REFEREE 1

Specific Comments

P2360L12-13 Were fluxes measured of the drained fen?

> Changed to: "However, fluxes measured in the field showed only occasional traces of CH_4 emissions (Danevčič and J. Hacin, personal communication, 2006)."

P2361 The samples were sieved and stored for maximal 2 weeks. During sieving and storage, soil samples were exposed to oxygen. Could such oxic conditions have caused an inactivation of methanogens and a re-oxidation of iron? Was the lag phase for methane production affected by handling and storage of fen soil?

> Re-oxidation of iron was not affected, because soil was sampled at the end of summer when the water table was low anyway and the topsoil well aerated. Methanogens and methanogenic potentials are pretty resistant to desiccation, see for example Fetzer et al. (1993), Joulian et al. (1996), Ueki et al. (1997), and Jäckel et al. (2001). Hence, the length of the lag for methanogenesis was most probably a sum of soil characteristics and microbial community at sampling time. No changes made.
>
> Fetzer,S., Bak,F., Conrad,R., 1993. Sensitivity of methanogenic bacteria from paddy soil to oxygen and desiccation. Fems Microbiology Ecology 12(2), 107-115.
> Jäckel,U., Russo,S., Schnell,S., 2005. Enhanced iron reduction by iron supplement: a strategy to reduce methane emission from paddies. Soil Biology & Biochemistry 37(11), 2150-2154.
> Joulian,C., Ollivier,B., Neue,H.U., Roger,P.A., 1996. Microbiological aspects of methane emission by a ricefield soil from the Camargue (France). 1. Methanogenesis and related microflora. European Journal of Soil Biology 32(2), 61-70.

P2362L9-10 Please clarify; on the first sight, it is puzzling to read that methane oxidation was measured after adding an inhibitor for methane oxidation.

> The standard-technique for measuring methane oxidation is by inhibition, comparing the increase of headspace methane concentrations with and without a specific inhibitor. Changed to: "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)."

P2362L25 What was the rational of using only temperatures above 25 °C, although soil temperatures ranged from 1-20 °C (P2361L23)?

> The rational for using temperatures above 25°C was based on previous results obtained from the temperature block experiment indicating that lag at lower temperatures would be too long to give measurable results within reasonable time. No changes made.

P2363 Please give details of the gas chromatographic determinations or cite appropriate references. What columns, oven temperatures, gas chromatographs, flow rates of carrier gas, etc. were used?

> 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."

P2364 Please give position in the protocol, number and volumes of guanidine thiocyanate solution washes or appropriate reference. Please indicate manufacturer, city, country whenever needed.

> Full addresses are now indicated.
>
> A reference has been added for the guanidine thiocyanate wash: "Humic acids were removed by additional washes with guanidine thiocyanate solution (5.5 M; Metje and Frenzel, 2005)."

P2365 What PCR-chemicals were used?

> Information added: "Enzymes and reagents for PCR reactions were obtained from Promega (Madison, WI, USA)."

P2366L5-13, Fig. 6-8. Verification of tree topology is needed. E.g., the authors could apply bootstrapping (>1000) or drawing of consensus trees based on 2 additional treeing algorithms (which may be more robust than bootstrapping).

> 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.

P2368L21-P2369L1-5 Was the affiliation of T-RFs to certain groups (as evidenced by literature references) also supported by clone sequences retrieved from the fen soil analyzed?

We 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, Y. Het al. 2005; Penning and Conrad, 2006). This affiliation is consistent with the clone library: archaeal 16S rRNA gene clone sequences from the original soil belonged exclusively to RC-VI, and *mcrA* clone sequences from a methanogenic slurry incubated at 35°C were represented by acetoclastic *Methanosarcina* and hydrogenotrophic RC-I at a ratio of 91:9."

P2369 and Table 1. What were the similarities of DGGE band derived 16S rRNA gene sequences to the closest related organisms? Such information should be included into Table 1. P2370 The discussion should take such similarities into account. Are those sequences indeed indicative of organisms with the suggested physiologies?

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.

We have changed in *Results* to:
"All sequences (*n*=74) clustered within the order *Desulfuromonadales,* including the the Fe(III) reducing genera *Geobacter*, *Desulfuromonas* and *Pelobacter.* One sequence was affiliated to the genus *Anaeromyxobacter*. The clones and the respective DGGE bands were assigned to groups I-XI (Figure 8). The vast majority of bands were positioned on the gradient gel between 40 and 55% denaturant. The bands positioned at denaturant concentrations >55% (not shown) belonged to the iron reducer *Anaeromyxobacter*. Most bands were present throughout the whole temperature range but differing in relative intensity."

In the discussion we write now:

"At low temperatures, one of the pronounced bands belonged to group XI that could be affiliated to the acetate oxidizing *Desulfuromonas acetoxidans*."