

General comments

Generally, the manuscript address scientific questions within the scope of BG; proving the source of methane in shallow aquifer is a relevant and important issue. The author's present data which indicate that methane detected in an alluvial aquifer is not produced in the aquifer itself but is produced in the underlying coal seam and subsequently migrates upwards to the aquifer. This finding would be of fundamental interest for the risk assessment regarding the occurrence of methane in shallow aquifers. However, three of the authors (including the first and last author) published already in 2015 a paper in which basically the same conclusion has been drawn (Iverach et al., 2015); moreover, essential data – the carbon isotope signatures of methane – shown in the present manuscript have been already published by Iverach et al. (2015). This reduces the originality and novelty of this paper.

The overall presentation is well structured and clear, including an accurate title, a proper abstract and introduction into the topic, and adequate citations of related work.

The applied methods and assumptions are valid; some of the used scientific methods are not clearly described and cannot be reproduced (see specific comments). Generally, the results are sufficient to support the main conclusion that the source of the methane detected in the alluvial aquifer was the underlying coal seam. Some interpretations based on the geochemical and microbiological data are certainly speculative (see specific comments) and need to be supported by literature/experimental data; if not possible, these parts should be condensed or deleted. On the other hand, one important result of this study, the oxygen concentrations of the investigated groundwater samples, is not seriously presented and discussed in the main manuscript (the data are somewhat hidden in the supplemental information). The oxygen data indicate that the studied aquifer zones are predominantly aerobic, a fact that could explain the absence of strictly anaerobic methanogens in the groundwater samples. Due to the presence of methanotrophs and availability of oxygen in the aquifer, the question arises to which extent methane is oxidized and whether aerobic oxidation of methane is trackable in the aquifer by compound specific stable isotope analysis, as this reaction is characterized by strong carbon and hydrogen isotope fractionation (Feisthauer et al., 2011). Unfortunately, this aspect is not discussed in the manuscript.

Specific comments

Lines 96-103: This statement is too strict. It's true that sulfate reducers generally outcompete methanogens but not always, see Struchtemeyer et al. (2005).

Lines 119-133: I suggest mentioning that the expression of the particulate and soluble methane monooxygenase is triggered by the amount of available copper ions.

Lines 208-212: For clarity, I suggest indicating the depth at which each well was sampled. I do not understand why the eight samples are representative of the aquifer, please explain in detail.

Line 226: How long were the DIC samples stored before measurement? Please indicate.

Lines 228-230: I wonder why samples for geochemical and microbiological analyses were not sampled at the same time, which would have strengthened the main conclusions of this paper.

Lines 232: Probably, any nanobacteria (prokaryotes smaller than 0.2 μm) were lost during this procedure?

Lines 241-259: Give references for the methods of $\delta^2\text{H-H}_2\text{O}$, $\delta^{18}\text{O-H}_2\text{O}$, $\delta^{13}\text{C-DIC}$, $\delta^{13}\text{C-DOC}$, $\delta^{18}\text{O-SO}_4^{2-}$ and $\delta^{34}\text{S-SO}_4^{2-}$ analysis or describe the methods in detail that they can be reproduced.

Lines 262 ff. A critical question is whether the microbial community of a groundwater sample will truly reflect the microbial community of the subsurface from which the groundwater was extracted from. This aspect should be briefly discussed (probably in the Results & Discussion section).

Figure 2: In the Figure, five ranges are shown (indicated by 5 different colors) whereas only four ranges are given in the legend. I recommend using different colors for each order of magnitude for higher resolution. A general drawback of Figure 2 is the lack of any statistics, what are the standard deviations of the data?

Line 420 ff. See comment above. It's true that sulfate reducers generally outcompete methanogens but not always, see Struchtemeyer et al. (2005). I recommend discussing with more caution.

Lines 425-428: It is very speculative to conclude that the detected phylotypes affiliated to sulfate or sulfur reducers will oxidize acetate (or outcompete methanogens). I suggest discussing with more caution. Deducing specific metabolic activities from partial 16S rDNA sequences is questionable.

Lines 428-432: I do not understand this argumentation. Methylocella are aerobic organisms, whether methanogens are strictly anaerobic. They probably do not exist in the same ecological niche.

Lines 448-450: What could be an alternative pathway for aerobic methane oxidation in an anaerobic environment? The initial methane oxidation reactions will always depend on molecular oxygen, hence aerobic methane oxidation cannot take place in the absence of oxygen. Why not discussing the detected (high) oxygen concentrations of the groundwater samples in this context?

Lines 460-462: I wonder why the oxygen data are not shown in more detail. Some wells seem to be fully aerobic, a result which does not correspond to the observation of the dominance of sulfate or sulfur reducing deltaproteobacteria in most of the samples. On the other hand, the presence of oxygen explains well the presence of methanotrophs and other aerobes in the groundwater samples. Probably, the discrepancy might be explained by the sampling artifacts; the pumped groundwater may contain strictly anaerobic organisms originally attached to the aquifer solids in which anoxic microenvironments exist.

Lines 470-476: This hypothesis is very, very speculative. Are there any indications for the presence of nitrate in the groundwater? Why Chloroflexi should convert denitrification products to oxygen? The hypothesis needs more arguments (support by literature or own experimental data); if no other arguments are available, I suggest deleting this passage.

Lines 487-488: Give references for this statement.

Lines 490-491: I doubt that the methane concentrations were high enough to allow sulfate-dependent AOM. Please discuss.

Cited literature:

Feisthauer S, Vogt C, Modrzynski J, Szlenkier M, Krüger M, Siegert M, Richnow HH (2011) Different types of methane monooxygenases produce similar carbon and hydrogen isotope fractionation patterns during methane oxidation. *Geochim. Cosmochim. Acta* 75: 1173-1184

Iverach CP, Cendón DI, Hankin SI, Lowry D, Fisher RE, France JL, Baker A, Kelly BFJ (2015) Assessing connectivity between an overlying aquifer and a coals seam gas resource using methane isotopes, dissolved organic carbon and tritium. *Sci. Rep.* 5: 1-11

Struchtemeyer CG, Elshahed MS, Duncan KE, McInerney MJ (2005) Evidence for acetoclastic methanogenesis in the presence of sulfate in a gas condensate-contaminated aquifer. *Appl. Environ. Microbiol.* 71: 5348-5353

Technical comments

Line 322: DSMZ, Braunschweig, Germany