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Microbial reduction of iron and porewater biogeochemistry in acidic peatlands

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Abstract

Temporal drying of upper soil layers of acidic methanogenic peatlands might divert the flow of reductants from CH_4 formation to other electron-accepting processes due to a renewal of alternative electron acceptors. In this study, we evaluated the in situ relevance of Fe(III)-reducing microbial activities in peatlands of a forested catchment that differed in their hydrology. Intermittent seeps reduced sequentially nitrate, Fe(III), and sulfate during periods of water saturation. Due to the acidic soil conditions, released Fe(II) was transported with the groundwater flow and accumulated as Fe(III) in upper soil layers of a lowland fen apparently due to oxidation. Microbial Fe(III) reduction in the upper soil layer accounted for 26.7 and 71.6% of the anaerobic organic carbon mineralization in the intermittent seep and the lowland fen, respectively. In an upland fen not receiving exogenous Fe, Fe(III) reduction contributed only to 6.7%. Fe(II) and acetate accumulated in deeper porewater of the lowland fen with maximum concentrations of 7 and 3 mM, respectively. Both supplemental glucose and acetate stimulated the reduction of Fe(III) indicating that fermentative, incomplete, and complete oxidizers were involved in Fe(II) formation in the acidic fen. Amplification of DNA yielded PCR products specific for *Acidiphilium*-, *Geobacter*-, and *Geothrix*-, but not for *Shewanella*- or *Anaeroromyxobacter*-related sequences. Porewater biogeochemistry observed during a 3-year-period suggests that increased drought periods and subsequent intensive rainfalls due to global climate change will further favor Fe(III) and sulfate as alternative electron acceptors due to the storage of their reduced compounds in the soil.

1 Introduction

Acidic wetlands (pH<5.0) represent a vast type of northern wetlands in Eurasia and North America (Harriss et al., 1993). Waterlogging, low temperatures, and low nutrient quality of plant litter impair decomposition of plant litter, favoring the accumulation of organic carbon. Emission estimates of the greenhouse gas methane (CH_4)

from wetlands range from 92 to 232 Tg CH_4 year^{-1} (Wuebbles and Hayhoe, 2002). Although rates of CH_4 production were shown to be correlated with water-table depth, peat chemistry and vegetation type (Verville et al., 1998), pathways of CH_4 production are still not well understood. Generally, two-thirds of the biogenic CH_4 produced in wetlands originates from acetoclastic methanogenesis (Conrad, 1999). However, H_2-CO_2 appears to be a significant precursor in northern peatlands (Avery et al., 2003; Horn et al., 2003). Acetate even accumulates in some peatlands as a terminal product of anaerobic decomposition indicating that it is not the primary source of CH_4 that is emitted from such habitats (Hines et al., 2001; Duddleston et al., 2002). Acetate consumption appears to occur in these peatlands after diffusion into oxic environments where it is oxidized to carbon dioxide (CO_2).

High-latitude regions are expected to experience a temperature increase as a result of global climate change, and climate models predict a decrease in annual precipitation in most European regions during the next decades (International Panel on Climate Change (IPCC) 2007). Thus, transient drying and oxidation of upper soil layers might divert the flow of reductants from CH_4 formation (Blodau et al., 2004) to other electron-accepting processes due to the renewal of alternative electron acceptors. Atmospheric nitrogen and sulfate depositions might further enhance the activity of sulfate and nitrate reducers under changed climatic conditions in northern peatlands similar to other wetlands (Gauci et al., 2002; Lamers et al., 2002; Vile et al., 2003). However, addition of alternative electron acceptors to ombrotrophic bogs and minerotrophic fens incubated in anoxic jars do not universally divert carbon and electron flow from CH_4 formation (Dettling et al., 2006).

δ^{34}S values and ^{35}S-labeling patterns indicate that the dissimilatory reduction of sulfate is an ongoing process in acidic seeps and fens of a forested catchment in northern Bavaria, Germany (Lehstenbach, Fichtelgebirge) (Alewell and Giesemann, 1996; Alewell and Novak, 2001) like in peat bogs (Wieder and Lang, 1988). In minerotrophic fens which are connected to the groundwater flow, ferric iron [Fe(III)] can be another potential electron acceptor. Fe(II) concentration profiles hint to microbial Fe(III) reduc-

tion in pH neutral fens adjacent to agricultural fields (Todorova et al., 2005), and Fe(III) reduction appears to parallel CH_4 formation in northern acidic wetlands (Metje and Frenzel, 2005). Fe(III) reduction might compete with methanogenesis for H_2 or acetate (Roden and Wetzel, 2003). Most Fe(III) reducers can use one or more alternative electron acceptors (Lovley et al., 2004), which might be advantegeous in upper peat layers that experience redox fluctuations due to water-table variations or oxygen release by plant roots. While Fe(III) exists predominantly in the solid phase as oxyhydroxide minerals at circumneutral pH, Fe(III) is more soluble under acidic conditions (Lovley et al., 2004). However, most cultured Fe(III) reducers are neutrophiles and have only negligible capacities to reduce Fe(III) under moderately acidic (pH 3–6) conditions. Thus, we have only a marginal understanding of the flow of carbon and reductant in acidic, Fe(III) rich habitats and of their inherent Fe(III) reducing microbial communities (Vile and Wieder, 1993; Cummings et al., 2000; Blodau et al., 2002; Cummings et al., 2003; Petrie et al., 2003; Adams et al., 2007; Blöthe et al., 2008).

The main objectives of this study were to (1) assess Fe(III) reducing activities of peatlands of the Lehstenbach catchment which differ in their hydrology, (2) to evaluate the importance of nitrate-, Fe(III)-, and sulfate-reducing processes by studying porewater depth gradients of electron acceptors or reduced compounds, (3) to elucidate the phylogeny of known groups of Fe(III) reducers which might be involved in the turnover of short-chain fatty acids detected in the porewater.

2 Material and methods

2.1 Field site description

The sites are located in the Lehstenbach catchment (Fichtelgebirge, Northeastern Bavaria, Germany), with a highest elevation of 877 m a.s.l. and an area of 4.2 km^2. Ninety % is covered by Norway spruce (*Picea abies* [L.] KARST.) of different age classes, and thirty % of the catchment soils are fens and seeps. Upland soils in the

catchment have developed from weathered granitic bedrock and are predominantly Dystric Cambisols and Cambic Podzols (WRB system). The annual precipitation in the catchment varies between 900 and 1160 mm yr^{-1} and the average annual temperature is 5°C. The site Schlöppnerbrunnen I (50°08′14″ N, 11°53′07″ E) is a fen which is located in the upper part of the catchment (upland fen), dominated by *Sphagnum* mosses alternately covered with patches of *Vaccinium myrtillus* (L.), *Juncus effusus* (L.), *Carex nigra* ((L.) Reichard)), *Carex rostrata* (Stokes), and *Carex canescens* (L.) (Table 1). The yearly mean groundwater table depth approximated 0.2 m. Some lower situated soils in the catchment Lehstenbach are only water saturated if the groundwater level increases during autumn and winter. In these seeps, represented by the site Köhlerloh (intermittent seep), the groundwater level reaches the soil surface during autumn, winter, and spring, whereas it is about 0.5-to-1 m below the surface during summer. Open areas at the seep are vegetated with *Sphagnum* mosses or are partly covered with dense layer of *Vaccinium myrtilus*. The fen Schlöppnerbrunnen II (50°08′38″ N, 11°51′41″ E) is completely overgrown by *Mollinia caerulea (L. Moench), Eriophorum vaginatum* (L.), *Carex canescens* (L.), and *Juncus effusus* (L.). It is located down slope in the catchment and close to the runoff (lowland fen). The mean groundwater table approximated 0.1 m.

Three intermittent seeps located upstream of the lowland fen were also sampled in October 2003. These seeps were covered with spruce, *Vaccinium myrtillus*, and some *Sphagnum* mosses.

2.2 Porewater collection

Porewater from the upper 40 cm of the fens site was sampled with dialysis chambers every two months during the time period from July 2001 to July 2004 with an interruption during the winter months due to coverage of the catchment with snow and ice as previously reported (Schmalenberger et al., 2007). The intermittent seep was only sampled during periods of water saturation.

2.3 Anoxic soil microcosms

For determining rates of anaerobic microbial activities, soil samples from three replicate sites and different depths (approximately 0–10, 10–20, and 20–30 cm) were obtained in September 2001 in sterile airtight vessels and transported to the laboratory. Replicates were pooled and processed within 4 h. Fourty g (wet wt) soil was placed into 125-ml infusion flasks (Merck ABS, Dietikon, Switzerland) inside an O_2-free chamber (100% N_2 gas phase). Bottles were closed with rubber stoppers and screw-cap seals, flushed with sterile argon for 15 min, and incubated in the dark at 15°C with an initial overpressure of 20–25 kPa argon at room temperature. Headspace gases were taken by sterile, argon flushed syringes from these bottles. Gas values were estimated by Henry's law and included the total amounts in both gas and liquid phases. To facilitate sampling of water soluble parameters, 40 ml anoxic, deionized water with a pH of 5.0 were added to another set of soil microcosms with 40 g (wet wt) soil. Adjustments of pH were performed with sterile solutions of 10 N HCl and 10 N NaOH. All microcosms were done in three replicates. At 8 time points, samples were taken during 15 days of incubation in the dark at 15°C. Activity rates were calculated by linear regression analysis during the time period of linear increase of reduced compounds or linear decrease of electron acceptors.

For determining the effect of supplemental electron donors on Fe(III)-reducing activities, soil samples (0–10 cm depth) were obtained from the lowland fen in March and October 2002. Thirtyfive g (wet wt) soil was mixed with 70 ml anoxic, deionized water with a pH of 5.0. Glucose (2.5 mM), acetate (2 mM), or lactate (2 mM) was added from sterile anoxic stock solutions; H_2 (10 ml) was added as sterile gas. At 10 time points, samples were taken during 15 days of incubation in the dark at 15°C.

2.4 Enrichments

Five g (wet wt) soil of the lowland fen (0–10 cm) obtained in October 2003 was mixed with 95 ml of dilution buffer (Küsel et al., 2001) and further diluted in a tenfold dilu-

tion series, which were used to inoculate an anoxic, undefined medium with a pH of 5.2 that was supplemented with 40 mM amorphous ferric hydroxide [$Fe(OH)_3$] and either acetate (5 mM) or H_2 (10 ml) as previously described (Blöthe et al., 2008). Bromoethanesulfonate (BESA) was added (15 mM) to inhibit methanogenesis. Tubes were incubated in the dark at 15°C for 3 months.

2.5 Analytical methods

The pH was measured with a U457-S7/110 combination pH electrode (Ingold, Steinbach, Germany). Headspace gases (H_2, CO_2 and CH_4) were measured with Hewlett Packard Co. (Palo Alto, CA, USA) 5890 series II gas chromatographs (Küsel and Drake, 1995). The reduction of Fe(III) was determined after acid extraction by the amount of Fe(II) formed in anoxic incubations. Aliquots (0.2 ml) of the media or of the soil suspension were taken by sterile syringes and transferred to 9.8 ml of 0.5 N HCl and incubated for 1 h at room temperature (Küsel et al., 1999). Fe(II) was measured after the addition of acetate by the phenanthroline method (Tamura et al., 1974). Pedogenic iron (Fe_d) was extracted with dithionite-citrate-bicarbonate solution (Mehra and Jackson, 1960). Poorly crystallized iron oxides, hydroxides, and associated gels (Fe_o) were extracted with acidic ammonium oxalate solution (Schwertmann, 1964). Extracted Fe was measured by atomic absorption spectrometry (Unicam 939 spectrometer, ThermoNicolet GmbH, Offenbach, Germany). Short chain aliphatic acids and alcohols were determined with Hewlett-Packard 1090 series II high-performance liquid chromatographs (Küsel and Drake, 1995). The detection limit for acetate approximated 5–10 μM at an injection volume of 200 μl. Dissolved organic carbon (DOC) was analyzed with a liquiTOC (Foss-Heraeus, Hanau, Germany). NH_4^+ and NO_3^-/NO_2^- were determined by flow injection analysis (QuickChemAE, Lachat Instruments, Milwaukee, WI). SO_4^{2-} was measured by ion chromatography (DX-100 with AS4 A column; Dionex, Sunnyvale, CA). Sulfide was measured by the Cline procedure (Cline, 1969).

2.6 DNA extraction, PCR amplification of 16S rRNA genes

DNA was extracted from lowland fen soil (0–10 cm depth) obtained in October 2003 and May 2007 using the MOBIO Power Soil DNA extraction kit according to manufacturer's instructions. Aliquots of DNA were PCR amplified using *Bacteria* domain-specific (GM3, GM4; Muyzer et al., 1995) and 16S rRNA gene primers specific for *Acidiphilium* (Acido594F, Acido1150R; Wulf-Durand et al., 1997), bioleaching-associated bacteria (Ferro458F/Ferro1473R; Wulf-Durand et al., 1997), *Geobacter* (GM3, 825R; Snoeyenbos-West et al. 2000), *Geothrix* (Gx182F, Gx472R; Snoeyenbos-West et al., 2000), *Shewanella* (Shw783F, Shw1245R; Snoeyenbos-West et al., 2000) as previously described (Blöthe et al., 2008). *Anaeromyxobacter*-specific PCR was performed according to the method described by Wu et al. (Ab112F, Ab227R; 2006).

2.7 Clone library construction

PCR amplicons produced with group-specific 16S rRNA gene primers were cloned using the pGEM®-T vector and *Escherichia coli* JM109 competent cells according to the manufacturer's instructions (Promega, Madison, WI USA). Clone libraries were screened by restriction fragment length analysis (RFLP) as previously described (Blöthe et al., 2008). All clones screened using RFLP were grouped into phylotypes according to RFLP banding patterns.

2.8 Phylogenetic and statistical analyses

Representative clones for each RFLP phylotype were sequenced bidirectionally using a Big-Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems 3100 Genetic Analyzer with Capillary Electrophoresis. Sequences were assembled using Sequencher v4.5 (Gene Codes Corp., Ann Arbor, MI) and prior to phylogenetic analysis, vector sequences flanking the 16S rRNA gene inserts were removed. Previously identified sequences with high sequence similar-

ity to the clones obtained in this study were determined using the BLAST algorithm against the GenBank database available from National Center for Biotechnology Information (NCBI) (Altschul et al., 1990). Clone sequences were checked for chimeras and aligned with reference sequences in the ARB software package as previously described (Blöthe et al., 2008). Dendrograms were constructed in the ARB software package by adding 16S rRNA sequences to the distance-matrix tree using PARSIMONY_INTERAKTIV without changing the overall tree topology (Ludwig et al., 2004). The coverage of the clone libraries were calculated (Singleton et al., 2001), and the sampling efficiency within clone libraries was assessed using Analytica Rarefaction 1.3 software (http://www.uga.edu/strata/software/) originally derived by Heck et al. (1975).

2.9 Nucleotide sequence accession numbers

The 16S rRNA gene sequences presented in this study have been deposited in the EMBL database under the accession numbers AM941453-AM941457 for *Acidiphilium*-affiliated 16S rRNA gene sequences, AM941458-AM941489 for *Geobacter*-affiliated 16S rRNA gene sequences, and AM941490-AM941491 for *Geothrix*-affiliated 16S rRNA gene sequences.

3 Results

3.1 Fe(III)-reducing activities

The intermittent seep and the lowland fen displayed high Fe(II) formation rates during the first 7 days of incubation and reached high amounts of Fe(II) formed compared to the upland fen (Table 2). The lowland fen was depleted in nitrate, and consumption of sulfate started after Fe(II) formation reached the plateau (Fig. 1). However, CH_4 formation paralleled Fe(III) reduction in all soil depths (Fig. 1, and data not shown). These parallel activities were not observed in the intermittent seep and the upland

fen. The upland fen displayed higher sulfate-consuming activities than the lowland fen (Table 2). The intermittent seep showed negligible in situ methane forming activities.

The initial rate of Fe(II) formation in the upper lowland fen was even slightly higher in March 2002 [1.84 μmol g (wet wt soil)$^{-1}$ d^{-1}]. Apparently, the upper soil layer was more oxidized in March, because the initial Fe(II) concentration approximated only 1.7 μmol g (wet wt soil)$^{-1}$ compared to 7.3 μmol g (wet wt soil)$^{-1}$ in September. However, both experiments yielded similar maximum concentrations of Fe(II) at the end of incubation (Figs. 1 and 2) which were equivalent to 70% of the Fe_d content of the soil.

3.2 Source of iron in the lowland fen

To detect the source of the high Fe_d concentrations in the lowland fen (Table 1), 3 intermittent seeps were sampled upstream of the lowland fen, and the upper 5 to 15 cm layer of each soil was incubated under anoxic conditions. These soils formed Fe(II) without delay with rates ranging from 90 to 1050 nmol g (wet wt soil)$^{-1}$ d^{-1}. Two of these soils had high initial concentrations of Fe(II) indicating that Fe(III) reduction was an ongoing process in these wetland soils. The amounts of pedogenic iron (Fe_d) and oxalate extractable iron (Fe_o in these acidic (pH 3.2) soils ranged from 2.3 to 13.9 g kg^{-1} and from 1.8 to 12.5 g kg^{-1}, respectively.

3.3 Porewater biogeochemistry

Porewater depth profiles at the intermittent seep showed typical biogeochemical gradients of redox sensitive compounds indicating the sequential utilization of nitrate, Fe(III), and sometimes of sulfate with increasing soil depth during water saturated conditions between autumn (November) and early summer (June) (data not shown; Küsel and Alewell, 2004). Fe(II) reached maximum concentrations of 50 μM in 25 cm depth. Declining sulfate gradients were only detected in early summer samplings. Formate (up to 650 μM), acetate (up to 260 μM), and lactate (up to 85 μM), but not propionate or butyrate were detected in the upper 20 cm of the porewater.

In general, the upland and the lowland fen showed stronger reduced conditions than the seep. Both fens showed similar qualitative biogeochemical porewater patterns during the three year sampling period. However, the lowland fen showed up to 35-fold higher concentrations of Fe(II), up to 3-fold lower concentrations of sulfate, and up to 2-fold higher concentrations of CH_4 in the porewater than the upland fen. The concentrations of Fe(II) in the lowland fen were highly variable (Figs. 3 and 4). During summer of 2001, 2002, and 2004, the depth integrated (0-to-40 cm) average Fe(II) concentrations approximated 2961, 216, and 92 μM. In general, maximum Fe(II) concentrations occurred below 30 cm depth.

In the lowland fen, the concentrations of nitrate (up to 55 μM), sulfate (up to 140 μM), and negligible concentrations of Fe(II) and ammonia indicated soil oxygenation down to a depth of 25 cm after the snowmelt in 2002, 2003, and 2004 (Fig. 3). Oxygenation occurred due to mixing of the porewater with lateral flowing, oxygenated surface water. Drying of the upper 5-to-10 cm occurred during the hot summer in 2003 followed by heavy rain falls prior to sampling in September. Approximately 50 μM nitrate and up to 420 μM sulfate (Fig. 3) were detected in the upper 20 cm of the fen soil, and Fe(II) concentrations did not exceed 30 μM. Fe(II) concentrations increased again in December 2003 to 370 μM. Sulfide never exceeded 5 μM in the porewater.

The concentrations of short chain fatty acids detected in the porewater of the lowland fen were much higher than in the upland fen. In general, concentrations increased with increasing soil depth (Fig. 3). Depth integrated average concentrations in the lowland fen approximated 82, 56, 44, 15, and 4 μM during 2001 to 2004 for acetate, formate, lactate, propionate, and butyrate, respectively. Highest concentrations were detected in November 2001 and December 2002, where concentrations of acetate, propionate, butyrate, and lactate reached up to 3160, 1600, 100, and 95 μM in the porewater (Fig. 3, and data not shown). In contrast, maximum concentrations of formate occurred in April 2002 (Fig. 3). Ethanol was never detected. Porewater pH did not decrease in the presence of high concentrations of short chain fatty acids. Acetate concentrations were positively correlated with Fe(II) (R^2=0.75; Fig. 4).

3.4 Effect of supplemental electron donors on Fe(III)-reducing activities

Since the high concentrations of acetate observed might be derived from fermentation, glucose and typical fermentative products were supplemented to lowland fen soil. The initial rate of Fe(II) formation was enhanced from 1.84 μmol g (wet wt soil)$^{-1}$ d^{-1} to 6.08 μmol g (wet wt soil)$^{-1}$ d^{-1} by supplemental glucose, but not with supplemental acetate, lactate, and H_2 (Fig. 2). However, a secondary Fe(II)-forming increase occurred after 4 days in acetate microcosms. Glucose and acetate supplemented microcosms yielded higher maximum Fe(II) concentrations of 24.7 and 26.2 μmol g (wet wt soil)$^{-1}$ compared to 15.4 μmol g (wet wt soil)$^{-1}$ of the control.

Glucose was rapidly consumed within 3 days and yielded CO_2 and acetate as end products, and H_2 and ethanol as transient products (Fig. S1, http://www.biogeosciences-discuss.net/5/2165/2008/bgd-5-2165-2008-supplement.zip). Supplemental lactate yielded acetate as main product. Both acetate and H_2 were not completely consumed during 15 days of incubation. H_2 but not acetate stimulated the formation of CH_4 (Fig. 2). Microcosms supplemented with either acetate, H_2 or glucose with soil obtained in October 2002 yielded similar results (data not shown). Formate was detected as a small transient product only in glucose amended microcosms of October 2002.

3.5 Molecular detection of Fe(III) reducers

PCR with lowland fen soil yielded products with 16S rRNA primers specific for acidophiles belonging to *Acidiphilium* and for neutrophiles belonging to *Geobacter* or *Geothrix*. No PCR products were obtained with a primer set specific for bioleaching-associated bacteria, *Shewanella* or *Anaeromyxobacter*. PCR products with *Geobacter* specific primers also were detected in enrichments obtained from a 10^{-3} soil dilution transferred to mineral medium at pH 5.5 supplemented with 40 mM amorphous ferric hydroxide [$Fe(OH)_3$] and 5 mM acetate or H_2. PCR products with *Acidiphilium* specific primers were detected up to a 10^{-3} soil dilution enrichment amended with acetate but

not with H_2, and PCR products with *Geothrix* specific primers were detected only in 10^{-1} soil dilution enrichments. Screening of 16S rRNA gene clones by RFLP revealed that all phylotypes detected in the enrichments also occurred in the fen soil.

A total of 45 *Acidiphilium*-16S rRNA gene clones were screened by RFLP, and 13 different phylotypes could be differentiated. Comparative sequence analyses indicated that 5 phylotypes were 95% similar to cultured *Acidiphilium* or *Acidosphaera* species. Clones were most closely related (96–98% sequence similarity) to a forest soil or sphagnum peat bog clone (Fig. 5). With the primer pair specific for *Geothrix*, 20 clones were obtained, but only 2 different phylotypes were obtained with a 96–97% sequence similarity to *Geothrix*-related sequences.

With the primer pair specific for *Geobacter*, a total of 83 clones were obtained and 40 different phylotypes were obtained. A number of non-*Geobacteraceae* sequences and chimeras between *Geobacteraceae* and non-*Geobacteraceae* were detected. Comparative sequence analysis revealed that 34 of the 40 sequences retrieved showed high sequence identity to *Geobacteraceae* sequences (Fig. 6); one was related to *Geobacter chapelleii* str. 172 (96% sequence similarity), three were related to *Geobacter bemidjiensis* and *Geobacter bremensis* (94-96% sequence similarity). Many sequences were similar to *Pelobacter* spp. (94–97%). *Acidiphilium* and *Geobacter* specific 16S rRNA gene clone libraries showed coverages of 57 and 35%, respectively.

4 Discussion

4.1 Mobilization and oxidation of Fe(II) in the catchment

At the upland fen, porewater concentrations of Fe(II) were low, similar to oligotrophic ombrogenic peatlands that receive most of their iron from atmospheric deposition. The low Fe(III) reduction rates corresponded to the low amounts of oxalate extractable Fe(III) oxides present in the upland fen and indicated that Fe(III) reduction was of minor significance for the oxidation of carbon in this fen similar to other northern peatlands

(Blodau et al., 2002). Porewater profiles of Fe(II) and sulfate in the intermittent seep were similar to pH neutral fens (Todorova et al., 2005; Dettling et al., 2006) or other wetlands (Roden and Wetzel, 2003). The high Fe(II) formation rate at the seep suggested a mobilization of Fe(II) during waterlogging from autumn to spring. Fe(II) was also spontaneously formed in other waterlogged seeps sampled upstream of the lowland fen further strengthening the suggestion that Fe(III) reduction is an ongoing process in many seeps of this catchment. The lowland fen is connected to a shallow groundwater layer receives water from these intermittent seeps and fens located in the north-east of the Lehstenbach catchment (Küsel and Alewell, 2004). Due to the acidic (pH 3.1) soil conditions of most seeps, the majority of the reductive dissolved Fe(II) will not adsorb to the solid phase and move with the groundwater flow. Indeed, concentrations of Fe(II) in a nearby groundwater well range from 9-to-143 μM (Küsel and Alewell, 2004). Thus, the lowland fen appears to receive continuously anoxic Fe(II)-rich groundwater. The high accumulation of iron in the upper soil of the lowland fen might have resulted from the oxidation of Fe(II) in oxidized peat surface layers. The high Fe_o/Fe_d ratios (~0.8) (Table 1) suggest that Fe(III) precipitated as amorphous oxides or as organic matter complexes. Microbial Fe(II) oxidation might yield colloidal or dissolved forms of Fe(III) readily available for microbial reduction (Roden et al., 2004). The high mean DOC concentration (77 mg L^{-1}) in the porewater would further favor Fe(III) reduction, because humic compounds can serve as electron shuttles between Fe(III) reducers and surface-bound Fe(III) sterically not accesible to microorganisms (Lovley et al., 2004).

4.2 In situ relevance of Fe(III)-reducing activities

According to the 1:4 ratio of CO_2 production to Fe(III) reduction (Roden and Wetzel, 1996), microbial Fe(III) reduction in the most active upper soil layer (0–10 cm) accounted for 26.7, 6.7, and 71.6% of the anaerobic organic carbon mineralization in the intermittent seep, the upland, and the lowland fen, respectively. The lowland fen showed sequential Fe(III)-reducing and sulfate-reducing activities but concomitant Fe(III)-reducing and methanogenic activities (Fig. 1). Overlapping or concomitant

Fe(III)-reducing and methanogenic activities were not observed in soil samples obtained after the snow melts indicating that prolonged anoxic or reduced conditions in the fen were necessary for the establishment of methanogenic activities. Short-term oxygenation of reduced surface soil during summer that leads to a rapid renewal of the Fe(III) pool might yield a partial shift of the electron flow from CO_2 to Fe(III) by methanogens and help to explain concomitant reduction processes in peatlands (Dettling et al., 2006; Metje and Frenzel, 2005; Paul et al., 2006) or in rice paddy soils after drainage (Krüger et al., 2001). The ability of some methanogens to interact with extracellular quinones, humic acids, and Fe(III) oxides has raised the possibility that methanogens contribute to Fe(III) and humic acid reduction (Bond and Lovley, 2002; van Bodegom et al., 2004). Concomitant activities can be also explained by the use of non-competetive substrates. Concomitant or reversed Fe(III) and sulfate reduction is reported from other fens (Todorova et al., 2005). It was suggested that concomitant reduction processes are due to the presence of more crystalline Fe(III) oxides like hematite or goethite in the soil (Postma and Jakobsen, 1996), which are reduced at lower redox potentials (Straub et al., 2001) than sulfate or CO_2 (Zehnder and Stumm, 1988). However, the high Fe_o/Fe_d ratios in the top soil hint to amorphous, easily reducible Fe(III) oxides.

4.3 Turnover of acetate

High short chain fatty acid and high Fe(II) concentrations were detected in deeper soil of the lowland fen during autumn to winter in 2001/2002 and 2002/2003 with maximum concentration of 3 and 7 mM for acetate and Fe(II), respectively. The high Fe(II) concentrations in this depth were surprising due to the relatively low amounts of iron in the solid phase. However, porewater biogeochemical gradients are not only diffusion limited in fens, and in situ microbial activities interfere with advective groundwater flow. Acetate could have been produced by fermentors using plant polysaccarides from dead root material. Accumulation of acetate up to 1.2 mM is reported from bogs in Michigan or Ontario (Shannon and White, 1996; Blodau et al., 2002) and appears to be due to

the absence of acetoclastic methanogenesis (Shannon and White, 1996; Hines et al., 2001). The decoupling of methanogenesis from carbon flow might be due to the direct inhibition of accumulated acetate on methanogens, because acetic acid can penetrate through the cell membrane and act as decoupler of the proton motive force under acidic conditions (Williams and Crawford, 1984; Goodwin and Zeikus, 1987) or due to transport and thermodynamic constraints (Beer and Blodau, 2007).

The positive correlation of acetate and Fe(II) in the lowland fen porewater during the 3-year-period (Figs. 3 and 4) suggests either a formation of acetate by incomplete oxidizing Fe(III) reducers or an accumulation of acetate after depletion of the Fe(III) pool by complete acetate-oxidizing Fe(III) reducers. Cultured *Geobacter* species, which were most closely related to clone sequences retrieved from this fen, like *G. chapellii*, *G. bemidjiensis*, and *G. bremensis* are complete oxidizers (Lovley et al., 2004). In contrast, the also closely related *Pelobacter venetianus* is an incomplete oxidizer. The low or negligible concentrations of acetate in upper soil suggests oxidation of acetate to CO_2 via aerobic respiration, other oxidative microbial processes or syntrophic oxidation of acetate to CO_2 by the concerted activity of acetate-oxidizing anaerobes and hydrogenotrophic methanogens (Horn et al., 2003; Metje and Frenzel, 2007).

4.4 Fe(III)-reducing microbial communities of acidic habitats

Most Fe(III)-reducing prokaryotes cultured to date are either neutrophilic or acidophilic and have only minor capacities to reduce Fe(III) or in a pH range from 4 to 6. *Acidiphilium cryptum* (ATCC 33463) can reduce small amounts of solid phase Fe(III) at pH 5 (Bilgin et al., 2004). Phylotypes related to cultured *Acidiphilium* or *Acidisphaera* species were detected in the lowland fen similar to slightly acidic coal mining lake sediments (Blöthe et al., 2008). *Geobacter* sp. CdA-3 that was isolated from a mining-impacted sediment can reduce Fe(III) at a pH range from 5.5 to 8.1 (Cummings et al., 2000), and *G. bremensis* can reduce Fe(III) down to pH 5 (Straub and Bucholz-Cleven, 2001). Members of the *δ-Proteobacteria* subdivision, including *Geobacter*- and *Anaeromyxobacter dehalogenans*-related sequences seem to be im-

portant metal-reducing organisms in biostimulated acidic subsurface sediments (North et al., 2004). However, other studies with acidic uranium contaminated sediments demonstrate that *Geobacteraceae* dominate only in sediment enrichment cultures incubated under neutral pH conditions (Petrie et al., 2003). Surprisingly, no PCR products of *Anaeromyxobacter*, or *Shewanella* related species were obtained from the lowland fen, although microorganisms from these genera are common to various metal-reducing environments. Due to our limited knowledge about Fe(III) reduction in moderate acidic habitats, phylogenetic analyses of Fe(III) reducers based on known 16S rRNA gene sequences are severely limited, and we might miss important genera. For example, *Acidobacteria* are discussed to be involved in the cycling of iron (Blöthe et al., 2008), which are also present in peatlands (Dedysh et al., 2006).

4.5 Anaerobic activities under changing environmental conditions

Ongoing atmospheric nitrogen and sulfur depositions might further enhance the activity of sulfate and nitrate reducers in peatlands. Lateral groundwater flow caused by the annual snow melt resulted in a temporal oxidation of the upper peat layers which favored the subsequent nitrate- Fe(III), and sulfate-reducing activities. Also drying of the top soil down to 10 cm depth in September 2003 followed by heavy rain falls lead to the appearance of nitrate and sulfate in the porewater and a renewal of Fe(III). The oxygenation and rewetting event of 2003 yielded even higher porewater sulfate concentrations in the lowland fen than in the upland fen despite the average 3-fold lower sulfate concentrations in the lowland fen (Fig. S2, http://www.biogeosciences-discuss.net/5/2165/2008/bgd-5-2165-2008-supplement.zip). The higher release of sulfate might be due to differences in the reduced sulfur pools, which were subjected to oxidation. The Fe-rich lowland fen contains higher contents of acid volatile sulfur (AVS, i.e. amorphous FeS) and total reduced inorganic sulfur (TRIS, i.e. amorphous FeS, S^0, and FeS_2) (Loy et al., 2004) but lower contents of organic sulfur compared to the upland fen (Paul et al., 2006). Synchrotron-based X-ray spectromicroscopy revealed that the fraction of organic reduced sulfur of these fens is more stable under alternating reduction-oxidation

processes than the FeS or FeS_2 pool (Prietzel, personal communication), simlar to results obtained with chemical S fractionations in peat bogs (Wieder and Lang, 1988; Wieder et al., 1990). The presence of Fe(II) seemed to affect both the storage and mobilization of sulphur. Thus, enhanced extreme weather conditions will not only shift the electron flow away from methanogenesis to alternative anaerobic processes, but also amplify the importance of iron for biogeochemical processes in iron-rich peatlands.

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Table 1. Characteristics of the seep and both fens from a forested catchment[a].

Field site	Vegetation	Groundwater table depth (m)	Soil type	Depth (cm)	Dry weight (%)	pH ($CaCl_2$)	C_{org} (%)	N_{total} (%)	Fe_d ($g\,kg^{-1}$)[b]	Fe_o ($g\,kg^{-1}$)[c]
Intermittent seep	*Sphagnum* mosses, *Vaccinium myrtilus*	0.1-to-1.0	Dystric Gleysol	0–10	17.8	3.2	37.2	2.0	2.1	2.0
				10–20	19.5	3.4	42.9	2.4	3.6	3.0
				20–30	23.5	3.6	34.7	1.9	2.7	2.3
upland fen	*Sphagnum* mosses, *Carex* sp., some spruce stockings	0.2	Fibric Histosol	0–10	10.6	4.1	42.4	1.8	0.5	0.5
				10–20	9.5	4.2	44.0	1.9	0.4	0.4
				20-30	9.6	4.4	39.4	2.1	0.3	0.4
lowland fen	*Molinia caerulea, Eriophorum vaginatum*	0.1	Fibric Histosol	0–10	5.7	4.6	38.0	1.9	18.9	14.2
				10–20	10.0	4.3	40.0	1.5	9.8	8.7
				20–30	13.6	4.4	34.3	1.6	3.4	2.7

[a] Presented are the averages from duplicate soil samples.
[b] Fe_d refers to pedogenic Fe-oxides.
[c] Fe_o refers to poorly crystallized Fe-oxides, hydroxides, and associated gels.

Table 2. Anaerobic activities of seep and fen soils in anoxic microcosms of September 2001.

Site	Depth (cm)	Rate of formation or consumption [nmol g (wet wt soil)$^{-1}$ d^{-1}][a] NO_3^-	Fe(II)	SO_4^{2-}	CO_2	CH_4	$Fe(II)^b_{max}$ [μmol g (wet wt soil)$^{-1}$]	Onset of CH_4 formation (days)
intermittent seep	0–10	−98.6	693	−21.0	649	1.6	8.1	11
	10–20	−48.0	487	1.2	215	0	5.5	n.a.[c]
	20–30	n.a.	544	4.8	198	0	3.5	n.a.
upland fen	0–10	−50.0	47	−19.0	175	25	0.7	1
	10–20	n.a.	19	−1.1	121	63	0.4	4
	20–30	n.a.	38	−0.2	44	30	0.4	4
lowland fen	0–10	n.a.	1177	−3.2	411	619	15.8	0
	10–20	n.a.	430	−0.34	253	244	8.5	0
	20–30	n.a.	379	−0.0	161	102	5.4	0

[a] Presented is the average rate observed in triplicate microcosms.
[b] Final Fe(II) concentrations after Fe(III)-reduction was completed.
[c] n.a. not applicable. No CH_4 was formed or no NO_3^- was present.

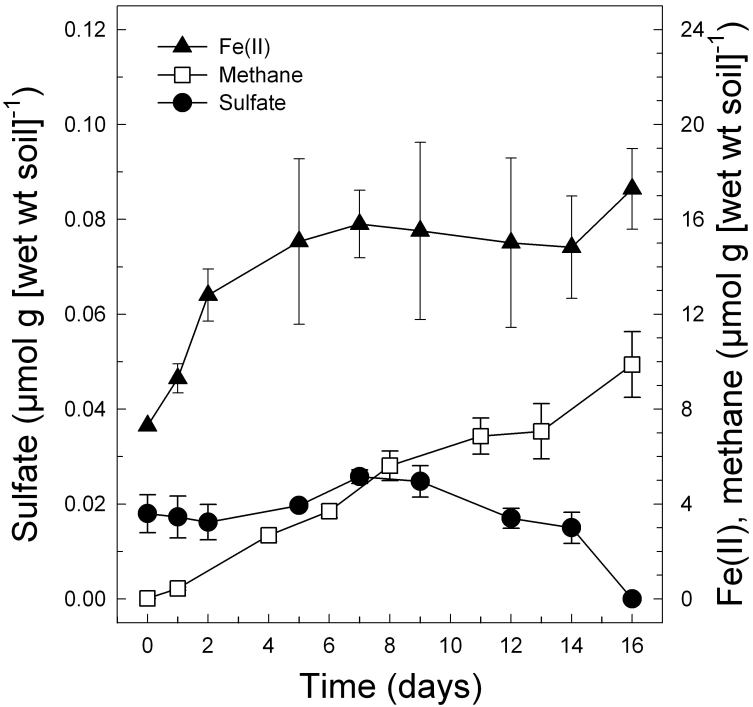

Fig. 1. Formation of Fe(II) and CH_4 and consumption of sulfate in anoxic microcosms of soil obtained from the lowland fen (0–10 cm depth) in September 2001. Presented are the averages ± standard deviations of triplicates.

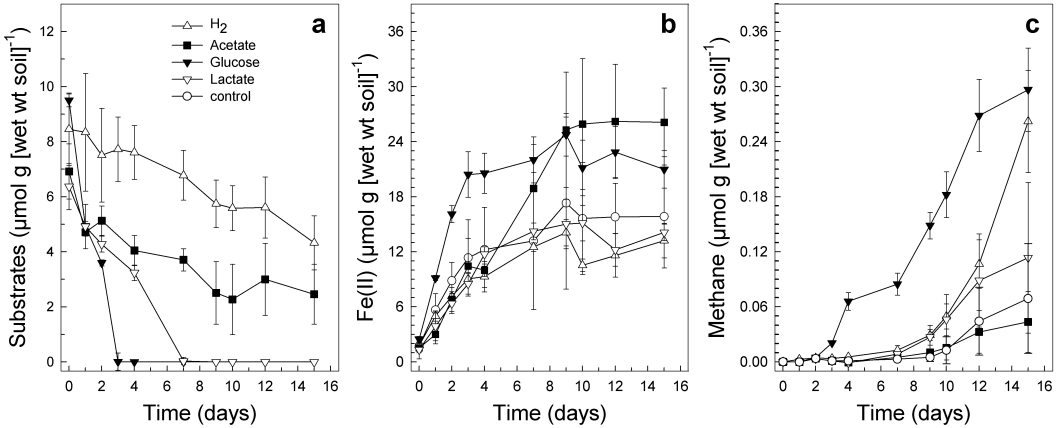

Fig. 2. Effect of the consumption of supplemental electron donors **(a)** on the formation of Fe(II) **(b)** and formation of CH_4 **(c)** in anoxic microcosms of soil obtained from the lowland fen (0–10 cm) in March, 2002. Presented are the averages ± standard deviations of triplicates.

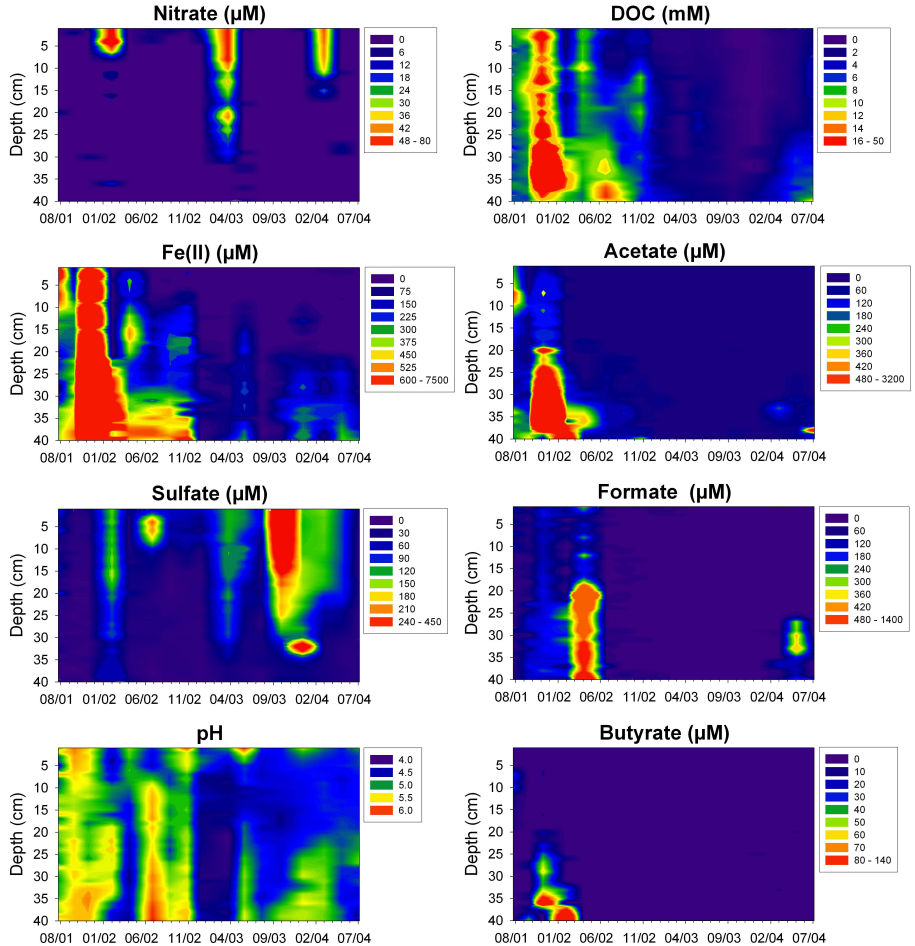

Fig. 3. Porewater concentrations of nitrate, Fe(II), sulfate, pH, DOC, acetate, formate, and buturate in the lowland fen sampled during July 2001 and July 2004.

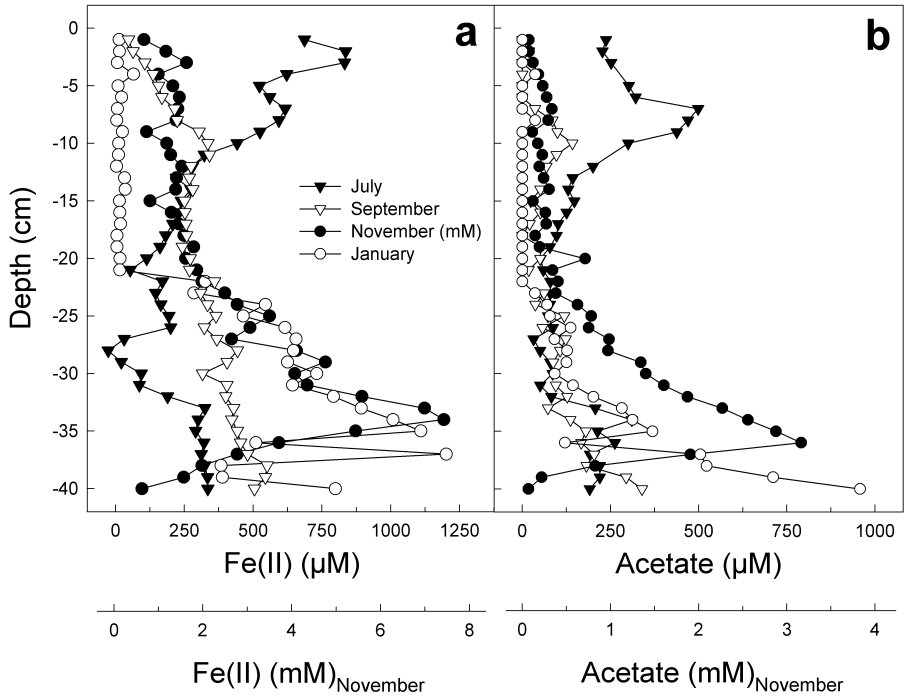

Fig. 4. Detailed porewater depth profiles of Fe(II) **(a)** and acetate **(b)** in the lowland fen sampled in July, September, and November 2001, and in January 2002.

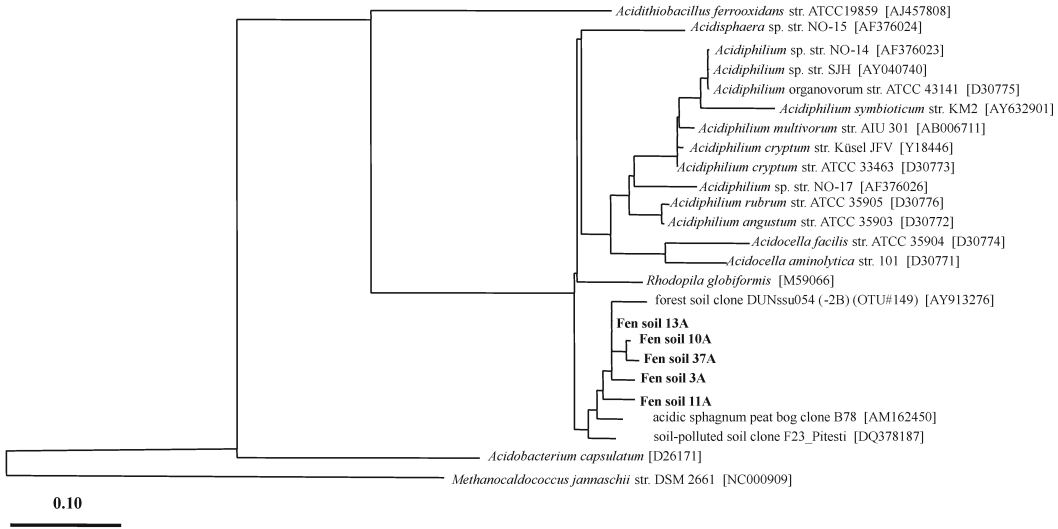

Fig. 5. Phylogenetic tree showing the relative positions of *Acidiphilium*-affiliated 16S rRNA gene sequences derived from the lowland fen soil (0–10 cm) obtained in October 2003. Sequences were added to the existing tree without changing the overall tree topology by using the ARB treeing tool PARSIMONY_INTERAKTIV. Names and accession numbers (between bracktes) for closest relatives 16S rRNA gene sequences are given. The bar indicates 10% sequence divergence.

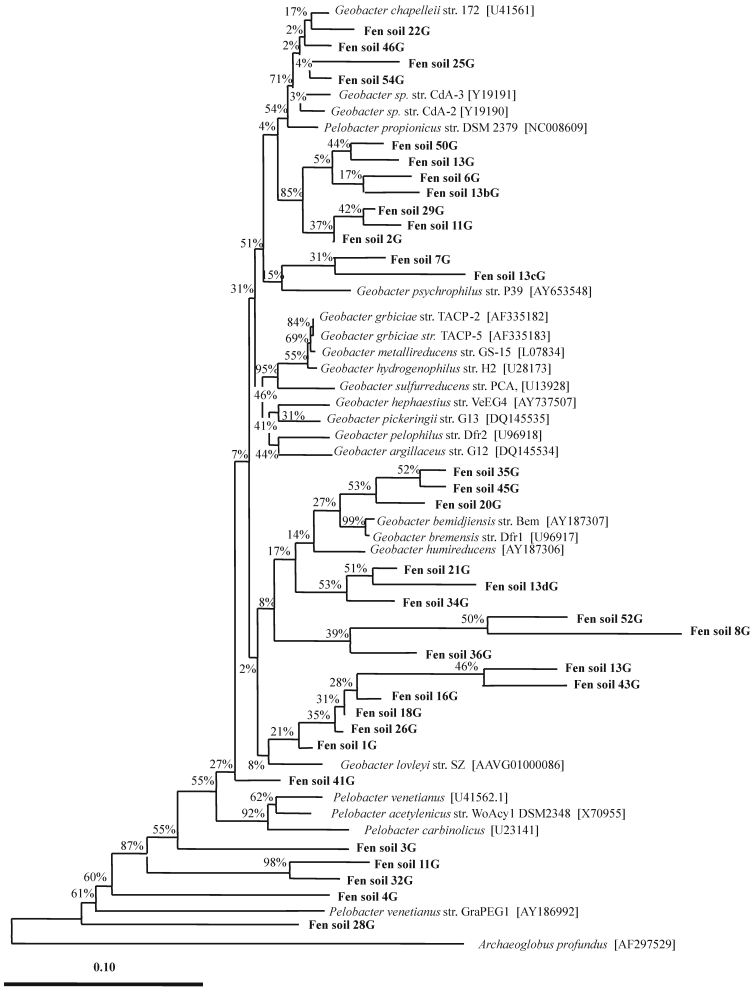
Geobacter chapelleii str. 172 [U41561]
Fen soil 22G
Fen soil 46G
Fen soil 25G
Fen soil 54G
Geobacter sp. str. CdA-3 [Y19191]
Geobacter sp. str. CdA-2 [Y19190]
Pelobacter propionicus str. DSM 2379 [NC008609]
Fen soil 50G
Fen soil 13G
Fen soil 6G
Fen soil 13bG
Fen soil 29G
Fen soil 11G
Fen soil 2G
Fen soil 7G
Fen soil 13cG
Geobacter psychrophilus str. P39 [AY653548]
Geobacter grbiciae str. TACP-2 [AF335182]
Geobacter grbiciae str. TACP-5 [AF335183]
Geobacter metallireducens str. GS-15 [L07834]
Geobacter hydrogenophilus str. H2 [U28173]
Geobacter sulfurreducens str. PCA, [U13928]
Geobacter hephaestius str. VeEG4 [AY737507]
Geobacter pickeringii str. G13 [DQ145535]
Geobacter pelophilus str. Dfr2 [U96918]
Geobacter argillaceus str. G12 [DQ145534]
Fen soil 35G
Fen soil 45G
Fen soil 20G
Geobacter bemidjiensis str. Bem [AY187307]
Geobacter bremensis str. Dfr1 [U96917]
Geobacter humireducens [AY187306]
Fen soil 21G
Fen soil 13dG
Fen soil 34G
Fen soil 52G
Fen soil 8G
Fen soil 36G
Fen soil 13G
Fen soil 43G
Fen soil 16G
Fen soil 18G
Fen soil 26G
Fen soil 1G
Geobacter lovleyi str. SZ [AAVG01000086]
Fen soil 41G
Pelobacter venetianus [U41562.1]
Pelobacter acetylenicus str. WoAcy1 DSM2348 [X70955]
Pelobacter carbinolicus [U23141]
Fen soil 3G
Fen soil 11G
Fen soil 32G
Fen soil 4G
Pelobacter venetianus str. GraPEG1 [AY186992]
Fen soil 28G
Archaeoglobus profundus [AF297529]
0.10

Fig. 6. Phylogenetic tree showing the relative positions of the *Geobacter*-affiliated 16S rRNA gene sequences derived from the lowland fen soil (0–10 cm) obtained in October 2003 as inferred by Parsimony method. Bootstrap values for a total of 100 replicates are shown at the nodes. Names and accession numbers (between bracktes) for all 16S rRNA gene sequences used for comparsion are given. The bar indicates 10% sequence divergence.