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A 'rare biosphere' microorganism contributes to sulfate reduction in a peatland

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Abstract

Methane emission from peatlands contributes substantially to global warming but is significantly reduced by sulfate reduction, which is fuelled by globally increasing aerial sulfur pollution. However, the biology behind sulfate reduction in terrestrial ecosystems is not well understood and the key players for this process as well as their abundance remained unidentified. Comparative 16S rRNA gene stable isotope probing in the presence and absence of sulfate indicated that a *Desulfosporosinus* species, which constitutes only 0.006% of the total microbial community 16S rRNA genes, is an important sulfate reducer in a long-term experimental peatland field site. Parallel stable isotope probing using *dsrAB* [encoding subunit A and B of the dissimilatory (bi)sulfite reductase] identified no additional sulfate reducers under the conditions tested. For the identified *Desulfosporosinus* species a high cell-specific sulfate reduction rate of up to 341 fmol SO_4^{2-} $cell^{-1}$ day^{-1} was estimated. Thus, the small *Desulfosporosinus* population has the potential to reduce sulfate *in situ* at a rate of 4.0–36.8 nmol (g soil w. wt.)$^{-1}$ day^{-1}, sufficient to account for a considerable part of sulfate reduction in the peat soil. Modeling of sulfate diffusion to such highly active cells identified no limitation in sulfate supply even at bulk concentrations as low as 10 μM. Collectively, these data show that the identified *Desulfosporosinus* species, despite being a member of the 'rare biosphere', contributes to an important biogeochemical process that diverts the carbon flow in peatlands from methane to CO_2 and, thus, alters their contribution to global warming.

Keywords

rare biosphere; sulfur cycle; peatlands; keystone species; global warming

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Introduction

Peatlands harbor up to one third of the world pool of soil carbon (Limpens *et al.*, 2008) and are estimated to be responsible for 10–20% of the global emission of the greenhouse gas methane (Houweling *et al.*, 1999; Wuebbles and Hayhoe, 2002). Although regarded as a primarily methanogenic environment, dissimilatory sulfate reduction contributes up to 36% of carbon mineralization in these ecosystems, depending on sulfur deposition by rain or groundwater (Blodau *et al.*, 2007; Deppe *et al.*, 2009; Vile *et al.*, 2003). Sulfate concentrations in peatlands are generally low being in the range of 10–300 μM (Blodau *et al.*, 2007; Deppe *et al.*, 2009; Schmalenberger *et al.*, 2007). However, the turnover time of the standing sulfate pool can be less than a day (Blodau *et al.*, 2007; Knorr and Blodau, 2009), indicating a rapid recycling mechanism. Recycling of sulfate can proceed by the aerobic oxidation of sulfide, e.g., in regions where oxygen penetration and anoxic microniches overlap (i.e., the zone above water saturation) (Knorr and Blodau, 2009; Knorr *et al.*, 2009), in the rhizosphere of aerenchym-containing plants (Wind and Conrad, 1997), or during drying-rewetting events (Knorr and Blodau, 2009; Knorr *et al.*, 2009; Reiche *et al.*, 2009). In addition, experimental evidence is gathering for a rapid anoxic recycling mediated by oxidation of sulfide mainly with quinone moieties of the large pool of humic matter in peatlands (Blodau *et al.*, 2007; Heitmann and Blodau, 2006; Heitmann *et al.*, 2007; Jørgensen, 1990a; Jørgensen, 1990b) or by electric currents spanning from the anoxic to the oxic zone (Nielsen *et al.*, 2010).

This sulfate recycling is important, as sulfate reducers compete for substrates with microorganisms involved in the methanogenic degradation pathway, resulting in a considerable diversion of the carbon flow in peatlands from methane to CO_2 (Gauci *et al.*, 2004). In the near future, this effect will become even more pronounced because population growth and increasing energy consumption in Asia as well as exploitation and combustion of oil sands are predicted to increase global sulfur deposition on peatlands by acid rain (Gauci *et al.*, 2004; Limpens *et al.*, 2008). Furthermore, proposed geo-engineering solutions to counteract global warming via SO_2 deposition into the stratosphere (Rasch *et al.*, 2008) would additionally increase terrestrial sulfur deposition and soil acidification. Despite the de-acidification function of terrestrial sulfate reducers (Alewell *et al.*, 2008) and their predicted suppression of global methane emission from peatlands by up to 15% (Gauci *et al.*, 2004), we know very little about these microorganisms. To identify active sulfate reducers, we studied a peatland that is part of a long-term experimental field site in the German-Czech border region (Fig. S1) and was exposed to extensive acid rain and sulfur deposition during the time of intensive soft coal burning in Eastern Europe (Berge *et al.*, 1999; Moldan and Schnoor, 1992). In anoxic peat soil incubations of this particular site, sulfate reduction can cause a decrease in methanogenesis by 68–78% (Loy *et al.*, 2004). Here, we present cumulative evidence that a low G+C, Gram-positive member of the ‘rare biosphere’ is an important sulfate reducer in this minerotrophic peatland.

Materials and Methods

Sampling site

The minerotrophic fen Schlöppnerbrunnen II (50°08′38″N, 11°51′41″E), which was used as a model habitat for peatlands in this study, is situated in the Fichtelgebirge Mountains in northeastern Bavaria, Germany (Fig. S1). The site has been studied extensively over the past decades (e.g., Loy *et al.*, 2004; Matzner, 2004; Paul *et al.*, 2006; Schmalenberger *et al.*, 2007). Briefly, the soil pH is typically at pH 4–5 (Küsel *et al.*, 2008; Loy *et al.*, 2004; Reiche *et al.*, 2009) and sulfate concentrations vary from 20–240 μM (Loy *et al.*, 2004; Schmalenberger *et al.*, 2007). Standing pools of lactate, acetate, and formate are generally in the lower μM-range (up to 100, 100, and 190 μM, respectively) but can reach peak concentrations of 3.2 mM (acetate) and 1.4 mM (formate). Propionate is only occasionally detected but can reach peak concentrations of 1.6 mM (Küsel *et al.*, 2008; Schmalenberger *et al.*, 2007). Sampling is detailed in *SI Methods*.

Pre-incubation and stable isotope labeling

Incubations were set up to mimic the conditions in the peatland as closely as possible with respect to *in situ* concentrations of sulfate and substrates. For this purpose, 30 g of soil from the 10–20-cm depth fraction were gassed in a 125-ml serum bottle with N_2 and mixed under the same N_2-stream with 60 ml of filter-sterilized (0.2 μm), anoxic fen water. Subsequently, serum bottles were sealed with butyl rubber septa and incubated without agitation at 14°C in the dark. The soil slurries had a pH of 4. For substrate turnover determination and stable isotope labeling an unlabeled or fully ^{13}C-labeled substrate mixture was added weekly to the mesocosms. The mixture consisted of lactate, acetate, formate, and propionate (end-concentration 50–200 μM each). In addition, sulfate was added weekly to an end-concentration of 100–200 μM. Soil slurries with substrate but without sulfate addition served as controls. Upon substrate addition, soil slurries were briefly shaken to ensure complete mixing. The turnover of added substrates and sulfate was measured by ion chromatography as detailed in *SI Methods*.

Stable isotope probing

For SIP analyses, total nucleic acids were extracted from frozen samples (−80°C) by grinding in liquid nitrogen and following thereafter the procedure described by Lüders *et al.* (2004). Minor modifications included a humic acid precipitation step with 7.5 M Na-acetate as described by Bodrossy *et al.* (2006). DNA was separated from RNA using the AllPrep DNA/RNA Mini Kit (Qiagen) and quantified using PicoGreen staining according to the manufacturer's protocol (Invitrogen). Density gradient centrifugation was performed as described by Neufeld *et al.* (2007). Gradients were fractionated into 20 equal fractions (ca. 250 μl); 50-μl aliquots of each fraction were used for density determination using a refractometer (AR 200, Reichert Analytical Instruments, Depew, NY, USA). DNA was extracted from fractions as described previously Lüders *et al.* (2004). T-RFLP analysis as well as amplification, cloning, and phylogenetic analysis of 16S rRNA genes and *dsrAB* are detailed in *SI Methods*.

Quantitative real-time PCR analysis

For qPCR analysis of pristine non-incubated soils samples, DNA was extracted from 250 mg of peat soil (wet weight) using the Power Soil™ DNA Kit (MoBio Laboratories, Solana Beach, CA, USA). *Desulfosporosinus*-targeted and total *Bacteria/Archaea*-targeted quantitative real-time PCRs (qPCR) of 16S rRNA genes were performed using the primer pairs DSP603F (5′-TGT GAA AGA TCA GGG CTC A-3′) / DSP821R (5′-CCT CTA CAC CTA GCA CTC-3′) [constructed based on clone libraries of this study and the Arb SILVA 96 database (Pruesse *et al.*, 2007)] and modified 1389F (5′-TG TAC ACA CCG CCC GT-3′) / 1492R (5′-GGY TAC CTT GTT ACG ACT T-3′) (Loy *et al.*, 2002), respectively. Primer 1389F has a weak mismatch to archaeal 16S rRNA genes at the third position from the 5′-end (T vs. C) but is not regarded to be discriminative against *Archaea*. Reactions were performed in triplicates using the Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen), fluorescein (10 nM), bovine serum albumine (5 μg μl^{-1}), 5 ng of template DNA, and the following annealing temperatures: 64°C for *Desulfosporosinus* and 52°C for *Bacteria*/*Archaea*. Further details are given in *SI Methods*.

Nucleic acid sequences

16S rRNA gene and *dsrAB* sequences obtained from the ‘heaviest’ PCR-amplifiable SIP fractions have been deposited at GenBank under accession numbers GU270657–GU270832 and GU371932–GU372082, respectively. The *dsrAB* sequence of *Desulfosporosinus* strain DB and the *Desulfosporosinus-dsrA* sequence from the SIP incubations have been deposited at GenBank under the accession numbers GU372083 and GU371931, respectively.

Results and Discussion

Substrate turnover in peat soil slurry incubations

Six anoxic peat soil slurries were pre-incubated at 14°C for 28 days. Electron acceptors such as nitrate, sulfate, and iron(III) are typically reduced after 16 days in Schlöppnerbrunnen II peat soil slurries (Küsel *et al.*, 2008), which is an important prerequisite for selective labeling. During this pre-incubation, lactate, acetate, and formate stayed in the lower μM-range (<20 μM) while propionate accumulated transiently up to 155 μM in individual slurries. Initial sulfate concentrations were 22 μM and dropped thereafter below 4 μM (Fig. 1A). After pre-incubation, an unlabelled substrate mix of lactate, acetate, formate, and propionate (50–200 μM each) was added twice over a period of two weeks to all soil slurries to determine the time needed for substrate depletion. In addition, three of the six soil slurries were supplemented once with 100–200 μM sulfate. In all incubations, lactate and formate were readily turned over within two days, while acetate and propionate needed four and six days for turnover after the first and second substrate addition, respectively (Fig. 1B, Table 1). Thereafter, soil slurries were incubated without any additions for 17 days to allow for complete depletion of added ^{12}C-substrates. After this post-incubation, the headspace of each mesocosm was flushed with 100% N_2 to remove accumulated $^{12}CO_2$ and the actual SIP incubations were started (pre-incubations and subsequent SIP incubations are detailed in Fig. S2).

As expected for methanogenic low-sulfate environments, sulfate turnover was slower than substrate consumption with sulfate reduction accounting for 12% of the total electron flow in incubations with sulfate addition. Sulfate turnover rates as determined by linear regression analysis over the first 9 days were 13.1 μmol $SO_4^{2-}L^{-1}$ day^{-1} [equal to 26.2 nmol SO_4^{2-} (g soil w. wt.)$^{-1}$ day^{-1}] (Fig. 1B) and, thus, in the range of radiotracer-measured *in situ* sulfate reduction rates of the studied peatland (0 to ca. 340 nmol (g soil w. wt.)$^{-1}$ day^{-1} (Knorr and Blodau, 2009; Knorr *et al.*, 2009). A possible underestimation of sulfate turnover due to anoxic re-oxidation of sulfide is not expected due to the 28 days-long depletion phase of endogenous electron acceptors before sulfate addition and the observed linearity of sulfate depletion.

A *Desulfosporosinus* species is the major sulfate reducer in the SIP incubations

To identify active sulfate reducers against the large background of microorganisms involved in methanogenic organic matter degradation, we applied DNA stable isotope probing (SIP) in a differential display format, which involved parallel incubations in the presence or absence of sulfate at *in situ* concentrations (100–200 μM). Incubations were amended weekly with *in situ* concentrations of ^{13}C-substrates (composition as for ^{12}C-substrates) with or without sulfate for 2 weeks, 2 months, and 6 months – each in a separate mesocosm (six in total). All provided substrates are well known to be utilized by sulfate reducers. In addition, formate is regarded as an equivalent to H_2, which some sulfate reducers use as sole energy source (Rabus *et al.*, 2006). Such autotrophic sulfate reducers can have higher doubling times than heterotrophic sulfate reducers (Rabus *et al.*, 2006) and were targeted in addition by $^{13}CO_2$, which stemmed from the degradation of the supplied ^{13}C-substrate mixture and was the major source of CO_2 available.

Incorporation of substrate-^{13}C into the biomass of active sulfate reducers was followed by pairwise comparison of incubations with and without sulfate using a 16S rRNA gene-based T-RFLP screening of density-resolved DNA. A clear difference in T-RFLP patterns between incubations with and without sulfate became apparent for the bacterial community after 2 months of incubation. In sulfate-amended incubations, a distinct T-RF at 140 bp dominated the ‘heaviest’ (^{13}C-labeled) PCR-amplifiable density fractions and was almost absent in the ‘light’ (unlabeled) fractions. In the control incubation without sulfate, the 140-bp T-RF was of very minor abundance in each fraction throughout the density gradient (Fig. 2). The same was true for the ^{12}C-control of non-incubated pristine peat soil (Fig. S3). Cloning of bacterial 16S rRNA genes from the ‘heavy’ fraction of the incubation with sulfate (Table S1, Fig. S4) revealed that the dominant 140-bp T-RF represents almost exclusively organisms within the genus *Desulfosporosinus* (*Firmicutes*) (16 out of 95 clones in the incubation with sulfate, Fig. 3) and one clone of the *Acidobacteria* subgroup 3. A differential T-RFLP analysis using the alternative restriction enzyme RsaI confirmed that the dominant 140-bp T-RF in the ‘heavy fraction’ represented exclusively *Desulfosporosinus* spp. (data not shown). Three additional *Desulfosporosinus* sp. clones had a T-RF at 171 bp (indicating that different *Desulfosporosinus* ecotypes may be present in the studied peatland) but a corresponding peak was not detected in the SIP-T-RFLP analyses. In a parallel clone library from the heavy fraction of the incubation without sulfate, no *Desulfosporosinus* sp. was detected (Table S1, Fig. S4). Again, one clone representing *Acidobacteria* subgroup 3 with a

T-RF of 140 bp was retrieved, explaining the very minor T-RF at 140 bp in the incubations without sulfate (Fig. 2) and most likely also in the ^{12}C-control of non-incubated pristine peat soil (Fig. S3).

The 6-month incubations corroborated that a *Desulfosporosinus* sp. was the main microorganism that incorporated ^{13}C-label in the presence, but not in the absence, of sulfate (Fig. S5). Incubating for 2 weeks was apparently too short to detect significant differences between incubations with and without sulfate using *in situ* substrate concentrations (Fig. S6). T-RFLP screening of archaeal 16S rRNA genes from the 2-month incubations revealed no differences between density-resolved DNA extracts of incubations with and without sulfate (data not shown), indicating that archaeal sulfate reducers apparently did not play an important role under the conditions tested.

DNA in the ‘heaviest’ PCR-amplifiable density fractions in the 2-month incubations had a density of 1.727 and 1.723 g ml^{-1} in the incubations with and without sulfate, respectively. This corresponds roughly to 60–70% ^{13}C-labeling based on a G+C-content of 50 mol%. In comparison, unlabeled ^{12}C-DNA of microorganisms with a high G+C-content such as *Micrococcus luteus* (G+C content 71 mol%) have a similar buoyant density of up to 1.725 g ml^{-1} (Lüders *et al.*, 2004). However, all described *Desulfosporosinus* spp. have a G+C content of 37–47 mol% (Alazard *et al*., 2010; Lee *et al.*, 2009a; Ramamoorthy *et al.*, 2006; Spring and Rosenzweig, 2006; Vatsurina *et al.*, 2008) and the 140-bp T-RF in the ^{13}C-control without sulfate (Fig. 2) as well as in the ^{12}C-control of non-incubated pristine peat soil (Fig. S3) was of very minor abundance and attributed to *Acidobacteria* subgroup 3. Therefore, the occurrence of the dominant 140-bp T-RF in the ‘heaviest’ PCR-amplifiable density fraction in the incubation with sulfate clearly reflects ^{13}C-incorporation into *Desulfosporosinus* sp. In addition, growth on other than the provided, easily-degradable ^{13}C-labeled substrates is unlikely due to the 28-days long pre-incubation phase before the actual substrate turnover and SIP incubations. A detailed analysis of microbial populations, which were identified in the ‘heaviest’ PCR-amplifiable density fractions of both incubation setups and contributed therefore to metabolic pathways other than sulfate reduction, e.g., fermentation in the methanogenic degradation pathway, is given in *SI Text*.

qPCR analysis confirms physiological activity of the *Desulfosporosinus* species in the presence of sulfate

DNA replication of *Desulfosporosinus* sp. in the sulfate-reducing mesocosms was confirmed by quantitative PCR. While the abundance of *Desulfosporosinus* sp. in SIP incubations without sulfate mirrored the natural abundance over time (0.006% of total *Bacteria* and *Archaea*), it steadily increased to 0.2% (2 weeks ^{12}C-substrate turnover determination and 2 weeks SIP incubation), 0.6% (2 weeks ^{12}C-substrate incubation and 2 months SIP), and 3.1% (2 weeks ^{12}C-substrate incubation and 6 months SIP) of total bacterial and archaeal 16S rRNA genes in the incubations with sulfate (Fig. 4). This result clearly corroborates the observations of the SIP study, which relies on the multiplication of active microorganisms to incorporate label into their DNA, and shows at the same time that the enrichment of *Desulfosporosinus* sp. in the intensively analyzed 2-month incubation was still minimal.

The low abundance *Desulfosporosinus* can sustain a high cell-specific sulfate reduction rate

Using the onset of our ^{12}C-substrate turnover experiments, where the number of *Desulfosporosinus* sp. equaled its natural abundance (also shown by the same abundance in incubations without sulfate, Fig. 4), we estimated the cell-specific sulfate reduction rate (cs-SRR) for this *Desulfosporosinus* sp. to be 341 fmol SO_4^{2-} cell^{-1} day^{-1}. The rate was calculated by dividing the measured SRR of the turnover experiments [26.2 nmol SO_4^{2-} (g soil w. wt.)$^{-1}$ day^{-1}, Fig. 1B] by the abundance of the natural *Desulfosporosinus* population at 10–20 cm depth. The natural *Desulfosporosinus* population was estimated from the quantified 16S rRNA gene abundance divided by an average of 4.4 16S rRNA copies per cell among *Peptococcaceae* for which genome data are available [no data available yet for *Desulfosporosinus* spp., (Lee *et al.*, 2009b); http://ribosome.mmg.msu.edu/rrndb/index.php]. We assumed no significant contribution of additional sulfate reducers to the measured SRR, as *Desulfosporosinus* sp. was the only recognized sulfate reducer in our SIP incubations. However, activity of other sulfate reducers at the very beginning of our turnover experiments cannot be completely ruled out and thus the determined cs-SRR might be overestimated.

The estimated cs-SRR of the identified peatland *Desulfosporosinus* sp. is at the upper end of cs-SRRs reported for pure cultures (Detmers *et al.*, 2001). In marine sediments, cs-SRRs are three orders of magnitude lower, which is explained by substrate limitation (Ravenschlag *et al.*, 2000; Sahm *et al.*, 1999). The studied peatland, however, is not regarded as substrate limited, which is supported by long periods of a high dissolved organic carbon content (average 18 mg L^{-1}) and the spatial and temporal co-occurrence of redox processes with differing energy yield (Alewell *et al.*, 2008; Küsel *et al.*, 2008). In addition, apparent sulfate half-saturation concentrations, K_m, for sulfate reducers from low-sulfate environments can be as low as 5 μM indicating no kinetic limitation from the electron acceptor side as well (Pallud and van Cappellen, 2006, and references therein). This is supported by the high radiotracer-measured SRRs of the studied peatland even at bulk sulfate concentrations <10 μM (Knorr and Blodau, 2009; Knorr *et al.*, 2009).

An intriguing question remaining is whether sulfate supply might hamper the small *Desulfosporosinus* population to sustain such high cs-SRR. In an extreme scenario, each cell of the highly diluted *Desulfosporosinus* population would rapidly turn over sulfate in its close vicinity and, thereafter, might run into sulfate limitation controlled by diffusion of sulfate to the cell. To test this, we calculated the diffusive flux *J* of sulfate to a single *Desulfosporosinus* cell using a diffusion coefficient for sulfate as determined experimentally for anoxic sediments ($D_S = 0.5 \times 10^{-5}$ cm^2 s^{-1}; Krom and Berner, 1980), a cell radius *r* for *Desulfosporosinus* cells of 0.4 μm (Spring and Rosenzweig, 2006), and a 3-dimensional diffusive flux model for spherical symmetries (Equ. 1; Koch, 1990).

$$J = 4 \cdot \Pi \cdot D_S \cdot r \cdot c_{max} \quad \text{Equ.1}$$

Using a low ambient sulfate concentration c_{max} in the peat of 10 μM (typically 10–300 μM), the diffusive flux into a single *Desulfosporosinus* cell would be 2 pmol SO_4^{2-} day^{-1}. Even

with this conservative estimate, the diffusive flux of sulfate would be one order of magnitude higher than the estimated cs-SRR of *Desulfosporosinus* sp. *in situ* (341 fmol SO_4^{2-} $cell^{-1}$ day^{-1}). This clearly shows that *Desulfosporosinus* cells or any other sulfate reducer will not be limited by sulfate diffusion and make such high cs-SRR also plausible in the natural peatland.

The low abundance *Desulfosporosinus* has the potential to drive a substantial part of sulfate reduction in the peatland

Desulfosporosinus sp. with its low natural abundance of 0.006% of the total bacterial and archaeal community is a member of the 'rare biosphere', which is defined as the sum of those taxa with an abundance of less than 0.1–1% (Fuhrman, 2009; Pedros-Alio, 2006; Sogin *et al.*, 2006). Based on its absolute abundance in the peatland over a depth profile of 0–30 cm (Fig. 5), the potential SRR of the natural *Desulfosporosinus* population was calculated using its estimated cs-SRR. The potential SRRs of the natural *Desulfosporosinus* population were 4.0–36.8 nmol (g soil w. wt.)$^{-1}$ day^{-1} between 0–30 cm soil depth (Fig. 5). In comparison, radiotracer-measured gross SRRs of the studied peatland ranged from 0 to ca. 340 nmol (g soil w. wt.)$^{-1}$ day^{-1} over a depth profile of 0–30 cm and a 300 days period, with sulfate reduction proceeding at >10 nmol (g soil w. wt.)$^{-1}$ day^{-1} in at least one of the analyzed depth fractions at each sampling day (5–10 cm depth fractions) (Knorr and Blodau, 2009; Knorr *et al.*, 2009). Even if cs-SRR of *Desulfosporosinus* sp. were overestimated by one order of magnitude and would therefore resemble average cs-SRR of cultured sulfate reducers (Detmers *et al.*, 2001) or if a subpopulation would have occurred as inactive spores, the natural *Desulfosporosinus* population would still have the potential to drive a considerable part of sulfate reduction compared to its abundance. The presence of mostly physiologically active *Desulfosporosinus* cells in water-saturated, anoxic soil pockets above the water-table and in the anoxic peat below the water-table is expected as sulfate reduction in peatlands is not only fuelled by allochthonous sulfate but also by an oxic (Deppe *et al.*, 2009; Knorr and Blodau, 2009; Knorr *et al.*, 2009; Reiche *et al.*, 2009) and anoxic sulfur cycle (Blodau *et al.*, 2007; Jørgensen, 1990b; Nielsen *et al.*, 2010) and constitutes an ongoing process in the studied peatland as evident from $\delta^{34}S$ measurements (e.g., Alewell and Novak, 2001; Alewell *et al.*, 2008) and the radiotracer studies described above. In addition, *Desulfosporosinus* spp. are known to switch under sulfate limitation to the fermentation of lactate and pyruvate (Spring and Rosenzweig, 2006), to reductive acetogenesis from formate, methanol, or methyl groups of aromatic compounds (Rabus et al., 2006), or to dissimlatory iron(III) reduction (Ramamoorthy et al., 2006). At the same time, *Desulfosporosinus* spp. are well adapted to persist throughout extended periods of droughts and subsequent complete oxygenation of the peat soil (Reiche *et al.*, 2009) by their ability to form endospores (Lee *et al.*, 2009a; Ramamoorthy *et al.*, 2006; Spring and Rosenzweig, 2006; Vatsurina *et al.*, 2008). In summary, *Desulfosporosinus* spp. appear well-adapted to the highly fluctuating conditions in low-sulfate peatlands.

dsrAB-based SIP identifies no substantial contribution of other sulfate reducers

As evident from gross SRRs (Knorr and Blodau, 2009; Knorr *et al.*, 2009) and previous diversity studies, several other sulfate reducers are present in this peatland, e.g., *Desulfomonile* spp. and *Syntrophobacter* spp. (Loy *et al.*, 2004). In addition, the presence of

potentially new taxa is indicated by the detection of novel deep-branching lineages of the functional marker genes *dsrAB* [encoding subunit A and B of the dissimilatory (bi)sulfite reductase] (Loy *et al.*, 2004; Schmalenberger *et al.*, 2007). Currently, it is not known whether microorganisms harboring these novel *dsrAB* are capable of dissimilatory sulfate/sulfite (Wagner *et al.*, 2005) or organosulfonate reduction (Laue *et al.*, 1997; Laue *et al.*, 2001), switch between a syntrophic and sulfate reducing lifestyle upon the availability of sulfate (Wallrabenstein *et al.*, 1994; Wallrabenstein *et al.*, 1995), or are purely syntrophic microorganisms (Imachi *et al.*, 2006). Comparison of *dsrAB* clone libraries from the 'heavy' fractions of the SIP incubations with and without sulfate (Fig. 6, Table 2) and *dsrAB* T-RFLP analyses for both incubations (Fig. S7) indicated that known sulfate reducers other than *Desulfosporosinus* or microorganisms harboring novel deep-branching *dsrAB* made no quantitatively important contribution (if any) to sulfate reduction (detailed in *SI Text*). However, it is very likely that additional sulfate reducers in the studied peatland use other substrates for energy metabolism or are adapted to other conditions than those provided in our SIP incubations and, thus, were not identified as active populations.

Interestingly, *Desulfosporosinus*-like *dsrAB* could not be detected in the 'heavy' fractions when the standard highly degenerated primers were applied for PCR, indicating that the labeled *Desulfosporosinus* sp. harbors *dsrAB* with mismatches in the primer binding sites as observed previously also for other closely related sulfate reducers (Zverlov *et al.*, 2005). Consistent with this hypothesis, a *dsrA* fragment closely related to *dsrAB* of *Desulfosporosinus* spp. (Fig. 6) and probably related to one of the detected *Desulfosporosinus* ecotypes (Fig. 3) could be amplified after using newly constructed primers targeting selectively the genera *Desulfosporosinus* and *Desulfitobacterium*. This also explains why this 'rare biosphere' member eluded previous *dsrAB*-based diversity studies of this peatland.

Conclusions

Microbial diversity surveys using high through-put sequencing of 16S rRNA gene amplicons revealed that any microbial community in the environment is typically composed of abundant taxa, which are considered to carry out most ecosystem functions, and very low abundant taxa (less than 0.1–1%), which are referred to as the 'rare biosphere' (Fuhrman, 2009; Pedros-Alio, 2006; Roesch *et al.*, 2007; Sogin *et al.*, 2006; Turnbaugh *et al.*, 2009; Webster *et al.*, 2009). Recent advances in data analyses allow more precise estimates of the actual extent of the 'rare biosphere' diversity (Kunin *et al.*, 2009; Quince *et al.*, 2009). However, we are only starting to learn which different ecological roles these rare microorganisms may play. In this study, we present cumulative and independent lines of evidence that a *Desulfosporosinus* species as member of the 'rare biosphere' is an important sulfate reducer in the investigated model peatland. This finding provides an example for a microorganism of numerical low abundance that can have an impact on (i) the carbon flow in terrestrial ecosystems (Blodau *et al.*, 2007; Loy *et al.*, 2004; Vile *et al.*, 2003; this study) and (ii) on globally relevant processes such as the decrease in emission of the greenhouse gas methane (Gauci *et al.*, 2004). A similar situation has been recently observed for marine and freshwater environments. In coastal marine surface-waters, a low abundant *Methylophaga* sp. was shown to be the major methanol oxidizer (Neufeld *et al.*, 2008) and

in the chemocline of a meromictic lake, a low abundance but large anaerobic phototrophic bacterium (0.1–0.4% of total cells) accounted for 40% of ammonium and 70% of inorganic carbon uptake (Halm *et al.*, 2009; Musat *et al.*, 2008). However, in contrast to our study the latter was explained by the large biomass (40% of the total microbial biomass) of these voluminous lake bacteria (Halm *et al.*, 2009; Musat *et al.*, 2008).

Two major advantages of a low abundance life strategy have been outlined before: protection against viral lysis and protection against protist predation due to lowered probabilities of encounters (Pedros-Alio, 2006). In addition, the low abundance of the peatland *Desulfosporosinus* sp. may be caused by the observed fluctuating conditions in peatlands such as de-coupled variations in substrate and sulfate concentrations (Küsel *et al.*, 2008), irregularly occurring disturbance events such as oxygen exposure by droughts or heavy rainfall (Deppe *et al.*, 2009; Knorr and Blodau, 2009; Knorr *et al.*, 2009; Reiche *et al.*, 2009), and continuous energy consumption for maintenance, e.g., by simultaneous sulfate reduction and oxygen detoxification at oxic-anoxic interfaces (Brune *et al.*, 2000) or by maintaining intracellular pH homeostasis in the acidic peatland. Such stresses were partially relieved in our SIP incubations, leading to a possible re-channeling of energy requirements from maintenance towards growth, and are a possible explanation for the observed slow increase of the *Desulfosporosinus* population over the incubation period. Alternatively, the identified peatland *Desulfosporosinus* might represent an r-strategist making use of the slightly elevated sulfate concentrations (100–300 μM) during and after oxygenation events, which often occur in the analyzed 10–20 cm depth fraction due to water-table fluctuations. At the same time, it would have to cope with oxygen and pH stress, again restricting its energy supply for growth. Interestingly, gross *in situ* SRR during such oxygenation events increase several-fold reaching peak rates of >600 nmol (g soil w. wt.)$^{-1}$ day^{-1}, which is explained by sulfate reduction in water-saturated, anoxic soil pockets above the water table (Knorr and Blodau, 2009; Knorr *et al.*, 2009).

Our findings highlight that microbial communities do not only consist of abundant microorganisms, which carry out the major ecosystem functions, and the dormant ‘rare biosphere’, which results from random dispersal and/or functions as a ‘microbial seed bank’ and insurance for the case of changing environmental conditions (Fuhrman, 2009; Hubert *et al.*, 2009; Patterson, 2009; Pedros-Alio, 2006; Turnbaugh *et al.*, 2009). In addition, microbial keystone species, like *Desulfosporosinus* sp. in the peatland, “whose effect is large, and disproportionately large relative to their abundance” (Power *et al.*, 1996) are apparently of considerable importance in certain ecosystems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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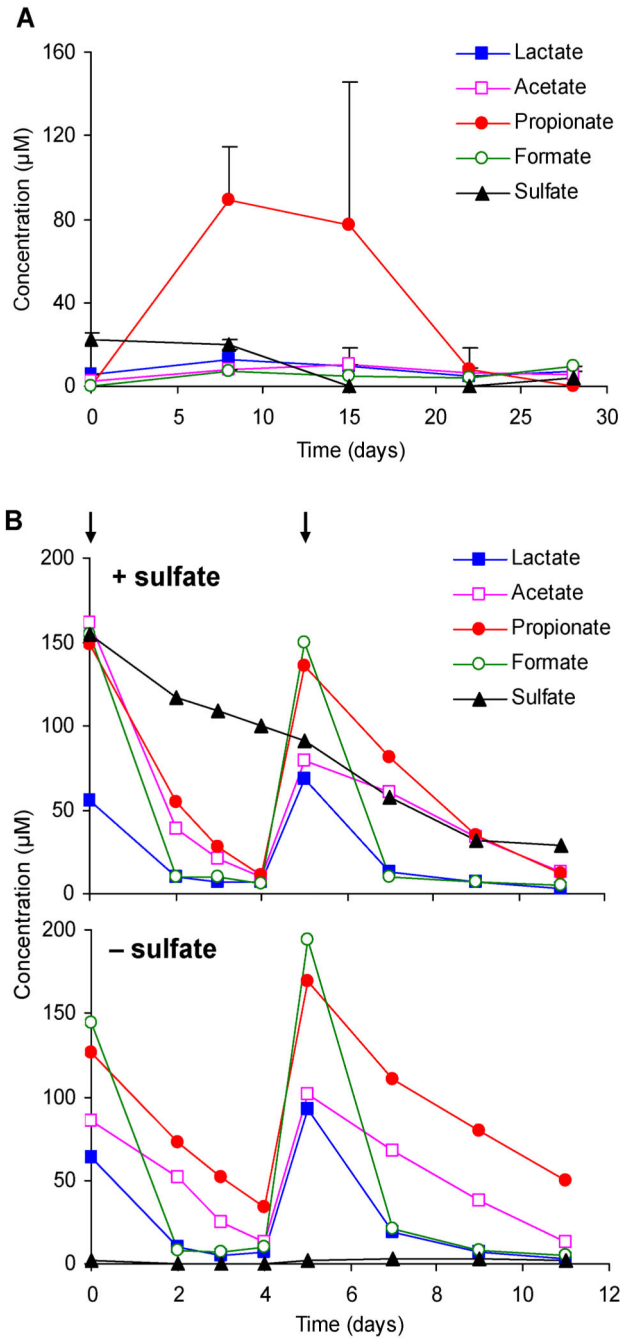

Fig. 1.

Substrate and sulfate measurements during pre-incubation and ^{12}C-substrate turnover determinations. (A) Monitoring of indigenous substrate and sulfate concentrations during 4 weeks of pre-incubation of anoxic non-amended peat soil slurries. Averages ± SD are shown (n=6). (B) Time course of ^{12}C-substrate turnover in anoxic peat soil slurries in the presence and absence of sulfate. Arrows indicate the time points of substrate additions; sulfate was added only once at the beginning of the experiment. Data points represent average values of three independent soil slurries; standard deviation bars were omitted for better visibility.

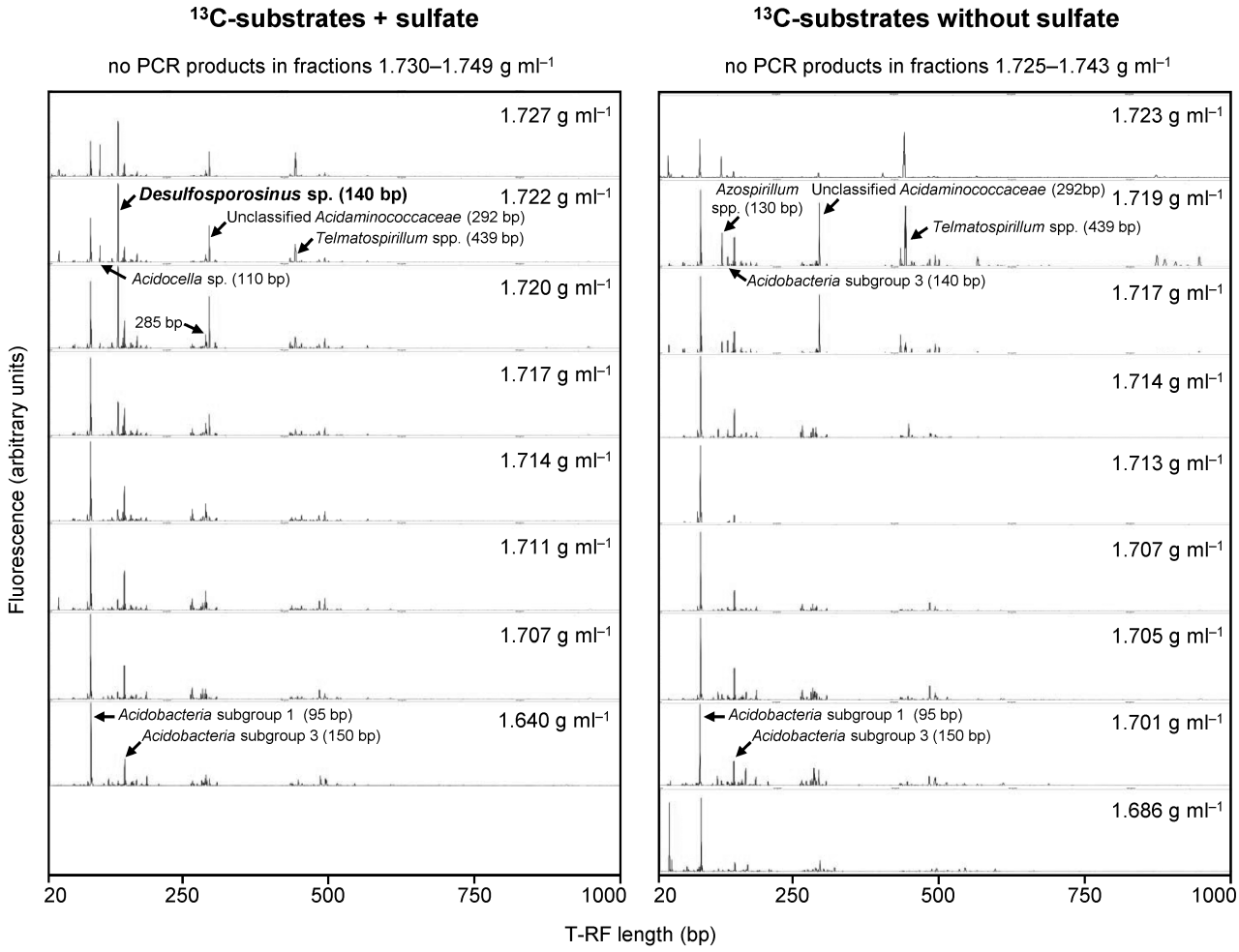

Fig. 2.

T-RFLP fingerprinting of density-resolved bacterial 16S rRNA genes after two months of SIP incubations in the presence and absence of sulfate. CsCl buoyant densities are given for each fraction. Major bacterial populations are indicated with their respective T-RFs, which were assigned using 16S rRNA gene clone libraries generated from fractions with buoyant densities 1.722 g ml^{-1} (incubation with sulfate) and 1.719 g ml^{-1} (incubation without sulfate), respectively (see also Table S1, Fig. S4). T-RFs, which had no assignment according to their respective clone library, are indicated by their length only. The range of 'heavy' fractions that yielded no PCR product is indicated above the T-RFLP profiles.

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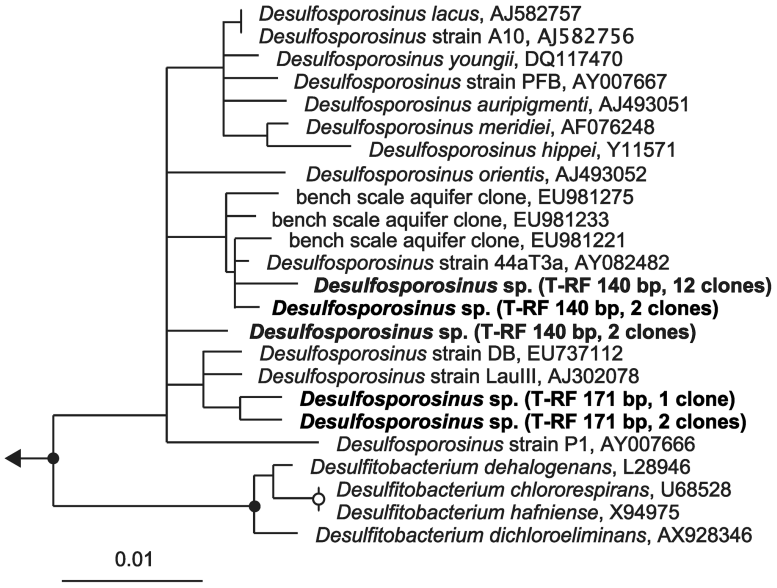

Fig. 3.

Phylogenetic consensus tree of 16S rRNA gene clones affiliated to the genus *Desulfosporosinus* (marked in bold). Clones were grouped according to ≥99% sequence identity; representing T-RFs and number of clones per group are indicated. With one exception, all *Desulfosporosinus* clones have a 16S rRNA sequence identity of >97% to each other. Parsimony bootstrap values for branches are indicated by solid circles (>90%) and open circles (75 to 90%). GenBank accession numbers of published 16S rRNA gene sequences are indicated behind the name of the respective sequences. The bar represents 1% estimated sequence divergence as inferred from distance matrix analysis.

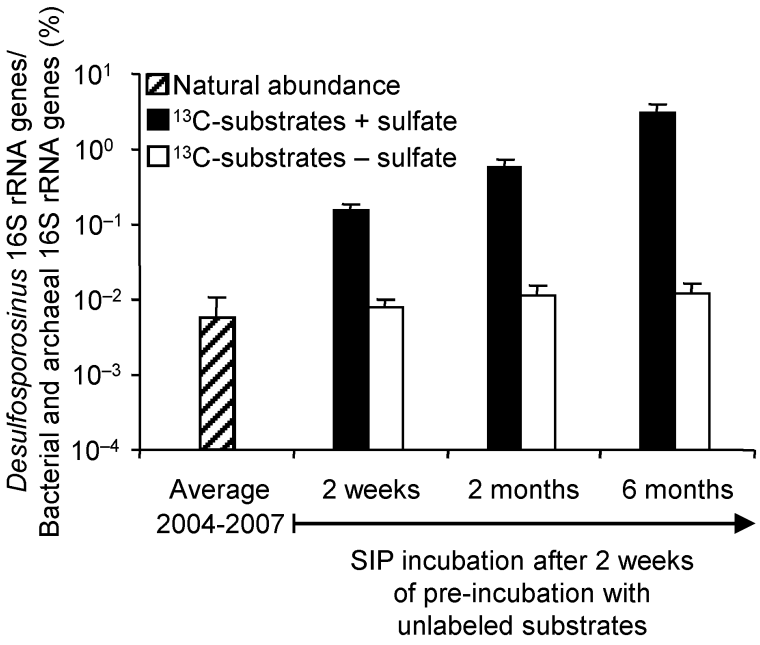

Fig. 4.

Quantification of *Desulfosporosinus* 16S rRNA genes relative to total 16S rRNA genes of *Bacteria* and *Archaea* by quantitative real-time PCR. The relative abundance ± SD of *Desulfosporosinus* sp. was determined for pristine peat soil samples over the years 2004, 2006, and 2007 (10–20 cm depth; biological replicates, n=3) in comparison to SIP incubations with and without sulfate (technical replicates, n=3). Peat soil of the 10–20-cm depth horizon was also used for the SIP incubations.

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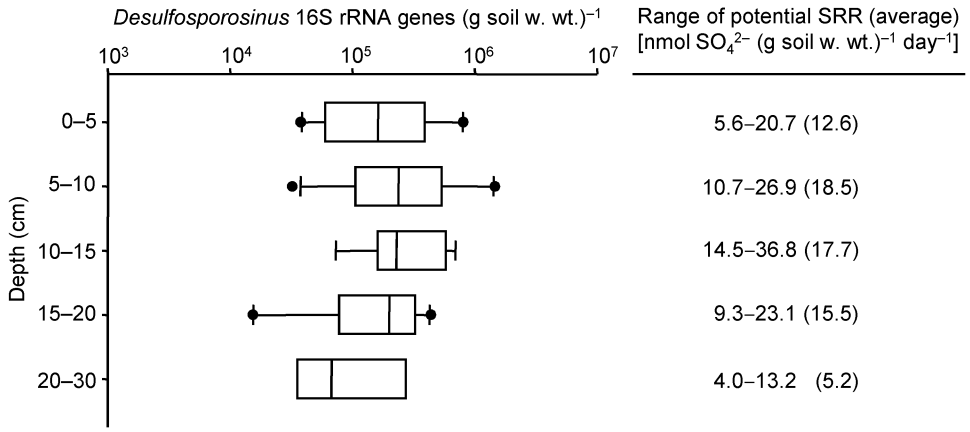

Fig. 5.

Quantification of *Desulfosporosinus* 16S rRNA gene numbers by quantitative PCR over a peatland depth profile of 0–30 cm and quantification of potential *Desulfosporosinus* sulfate reduction rates (SRR). 16S rRNA gene numbers were determined in triplicate cores over the years 2004, 2006, and 2007, with the exception of the 20–30-cm depth, where samples were only available for the year 2007. The distribution of gene numbers is represented in boxplots showing the interquartile range and the median. Whiskers (maximum 1.5-fold interquartile range) represent the data distribution outside the interquartile range; outliers are depicted as black circles. Potential SRR of the *Desulfosporosinus* population were determined using the estimated cell-specific SRR of the identified peatland *Desulfosporosinus* sp., the interquartile range of *Desulfosporosinus* 16S rRNA genes per depth, and an average of 4.4 16S rRNA gene copies per cell (for details see text).

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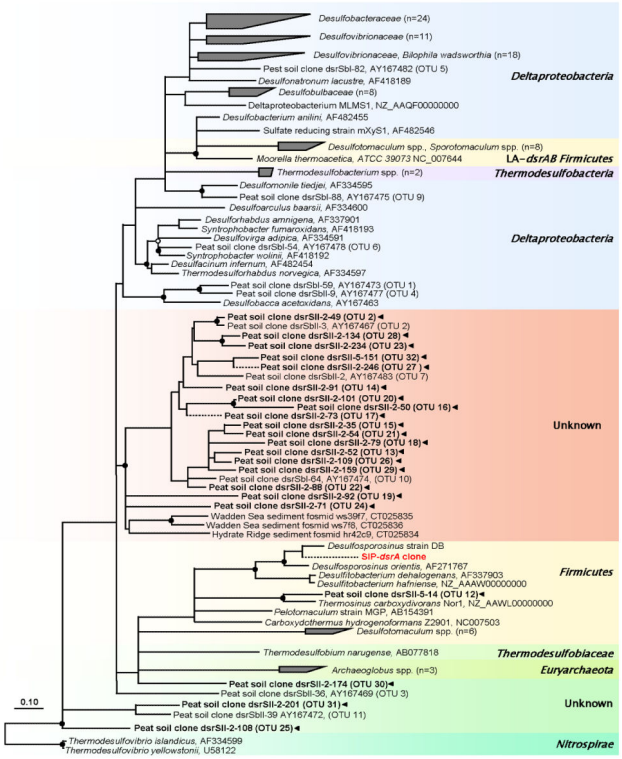

Fig. 6.

Phylogenetic consensus tree of deduced DsrAB amino acid sequences longer than 500 amino acids, showing the affiliation of OTUs retrieved from the 'heavy' SIP fractions (indicated by a triangle) in comparison to known sulfate reducers and peat soil OTUs retrieved in a previous study from the same and a neighboring peatland (Loy *et al.*, 2004. An OTU comprises all sequences having ≥90% amino acid sequence identity. Deduced DsrAB sequences shorter than 500 amino acids (indicated by dashed branches) were individually added to the distance matrix tree without changing the overall tree topology by using the ARB Parsimony_interactive tool. Parsimony bootstrap values for branches are indicated by solid circles (>90%) and open circles (75 to 90%). The *Desulfosporosinus*-related *dsrA* clone (shown in red) was retrieved from the 2-month incubation with sulfate using *Desulfosporosinus*/*Desulfitobacterium*-selective primers. GenBank accession numbers of published DsrAB sequences are indicated behind the name of the respective sequences. The bar represents 10% estimated sequence divergence as inferred from distance matrix analysis.

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Table 1

Substrate turnover rates during incubation with ^{12}C-substrates.

	Substrate turnover rate ± st. dev. (µmol L^{-1} day^{-1})			
	^{12}C-substrates + sulfate		^{12}C-substrates only	
^{12}C-substrate	1. week	2. week	1. week	2. week
Lactate	22.8 ± 6.1	27.5 ± 1.7	27.0 ± 1.8	28.4 ± 4.6
Acetate	14.3 ± 8.4	11.3 ± 1.2	18.8 ± 3.5	14.7 ± 1.2
Formate	72.3 ± 4.5	69.7 ± 3.2	68.2 ± 5.8	70.3 ± 4.9
Propionate	35.2 ± 6.1	20.9 ± 2.4	23.6 ± 2.9	19.5 ± 7.7

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Table 2

Phylogenetic affiliation and abundance of *dsrAB* clones retrieved from 'heavy' fractions of density-resolved DNA extracts from the 2-month incubations. Clones were grouped into operational taxonomic units (OTUs) using a 90% amino acid sequence identity threshold. The numbering of OTUs is based on a previous study of the same peatland (Loy *et al.*, 2004). Terminal restriction fragments (T-RFs) of retrieved *dsrAB* sequences are given for a double digest with MspI and MboI as well as for MboI only. Multiple T-RFs represent different clones of the same OTU with different locations of the restriction site in their sequence.

OTU	Number of clones from incubations		Representative clone	Closest relative (NCBI accession number; amino acid sequence identity in %)	T-RF (bp)	
	with sulfate [a]	without sulfate [b]			MspI + MboI	MboI
Schlöppnerbrunnen peat 2	2	2	dsrSII-2-49	Peat soil clone dsrSbII-3 (AY167467; 97%)	69	69
Schlöppnerbrunnen peat 12	1	1	dsrSII-5-14	Anoxic paddy soil clone 20 (FJ472883; 84%)	76	297, 515
Schlöppnerbrunnen peat 13	4	1	dsrSII-2-52	Metalliferous organic soil clone W17 (DQ855255; 84%)	114, 131	297
Schlöppnerbrunnen peat 14	19	11	dsrSII-2-91	Peat soil clone dsrSbII-3 (AY167467; 76%)	131	164
Schlöppnerbrunnen peat 15	14	19	dsrSII-2-35	Metalliferous organic soil clone W3 (DQ855249; 89%)	131, 127	313
Schlöppnerbrunnen peat 16	1	–	dsrSII-2-50	Metalliferous organic soil clone W6 (DQ855250; 67%)	76	494
Schlöppnerbrunnen peat 17	1	–	dsrSII-2-73	Metalliferous organic soil clone W6 (DQ855250; 73%)	60	190
Schlöppnerbrunnen peat 18	3	–	dsrSII-2-79	Metalliferous organic soil clone W3 (DQ855249; 84%)	47, 53	53, 116
Schlöppnerbrunnen peat 19	2	15	dsrSII-2-92	Metalliferous organic soil clone W3 (DQ855249; 72%)	53	53
Schlöppnerbrunnen peat 20	1	–	dsrSII-2-101	Metalliferous organic soil clone W3 (DQ855249; 72%)	76	494
Schlöppnerbrunnen peat 21	1	2	dsrSII-2-54	Metalliferous organic soil clone W3 (DQ855249; 85%)	127	313, 455, 507
Schlöppnerbrunnen peat 22	10	1	dsrSII-2-88	Aarhus bay sediment clone DSRIV-4 (FM179973; 87%)	116, 131	116, 455, 518
Schlöppnerbrunnen peat 23	1	1	dsrSII-2-234	Peat soil clone dsrSbII-34 (AY167468; 75%)	53	53
Schlöppnerbrunnen peat 24	2	1	dsrSII-2-71	Peat soil clone dsrSbII-3 (AY167467; 76%)	190	190
Schlöppnerbrunnen peat 25	2	–	dsrSII-2-108	Anoxic paddy soil clone OTU-10 (FJ472873; 88%)	87, 88	115, 116
Schlöppnerbrunnen peat 26	6	1	dsrSII-2-109	Metalliferous organic soil clone W3 (DQ855249; 85%)	53	53
Schlöppnerbrunnen peat 27	3	1	dsrSII-2-246	Peat bog clone 26c-40 (AM179475; 83%)	127	214
Schlöppnerbrunnen peat 28	4	1	dsrSII-2-134	Peat soil clone dsrSbII-34 (AY167468; 76%)	76	297, 276
Schlöppnerbrunnen peat 29	2	8	dsrSII-2-159	Metalliferous organic soil clone W3 (DQ855249; 73%)	76, 77, 88, 131	116, 190, 313
Schlöppnerbrunnen peat 30	1	–	dsrSII-2-174	Peat bog clone 26c-40 (AM179475; 87%)	53	53
Schlöppnerbrunnen peat 31	1	1	dsrSII-2-201	Polluted ground water clone LGWI09 (EF065057; 90%	69	69
Schlöppnerbrunnen peat 32	–	1	dsrSII-5-151	Peat soil clone dsrSbII-34 (AY167468; 74%)	127	252

[a] Good's Coverage 84%

[b] Good's Coverage 92%