. Labeled methane was added to the overlying water of both cores. One incubated control was kept untreated, and amorphous Fe(III)-oxides were added to the other core. (A) The change with time of $\delta^{13}C_{DIC}$ of the overlying water. (B) The change with time of Fe(II) of the overlying water. The error bar of duplicates is smaller than the symbol.

wt) oxidation and/or AOM. The latter process is not indicated by the methane profiles, however. Methane profiles show a diffusion curve from its production zone in the sediments toward the thermocline, where the major fate is aerobic oxidation. Methanogenesis ends at about 20 cm in depth (Adler et al. 2011), and below that zone there is a clear decrease in methane concentrations (Fig. 1A). Although it is known that methane sampling suffers from errors due to core handling and pressure release, in these shallow sediments underlying ~ 40 m of the water column, methane losses should be small. The observed decrease in methane concentrations below 20 cm appears robust, having been measured on several independent cores spanning all seasons. Lateral advection of pore fluids also is not a likely confounding factor in LK sediments, based on our conservative ion profiles (e.g., Na^+). Finally, the sediment profiles are assumed to be in quasi–steady state, experiencing only slight seasonal changes. Thus, the methane decrease below ~ 20 cm indicates an oxidative anaerobic sink.

Further evidence for AOM below the main methanogenesis zone comes from the $\delta^{13}C_{CH4}$ profile (Fig. 1B). Since methanogenesis is not significant in deeper sediments (below ~ 20 cm)—based on the leveling off or even slight decrease of $\delta^{13}C$ of the DIC values and on the methanogenesis rate calculations (Adler et al. 2011)—such heavy $\delta^{13}C_{CH4}$ values can only be explained by methanotrophy. This process results in ^{13}C-depleted values of DIC and enrichment of ^{13}C in the residual CH_4. The small variations (maximum 2‰ change) in $\delta^{13}C_{DIC}$ profiles in the zone of deep AOM are of the magnitude that would be expected from partial to complete oxidation of ~ 0.5 mmol L^{-1} of CH_4, with its $\delta^{13}C$ values in the presence of 10–12 mmol L^{-1} DIC with a $\delta^{13}C$ value of ~ + 12‰, when also considering the effects of carbonate mineral dissolution and precipitation. Thus, the profiles of both DIC and CH_4 are consistent with methane oxidation below 20 cm.

AOM is mediated by microbes, and the biomass of both primary and secondary CH_4 consumers is depleted in ^{13}C (Hinrichs et al. 1999; Orphan et al. 2001). Although TLEs integrate many extractable organic constituents of sediments, and although other interpretations of the TLE profile are possible, the decrease in its isotopic composition from −27‰ at the methanogenesis zone down to −31‰ at 27-cm depth is consistent with a greater fraction of lipidic material below 20 cm deriving from biomass participating in an active AOM cycle. The additional presence of ^{13}C-depleted lipids near the sediment–water interface could be consistent with both the biomass of microbial sulfate reducers and a small population of archaea mediating AOM.

The negligible concentration of sulfate, manganese, and nitrate (dissolved inorganic nitrogen in the sediments appears as ammonium [Fig. 1G]) in the deep sediments makes Fe-oxides the most probable terminal electron acceptors. This is supported by the increase in pore-water concentrations of Fe(II) (Fig. 1D) and the decrease in its isotopes (Fig. 1E). Both the AOM process and the observable Fe(II) increase in the deeper horizons are favored by the low concentrations of sulfide at depth. This is because the presence of sulfide in the upper part of the sediments causes precipitation of Fe(II) as FeS and pyrite, but it also encourages methanogenesis, suppressing and masking Fe(III)-driven AOM.

The relatively small increase of Fe(II) relative to the methane decrease in the deep AOM zone reflects the sink of Fe(II) with sulfide in the upper 10 cm of the sediment column. This sink shifts the Fe(II) profile to lower concentrations than would be expected in the absence of such precipitation. Additional loss of Fe(II) throughout the deeper sediments may occur as a result of the precipitation of minerals such as vivianite ($[Fe(II)]^3[PO_4^{3-}]^2$) or siderite ($FeCO_3$). Calculations on other lake sediments have indicated that these minerals should precipitate (Emerson 1976, 1978; Nembrini et al. 1982). Using estimated saturation values of solubility product constant (Ksp) for these minerals at 15°C (after Emerson [1976]), LK pore waters also are at or above saturation for these minerals. For siderite (Ksp ~ 10^{-10}), considering the pH (~ 7) of the sediments and DIC concentrations, the carbonate concentrations range from 1×10^{-5} mol L^{-1} in the upper part of the sediments to 1×10^{-4} mol L^{-1} in the deep part of the sediments. The measured Fe(II) concentrations are also between 1×10^{-5} mol L^{-1} and 1×10^{-4} mol L^{-1}. This yields multiplication $\geq 10^{-10}$. Similarly, for vivanite (Ksp ~ 10^{-35}), considering Fe(II) and PO_4^{3-} profiles (Fig. 1D,G), the multiplication is between 1×10^{-22} and 1×10^{-23} (mol L^{-1})2, which also is above the saturation levels. Together these effects indicate that the Fe(II) profiles should be interpreted qualitatively as reflecting the accumulated product of Fe(III) reduction but that they cannot be modeled quantitatively (for rates) or in stoichiometric ratio to the amount of CH_4 consumed, since sinks for Fe(II) into solid-phase products would skew these values.

The potential of Fe(III) oxides to support AOM in LK was tested by the mesocosm incubation studies. The ratio of Fe(II) increase to methane decrease in the first experiment (Fig. 2) indeed indicates that there are sinks for Fe(II), as suggested for the pore-water profiles. These sinks could be as vivanite or siderite, or the unusual ratio could reflect incomplete oxidation of the methane (complete oxidation to CO_2 would yield 8 : 1)—for example, to the equivalent of $CO_2 : C_2H_6$. This is consistent with suggestions from deep-sea sediments that oxidation may yield ethane or higher molecules (Hinrichs et al. 2006; Sivan et al. 2007) via a pathway that may involve acetate (Hinrichs et al. 2006). The observed methane decrease in the first experiment was small, possibly as a result of the low concentration of added Fe(III). However, in the second experiment, in which higher concentrations of amorphous Fe(III)-oxides were added, a significant difference between the control and the treated core was observed, indicating that the iron served as the terminal electron acceptor for the AOM process (Fig. 3).

The AOM rates calculated from both of the first two experiments were $4 \times 10^{-14} \pm 2 \times 10^{-14}$ mol cm^{-3} s^{-1}. These rates are close to our model results and about 15% of the calculated maximum methanogenesis rate in these sediments (Adler et al. 2011). The rates are also close to those rates estimated by Beal et al. (2009). The latter performed incubation experiments on Eel River Basin sediments and suggested that marine methanotrophs in these sediments are capable of using manganese (birnessite) and iron (ferrihydrite) to oxidize methane under anaerobic conditions with AOM rates of 4.4×10^{-13} and 2×10^{-13} mol cm^{-3} s^{-1}, respectively. The order of magnitude difference between our rates and theirs may be due to the difference in solid mineral phase or to differences in the marine vs. lacustrine microbial communities.

The third experiment (Fig. 4), with ^{13}C methane added to the overlying water, provides further evidence for this iron-dependent AOM. Whereas other interpretations—such as inhibition of methanogenesis—are possible for the two first experiments, our observation that the ^{13}C label appears in the DIC pool indicates that it must come from methanotrophy. In all of these experiments, sulfate was titrated to form highly insoluble barite, and all other potential electron acceptors for AOM were below detection limits. Therefore, the evidence indicates thermodynamic coupling of Fe(III) reduction to oxidation of CH_4, of which at least some of this AOM goes to completion (CO_2).

The rough estimation of AOM rates and the ratio of the Fe(II) increase to the methane decrease enable calculation of the required Fe(III) to sustain this iron-dependent AOM. It should be noted, however, that the Fe(III) requirement calculated in this way represents a minimum, because of the possible higher $Fe:CH_4$ ratio [without any Fe(II) loss]. Using the observed ratios of between 2 : 1 and 6 : 1, the sediment porosity ($\sim$ 0.7 in these depths), the sedimentation rate (4 mm yr^{-1}), and the AOM rates, we calculate that $\sim$ 50–150 μmol g dry wt^{-1} of Fe(III) would be required to sustain this AOM along its depths (porosity $\times$ AOM rate $\times$ $Fe:CH_4$ ratio $\times$ time in the AOM zone $\times$ $(1 - \text{porosity})^{-1} \times \text{sediment density}^{-1}$). This calculation is based on an estimate of $\sim$ 20 yr of time in the AOM zone (depth range of AOM $\times$ sedimentation rate). It can be seen that the required Fe(III) is certainly within the range of the available iron in the sediments.

The probability that the terminal electron acceptor for this AOM is Fe(III) is based on low concentrations of the other possible redox coupled species, such as sulfate. In the presence of accessible Fe(III), AOM by Fe(III) reduction is the thermodynamically expected outcome, yielding more free energy than both sulfate-dependent methanotrophy and methanogenesis (Zehnder and Brock 1980; Valentine 2002). The low potential for sulfate-dependent AOM in LK also was shown through microbial work (Schwarz et al. 2007). However, the direct mechanism of this methane oxidation is not yet clear. There are several different mechanisms possible for coupling iron reduction to methane oxidation. There may be direct methane-dependent iron reduction by one organism or by microbial consortia, similar to sulfate-dependent AOM. Another option may be the involvement of oxidized sulfur species via secondary sulfur cycles: for example, through oxidation of sulfide to sulfate by Fe(III) (despite actively removing both sulfate and sulfide from the experiments). It is also possible that H_2 in the system is consumed by Fe(III)-reducing bacteria to very low concentrations, at which methane oxidation to CO_2 and H_2 (''reverse methanogenesis'') is thermodynamically feasible. This was suggested as the original mechanism for coupling sulfate-reducing bacteria to AOM (Hoehler et al. 1994). Regardless of the actual mechanism operating here for Fe-AOM, the identity of the organism involved remains unknown.

We believe these findings clearly indicate the presence AOM in LK deep sediments. Anaerobic (heterotrophic) oxidation of organic matter by bacterial iron reduction occurs in LK in the water column just below the thermocline. Accessible reactive iron should be consumed higher in the sediment column or even the water column according to the large energy yield of its reduction; however, it is present throughout the sediments (Fig. 1H). This has long been regarded as paradoxical in the literature. It is known that there are bacteria that can use solid Fe(III) minerals and that iron-reducing bacteria can successfully compete with sulfate-reducing bacteria. However, there are several lake sediments similar to those of LK in which sulfate reduction completely consumes pore-water sulfate, even when there are still significant amounts of Fe(III) oxides. As is the case in LK, these sediments show high concentrations of Fe(III) and an increase in pore-water Fe(II) below the sulfate and sulfide zone (Nembrini et al. 1982; Wersin et al. 1991; Widerlund and Ingri 1995). The conclusion of these studies was that their observations reveal the importance of slow iron transformations, probably due to kinetic control on iron transformation. Lovley and Phillips (1986) suggested that the limitation of Fe(III) reduction and the persistence of Fe(III) to great depths may be typical of freshwater environments.

The low availability of Fe(III)-oxides for reduction was attributed to the form of the solid Fe(III) and the slow transfer of electrons between the solid phase and the organic acceptor. Lovley and Phillips (1986) showed that while amorphous Fe(III) oxyhydroxides are readily reducible by microbes, magnetite and the mixed Fe(III)–Fe(II) compounds, as well as most of the other oxalate-extractable Fe(III) minerals, are not available for microbial reduction. They suggested that as soon as there is production of Fe(II) from reduction of amorphous iron and subsequent precipitation of this Fe(II), the mixture of Fe(III) and Fe(II) becomes recalcitrant. We provide here a mechanism for the increase of the Fe^{2+} 'excess' with depth. Given these physical or kinetic barriers, iron reduction may become biologically competitive only after methanogenesis slows down or ends. Only then are iron reducers not outcompeted by organisms with higher substrate availability. The iron isotope profiles support the suggestion that there is active, microbial iron reduction in the deeper part of the sediments (Fig. 1E). It is not surprising that deep iron reduction is coupled to the oxidation of methane rather than residual total organic matter. Indeed, it is analogous to the fate of sulfate in marine sediments. In many marine sediments the majority of sulfate reduction is coupled to AOM rather than traditional BSR, with sulfate reduction rates significantly increased at the AOM zone (which is found deeper than the BSR zone). This can be seen in the linear pore-water profiles that show sulfate diffuses from bottom waters—without net loss—to the zone of AOM (Niewöhner et al. 1998; Sivan et al. 2007). Poor availability and reactivity of residual total organic matter may in both cases make CH_4 a better reactant. Recent work on pore-water profiles of sulfur cycling (Turchyn et al. 2006) also indicates microbial coupling of iron to sulfur or methane cycles far below the depth at which bacterial iron reduction would be expected to cease based on traditional pore-water reaction–diffusion modeling.

The results of this study indicate that respiration below the methanogenesis zone is a sink for methane and that it is likely driven by the reduction of solid-phase iron oxides. Thus, the data from Lake Kinneret provide the first in situ evidence for quantitatively significant iron-dependent AOM. Our estimated rate of this iron-dependent AOM process is about 15% of the maximum methanogenesis rate in these sediments. Similarly, the rate of iron-dependent AOM was speculated to be around 10% of the methanogenesis rate in marine sediments (Sivan et al. 2007). Slowly reacting Fe-oxides are abundant in many anoxic sediments. Hence, iron-dependent AOM could be a globally significant sink for methane in marine and freshwater environments. Total estimated marine sedimentary AOM is $\sim$

380 Tg yr^{-1} (Reeburgh 2007). If iron-mediated AOM conservatively contributes about 10% of this oxidation (38 Tg yr^{-1}), the size of this sink is nearly twice the estimated marine methane flux to the atmosphere. Importantly, it is likely to be the dominant mechanism of AOM in freshwater environments, which are the major source of methane to the atmosphere (70%, as compared to ~ 6% from the ocean).

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