We thank the reviewer for their time and constructive comments on our manuscript. We have addressed all concerns raised below.

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 microbiological data presented in this paper are unique and vastly improve our understanding of this aquifer system. A small portion of the geochemical data from the previous manuscript was reproduced here for ease of reading the 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.

We have added citations to all mentioned methods, and we have addressed the specific speculative comments below.

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.

The dissolved oxygen data in the groundwater were measured using a YSI probe on the surface that was also measuring the pH, EC, TDS, temp. As such, it is not a completely accurate representation of the DO conditions in the aquifer, as the degassing caused by pumping and the effect of the barometric pressure needs to be considered. However, we have mentioned the high DO concentration (line 541), addressing the comments above, as well as

the DO concerns raised below. Unfortunately, tracking methane oxidation was outside the scope of this study, which aimed at characterising for the first time the microbial community in this freshwater aquifer and seeing if it was possible to use microbes to help elucidate the source of CH_4 detected in the aquifer. It would be a very useful future study, but we have not mentioned it in the text because it is outside the scope of this investigation.

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).

This statement has been softened: "…because SRB often outcompete methanogenic archaea…" and the suggested reference has been included.

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.

This has been mentioned at the suggested location in the text.

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.

A table indicating the slotted interval for each sample has been included in the methods now. We understand that eight samples are a small dataset, however they are at varying depths and locations throughout the aquifer. Physico-chemical parameters and the spread of geochemical data indicate that the samples are representative of the spread of the conditions of the aquifer as a whole.

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

The DIC samples were analysed within one month and this information has now been included in the manuscript. They were also filtered through a 0.22 μm filter in the field, which is the best way to maintain the sample (provided refrigeration and proper storage) (Doctor et al. 2008). In addition, DIC samples from another field site were analysed 1 week after collection, and then re-analysed 6 months later and were found to have no difference in measurement.

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.

Insights from the original hydrogeochemical survey indicated that microbiological data would refine our understanding of the processes. Therefore we returned and collected microbiological data (at a limited number of sites due to budget constraints). In December of the same year (when the aquifer is under the same stress as in January), additional funding was granted and we were able to sample for the microbiology.

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

A 0.2 µm filter is standard for filtering microbial communities. The filtrate was also screened using SYBRGREEN I staining and microscopy and there was no detection of cells.

Lines 241-259: Give references for the methods of d2H-H2O, d18O-H2O, d13C-DIC, d13C-DOC, d18O-SO4, d34S-SO4 analysis or describe the methods in detail that they can be reproduced.

References for the methods of analysis for all geochemical data have been provided in this section.

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).

We do believe that the microbial community of the groundwater is reflecting the microbial community of the subsurface. Maamar et al. (2015) found that the microbial community composition of groundwater was controlled by groundwater residence times and the location of samples along the groundwater flow path, independent of the geology, stating that "hydrogeologic circulation exercises a major control on microbial communities". They also state: "…Thus, geochemical conditions, and in particular the availability of electron donors and acceptors, are a major driver of microbial community composition and diversity in groundwater and the geological substratum".

Additionally, when we sample the groundwater, we are also sampling fine particles with biomass attached. Further, the Condamine production wells are drawing water that is representative of the sampled formations and the intense purging ensures that this is the case. The ^{14}C and ^{3}H activities suggest that we are not drawing a modern/old mixed groundwater component, therefore whatever water is sampled is representative of the formation, and we presume the microbial communities within it.

A small paragraph explaining the above has been included in the discussion (line 507).

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?

Figure 2 has been changed - 4 different colours have been used for 4 different ranges. Standard deviations have been added to the figure legend and qPCR specific validations are in the methods (line 366-370).

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.

We have clarified the language above, however in the text at this location we do already say "These SRB are potentially outcompeting methanogenic archaea…", implying that this may not be the case. We then proceed with additional evidence as to why the lack of methanogenic archaea could be a result of this competition.

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.

We have clarified our discussion. Because most of the Deltaproteobacteria sequences detected in the groundwater were closely related to acetate-oxidising sulfate/sulfur reducing bacteria (*Desulfovibrionales, Syntrophobacterales, Desulfuromonadales*), it is reasonable to assume that the lack of methanogenic archaea could potentially be a result of competition from sulfate reducers taking the acetate, which is the methanogenic substrate required.

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.

Aerobic and anaerobic microorganisms can exist in the same environment. They are not strictly separated; e.g. anaerobic methanogens can occur in anoxic or suboxic microniches in mainly aerobic environments (Kato et al., 2007; Dimikić et al., 2011).

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?

As previously mentioned, the detected high concentrations of dissolved oxygen in the groundwater have been discussed now and it has been stated that these are most likely the reason for abundant aerobic methanotrophs in the groundwater. Therefore, an alternative pathway for aerobic methanotrophs, potentially using other electron acceptors, has not been discussed.

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.

As mentioned previously, the DO data are not a completely accurate representation of DO concentration within the aquifer - this is why they were included in the supplementary material but not highlighted in the text. If the discrepancy between DO and deltaproteobacteria is to be explained by sampling artifacts, it would probably be this, not microbial sampling methods.

Aerobic and anaerobic microorganisms can live alongside each other in many habitats in microniches. Sulfate reduction under oxic conditions has been observed and previously published; e.g. in cyanobacterial mats or periodically in activated sludge (Kjeldsen et al. 2004; Fike et al. 2008).

We have now explicitly referred to the role that the high concentration of DO is potentially playing in the absence of methanogenic archaea and abundance of aerobic bacteria (line 541). In addition, we have explained why the deltaproteobacteria are dominant in most samples despite the presence of O2.

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.

We have removed this hypothesis.

Lines 487-488: Give references for this statement.

A reference has been given for this statement (Pester et al. 2011).

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

We agree that methane concentrations were most likely not high enough to allow sulfate-dependent AOM in this groundwater. However, at this location in the manuscript we are going step-wise through our data providing evidence either for or against potential processes affecting the occurrence of CH4 in this groundwater – at this particular point, it is the possible occurrence of AOM in the groundwater. Hence, we state that the sulfate concentrations are potentially high enough to mediate AOM at 2 locations, however, we go on to state that further geochemical evidence (including lack of detected ANME's) indicate that this process is not occurring.

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 aceticlastic 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

This has been corrected.

References:

Dimikić, M., Pušić, M., Majkić-Dursun, B. & Obradović, V. Certain implications of oxic conditions in alluvial groundwater. *Water Res. Manage.* **1(2)**, 27-43, (2011).

Fike, D.A., Gammon, C.L., Ziebis, W. & Orphan, V.J. Micron-scale mapping of sulfur cycling across the oxycline of a cyanobacterial mat: a paired nanoSIMS and CARD-FISH approach. *ISME J.* **2**, 749-759, (2008).

Kato, M.T., Field, J.A. & Lettinga, G. Anaerobe tolerance to oxygen and the potentials of anaerobic and aerobic cocultures for wastewater treatment. *Braz. J. Chem. Eng.* **14(4)**, (1997).

Kjeldsen, K.U., Joulian, C. & Ingvorsen, K. Oxygen tolerance of sulfate-reducing bacteria in activated sludge. *Environ. Sci. Technol.* **38(7)**, 2038-2043, (2004).

Maamar, S.B., Aquilina, L., Quaiser, A., Pauwels, H., Michon-Coudouel, S., Vergnaud-Ayraud, V., Labasque, T., Roques, C., Abbott, B.W. & Dufresne, A. Groundwater Isolation Governs Chemistry and Microbial Community Structure along Hydrologic Flowpaths. *Fron. Microbiol.* **6**: 1457, (2015).

Pester, M., Schleper, C., Wagner, M. (2011) The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Current Opinion in Microbiology, 14: 300-306.