Referee 2

(1) The paper is in part a bit sloppy, i.e., missing parenthesis at citations in the text,

The missing parenthesis () has been added.

inconsistent figure layout concerning the order of accession numbers and species name (Fig. 7 vs. Fig. 6 and 8), the obtained accession numbers should be included in the trees, although they are given in the material and methods section,

The layout of the trees has been adjusted as requested.

the figure order has to be changed (Fig. 5 is mentioned after Fig. 6).

Figure numbers have been corrected.

(2) The length, particularly of the site description and site history in the introduction and methods section, needs to be shortened and combined. As your samples were obtained at just one sampling site and your investigations focused on process studies, a detailed explanation of the Ljubljana marsh area is redundant.

Site description has been condensed to:

"Ljubljana Marsh is a 16 000 ha fen region located in central Slovenia (45°58´N, 14°28´E). It is characterized by mineral-rich ground water and neutral pH. The average annual precipitation is 1400 mm, and the mean annual temperature is 10°C. A drainage-channel system was established in the 19th century and the landscape has been shaped ever since by peat extraction and agriculture. Today, 75% of the area is covered by grassland, 10% by forest, and 15% by cornfields. The experimental site is located on a grassland dominated by an *Arrhenatherion* (Seliškar, 1986). The water table is on average 50 cm below the surface, with pronounced seasonal fluctuations from flooded to 1 m below ground. The soil temperature in the upper 30 cm varies annually from 1°C to 20°C.

Samples were collected at the end of August in 2005 with ten individual soil cores (h=30 cm; d=15 cm) within a 10×5 m^2 area covering the upper 30 cm of soil. Soil samples were pooled, and plants and roots were removed to avoid litter input. The soil was homogenized by passing through a 3.15-mm sieve. Immediately after homogenization, aliquots (0.5 g) for molecular analysis were frozen and stored at –20°C. The remainder was kept at 4°C for maximally two weeks until the experiments were set up. The pooled soil contained 32% organic matter, and had a pH (H_2O) of 7.6. For a more detailed description of this soil see (Hacin et al., 2001)."

(3) As the main focus in the introduction section is on methanogenesis, the competition with Fe(III) reducers for substrates has to be introduced in more detail and supporting literature would be helpful.

See below (4).

In addition, there are suggestions that methanogens may transfer electrons to Fe(III).

See below (4).

(4) A brief introduction of Fe(III) reducing processes in hydrological unstable environments would support your story. It is not clearly stated in the introduction why *Geobacteraceae* were investigated as a representative group of Fe(III) reducing microorganisms.

Change made in the introduction: "In addition, the community structure of Archaea and *Geobacteraceae* was analyzed, taking the latter as representatives of mesophilic iron reducers."

(5) Please provide more information about GC and HPLC analysis (column material; especially for measurement of organic acids and alcohols).

Changed to: "Gases were measured as described previously (Bodelier et al., 2000; Metje and Frenzel, 2005). In short, CH_4 and CO_2 concentrations were measured on a SRI-8160A GC (SRI Instruments, Torrance, CA) with H_2 as carrier gas equipped with a methanizer and a flame ionization detector. H_2 concentrations were measured with a reducing gas detector (RGD2, Trace Analytical, Stanford, CA, USA). When H_2 concentrations were >200 ppm_v, a Shimadzu GC8A with N_2 as carrier gas and a thermal conductivity detector was used."

(6) I have concerns with your general conclusion that the formation of CH_4 was limited by competition for substrates, mainly for acetate (2372:20-21). From your accumulation data obtained 115 days after incubation (Fig. 2) it can not be stated that Fe(III) reduction and methanogenesis are competing processes. I agree that the correlation for acetate and CH_4 is quite as good. However, concomitant accumulation of acetate and Fe(II) at around 15°C suggesting a) no potential substrate limitation for methanogens and b) low acetate consumption of Fe(III) reducers. In addition, the pool of microbially available Fe(III) can be reduced within 70 days at 25°C (Fig. 4). Thus, accumulation data of redox sensitive processes and metabolic intermediates obtained after an incubation period of 115 days could be misinterpreted. Data for acetate during your incubations presented in parts in Fig. 4 could be strengthening the suggestion for competing processes. In addition, incubation experiments with additional acetate or H_2/CO_2 could also support this conclusion. Right now, I would suggest weakening your statement or add supportive data.

Looking at the biogeochemical data alone would indeed request weakening the argument. However, the molecular data strongly support our view:
(a) No methanogenes were detectable in the original soil,
(b) but in a methanogenic sample after incubation.
Hence we argue that methanogens didn't have suitable growth conditions due to substrate concentrations below their respective thresholds (= competition) until the competing iron reducers had exhausted their electron acceptor. This is consistent with the transient increase of hydrogen and acetate (Figure 2C & F)

indicating that at that time point and temperature the methanogenic population still had to grow up. Please note that the temperature axis can be treated analogously to a time axis. To clarify, we have added:

"We have shown that re-establishment of a methanogenic population and, hence, production of CH_4 was limited by competition for substrates, mainly for acetate."

(7) The authors suggest that a fluctuating water table would enhance the redox cycling of iron for this peatland and decrease potential emission of CH4 (2372:20-2373:2). I agree with this statement, but I think it would be worthwhile to consider the implications of the findings beyond this study site. Would these results apply to all peatlands of the same type that are intended to be reflooded? As methanogenesis was restricted to temperatures above 15°C do you expect any methanogenic activity in-situ (or maybe just in the summer season)? Do you think that changing redox conditions would enhance the mineralization and emission of carbon for this peatland?

These are very interesting points, but discussing them in depth would go far beyond the scope of our paper. Peatlands are far too different to derive a general prediction from our data: *Sphagnum*-peat in raised bogs will contain virtually no reducible iron, ruling out any effect like that observed here, and the same will be true for tropical wood peat. No additions made.

2358:11 How long was the lag period? In general, values or ranges for lag periods should be mentioned in the manuscript.

Changed to: "Methane production in anoxic soil slurries started only after a lag period of 84 d at 15°C and a minimum of 7 d at 37°C, the optimum temperature for methanogenesis. This lag was inversely related to iron reduction,..."

2358:18 I like that the abstract is succinct but a link from methanogens to Geobacteraceae as a representative group of Fe(III) reducers would be helpful.

We have already mentioned in the abstract that a diverse community of iron-reducing *Geobacteraceae* was found. No additions made.

2359:1-2 Since you focused on a peatland and the potential release of methane you should mention the important function of peatlands to store carbon than the ability to store and clean water.

We have amended the following paragraph:

"Peatlands are characterised by soils rich in organic matter. Peat accumulated since the last glaciations corresponds to 20-30% of the global soil carbon pool (Frolking et al. 2001; Gorham 1991)."

2359:27 Add the temperature optimum for mineralization.

There is no general optimum, hence we have changed to:

"...methanogenesis may dominate at low temperatures, while electrons are diverted to iron reduction at the respective temperature optimum of mineralization..."

2361:22 Add the range of these fluctuations.

It has been added to the site description.

2361:24 How were the cores pooled? Were all of the ten cores pooled together or just the individual cores? Be more specific.

Has been amended, see above: site description.

2362:5 Did you measure the moisture content of your soil incubations during the first incubation period of 65 days? Was it still at 90% water holding capacity although you used open microcosms? Add a comment.

Information added:

"The water content was checked regularly by weighting the microcosms and adjusted with demineralised water, if necessary."

2362:9-12 When did you add the difluormethane? Did you add this inhibitor to all incubations at every sampling or just at the end of incubation? Add this information to your material and methods section.

We have added more information and references:

"Methane oxidation was measured comparing fluxes with and without difluoromethane (CH_2F_2), a specific inhibitor of CH_4 oxidation (Miller and Oremland, 1998). Difluoromethane was added to a headspace concentration of 1% as described previously (Eller and Frenzel, 2001; Krüger et al., 2002)."

2362:13 What anaerobic processes are you referring to? Be more specific.

It is intentionally unspecific, because the experiment deals with all processes from fermentation through iron reduction to methanogenesis. No changes made.

2362:13-15 The writing needs to be improved! Separate both experiments to be more specific. Right now, it is difficult to follow your instructions.

We have specified:

"Homogenized soil in 15 or 25 ml pressure tubes was mixed with sterilized anoxic distilled water at a ratio of 1:1.5 (vol/vol) to a total volume of 6.5 (effect of temperature, see below) and 10 ml (methanogenic pathways, see below)."

2362:21 Add temperature steps (2.5°C?).

We have changed to:

"*The effect of temperature* was measured in 15-ml tubes incubated in a linear 0 to 80°C temperature gradient in the dark (Fey et al. 2001; Schulz et al. 1997). Tubes were incubated in duplicate at 30 temperatures for 115 days."

2362:22-24 Replace "after the tubes were opened" with "at the end of the experiment".

Changed as requested.

2362:25 Add "potential" methanogenic activity/pathways, because incubations temperatures were not representative for field conditions.

Changed as requested.

2363:3 Replace the first sentence.

See below

2363:3-9 Add more details, i.e. detection limit for gases (concerning 2362:7), column material. Add a definition for "low" concentrations?

Has been changed to:

"Gases were measured as described previously (Bodelier et al., 2000, Metje and Frenzel, 2005). In short, CH_4 and CO_2 concentrations were measured on a SRI-8160A GC (SRI Instruments, Torrance, CA) with H_2 as carrier gas equipped with a methanizer and a flame ionization detector. H_2 concentrations were measured with a reducing gas detector (RGD2, Trace Analytical, Stanford, CA, USA). When H_2 concentrations were >200 ppm_v, a Shimadzu GC8A with N_2 as carrier gas and a thermal conductivity detector was used."

2363:12 Again, add more details (HPLC, GC).

No changes made, the methods have been described in full detail before. A reference is given.

2364:8 Add a definition of labile and recalcitrant organic fractions in this case (extractable, thermal, microbial degradable)?

Experiment and model deal only with microbially degradable organic matter. We have changed to "..rate constants for the decomposition of labile and more recalcitrant organic fractions, respectively. Microbially not degradable fractions are not considered here."

2365:3-5 Add primer names.

Changed to: „The methanogenic communities were characterized by PCR amplification using three different *mcrA*-specific primer sets (Hales et al., 1996: ME1 and ME2; Luton et al., 2002: mcrAf and mcrAr; Springer et al., 1995: MCRf and MCRr)."

2365:26 Is it important to give the number (two) of the DGGE gels? It's confusing because you never mentioned different DGGE gels. Which bands from which incubation

Changed to: "Bands were excised...".

2368:23-24 Be consistent (see value for PCR products at 2368 15-16).

Checked and inconsistancy removed.

2368:27-2369:2 The interpretation for the T-RF 185bp fragment should be combined in one sentence.

Changed to: "It is suggested that the T-RFs of 185 bp represents members of *Methanosarcinaceae* or RC-VI (Kemnitz et al. 2004), 382 bp members of the euryarchaeal RC-III, and the T-RF of 394 bp members of the methanogenic RC-I (Kemnitz et al. 2004; Lu et al. 2005; Penning and Conrad, 2006)."

2369:3 What is the optimal temperature you are referring to? Did you mean the optimum temperature for acetoclastic methanogenesis or methanogenesis in general? Which methanogenic slurry did you use for this analysis (incubation temperature and time)?

Information is already given in the figure legend. In addition, we changed to: "In accordance with acetoclastic methanogenesis prevailing at optimal temperature (37°C, Figure 2), 46 *mcrA* clone sequences from a methanogenic slurry incubated at 35°C were represented by acetoclastic *Methanosarcina* sp. and hydrogenotrophic RC-I at a ratio of 91:9."

2369:6 Since not all PCR products belonging to Geobacteraceae (in comparison with 2369:13-15), be careful using "Geobacteraceae-specific PCR".

Changed in line 6 to "*Geobacteraceae*- targeting PCR..."

2370:13 As far as I know, some Archaea are also able to reduce Fe(III). Use a more general term instead "bacteria".

We are explicitly addressing the phylogeny of bacterial iron reducers – no changes made.

2371:6 Change "onset of iron reduction and methanogenesis" to "ongoing iron reduction and onset of methanogenesis".

Changed to: “The higher levels of H_2 and acetate in 15°C-samples coincided with the onset of methanogenesis.”

2371:9 Which sequences are you referring to? Be more precise!

Changed to: “The archaeal 16S rRNA gene sequences retrieved from the original soil fell all into rice cluster VI, a group of non-methanogenic Crenarchaeota.”

Table 1 For better comparison, add corresponding sequence similarities and names of clones, representative for the groups or bands at the specific incubation temperature.

We have amended the tree with accession numbers and bootstrap values, as requested. Adding group numbers (Roman numerals) and using the same numbering in the DGGE-gel allows linking gel to the tree. Table 1 is now redundant and has been deleted.

Phylogenetic trees: Add bootstrap values for the trees and mention the number of replicates for the phylogentic analyses.

In material and methods, we have changed to:

“Sequences were aligned and phylogenetically analyzed with the ARB software package (Ludwig et al., 2004) using neighbour-joining and Tree-Puzzle (Schmidt et al., 2002).”

Details are given in the respective figure captions,:

"Methanogenic *Archaea*: Tree-Puzzle tree of *mcrA*-sequences. The tree was calculated with 10,000 puzzling steps, the Whelan-Goldman substitution model, parameter estimation using neighbor-joining, a filter 20-100%, and 160 valid columns. Sequences retrieved after incubation at 35°C for 115 days are printed in bold. Scale bar: estimated number of changes per amino acid position. Root: *Methanopyrus kandleri* (AF414042). Clone sequences were aligned against an ARB-database with ~2500 *mcrA* sequences. Nearest cultivated and environmental neighbors were identified after adding the clone sequences with the quick add tool (parsimony) to an existing working tree with ~2500 sequences. Since clone sequences were forming coherent clusters, only 17 representatives out of 45 sequences were subsequently used together with the nearest cultivated and environmental sequences to generate the initial maximum-likelihood tree."

"*Geobacteraceae* and related *Desulfuromonadales*: Neighbour-joining tree of 16S rRNA gene sequences retrieved from the original soil, or recovered from DGGE bands. A bootstrap tree with sequences from cultivated species (>1,300 bases) was constructed and clone sequences were added afterwards by quick add (parsimony) as implemented in ARB, considering a total of 313 base positions. Scale bar: estimated number of base changes per nucleotide position. Root: *Escherichia coli* (AJ567617)."

No changes have been made in the tree showing the Crenarchaeota, because it's just meant to document the affiliation of the retrieved sequences with cluster VI. No cultivated members are known so far.