

Response of the methanogenic microbial community of a profundal lake sediment (Lake Kinneret, Israel) to algal deposition

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Abstract

An algal bloom of *Peridinium gatunense* generally precedes the annual maximum of methane release from the profundal sediment of Lake Kinneret. Therefore, we investigated the response of the sediment methanogenic microbial community to simulated algal deposition. Addition of algal biomass on top of sediment cores resulted in increased CH₄ production rates and concentrations of the fermentation products acetate and propionate in the upper 4-cm layers with maximum values at 1-cm depth. Addition of algae to sediment slurries also resulted in increased CH₄ production rates and a transient increase of H₂, propionate, and acetate concentrations within the first 10 d after addition. The composition of the active microbial community was determined by analysis of terminal restriction fragment polymorphism (T-RFLP) targeting ribosomal RNA and cloning and sequencing of reverse-transcribed 16S rRNA. Analysis of the sediment in the presence and absence of algae indicated that among the *Bacteria*, members of *Deltaproteobacteria* and *Clostridiales* responded by synthesis of ribosomes after 1 d of incubation and those of *Bacteroidetes* after 6 d. Among the *Archaea*, ribosomal RNA of the *Methanosaetaceae* (i.e., acetate-utilizing methanogens) slightly increased after 6 d. Algal deposition apparently stimulated ribosomal synthesis in these sediment microorganisms, thus resulting in increased activity. We conclude that these microorganisms were involved in degradation of the algal biomass resulting in transient release of acetate and other fermentation products and increased production of CH₄.

Availability of organic material is one of the key factors controlling structure and activity of microbial communities in anoxic freshwater lake sediment (Graf et al. 1982; Sander and Kalf 1993). Therefore, input of organic material into the sediment associated with annual algae blooms results in a response of the microbial community within the sediment (Goedkoop et al. 1997; Tornblom and Rydin 1998), for example, in the microbial production of CH₄ (Schulz and Conrad 1995). In freshwater sediments, CH₄ is usually the most important end product of the degradation of organic matter (Rudd and Taylor 1980). Previous studies of the decomposition of algal biomass in freshwater sediments have been limited to activity measurements and determination of total bacterial biomass (Schulz and Conrad 1995; Zohary et al. 2000a). However, the phylogenetic diversity, composition, and dynamics of the microbial community involved in the decomposition of sedimenting algal material have not been yet investigated.

In subtropical, meso-eutrophic, and monomictic Lake Kinneret, CH₄ production is one of the dominating biogeochemical processes involved in carbon cycling in

the lake sediment (Eckert and Conrad 2007). The seasonal plankton succession in Lake Kinneret is characterized by an algal bloom formed almost exclusively by *Peridinium gatunense* Nygaard during winter and spring (Hickel and Pollinger 1988). The highest input of organic matter into the profundal sediment occurs during the decline of this bloom between May and July (Pollinger 1986). Subsequently (July–November), high CH₄ emission from the profundal sediment is observed (Ostrovsky 2003). The increase in CH₄ bubbling intensity is probably triggered by the settling of organic matter onto the sediment (Eckert and Conrad 2007), similarly as observed in Lake Constance (Schulz and Conrad 1995).

Therefore, we studied the response of the microbial community in Lake Kinneret sediment to the input of algal biomass. We investigated both activity and composition of the microbial communities. We targeted ribosomal RNA as marker for microbial populations since 16S rRNA not only allows phylogenetic characterization but also is a good predictor for the size and activity of individual populations (Kemp et al. 1993). By combining biogeochemical process measurements with molecular analysis of 16S rRNA phylogenetic markers, we aimed to enhance our understanding of the processes operating during anaerobic degradation of algal deposits in freshwater sediment, which is an important event in the annual carbon cycle of a lake.

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Experimental procedures

Study site and sampling procedures—Lake Kinneret is a warm monomictic and meso-eutrophic lake located in the northern part of the Afro-Syrian Rift Valley in Israel (32°5'N, 35°4'E). The lake has a surface of 170 km² and a volume of 4 × 10⁹ m³. It is thermally stratified from March until December and exhibits a constant sediment temperature of 15°C at the central lake station A (about 42 m deep) with about 2°C interannual variation. With the onset of stratification, the hypolimnion turns gradually anoxic. Information on seasonal dynamics of nutrients and carbon is described in Serruya (1971). The highest input of organic material occurs with the sedimentation of dead algae after the annual bloom of *P. gatunense* from May to July (Pollinger 1986). Although patterns of annual blooms changed recently, *Peridinium* is still the most important primary producer (Zohary 2004).

Sediment cores were sampled in July (five cores) and in October (two cores) 2004, respectively, at the central lake station, using a gravity corer (Nüsslein et al. 2001). In the laboratory, the upper 0–10 cm of the five cores sampled in July 2004 were pooled in gastight bottles and kept under an argon atmosphere. The bottles were stored at 4°C, transported to Germany, and processed within 2 weeks. Transport and storage at 4°C had no effect on the methanogenic activity (Nüsslein et al. 2001). Sediment cores sampled in October 2004 were directly processed in the laboratory in Israel.

Preparation of algal blooms—*P. gatunense* Nygaard (a freshwater dinoflagellate isolated from Lake Kinneret (Hickel and Pollinger 1988) was cultured in a medium according to Lindstrom (1985) with slight modifications (4× concentrated) at 20°C under a 6 L:6 D regime. *P. gatunense* cells were harvested when growth reached a stationary phase, that is, after approximately 4 weeks. Harvesting was carried out by a vacuum pump through a 2.5-μm filter, subsequently followed by freeze drying of the cells at –53°C for 24 h by a dry freezer (HETO LyoLab 3000). Since the settling algal biomass consists mainly of dead cells and theca (Viner-Mozzini et al. 2003), decomposition of algal biomass was studied using freeze-dried *P. gatunense* cells to approximate the actual algal material that is deposited onto the sediment surface in situ.

Sediment core experiment—The effect of the added algal biomass on fatty acid concentration and methane production within different sediment depths was followed by adding algal cells (freeze-dried) equivalent to the annual sedimentation (8 mg per sediment slurry) to one sediment core and using a second core without addition of algal biomass as control. The cores were incubated at 15°C in the dark for 6 d. To determine methane production rates within different sediment depths, cores were sliced in layers 1 cm (0–4 cm), 2 cm (4–6 cm), and 3 cm (6–12 cm) thick. Sediment slurries were prepared in 26-mL pressure tubes using 5 mL of sediment and 5 mL of sterile anoxic deionized water. The tubes were incubated in triplicates for each layer at in situ temperature (15°C) in the dark. The

mixing ratios of CH₄ in the gas phase were repeatedly determined by taking gas samples (0.1 mL) with a gastight syringe and analyzing them with a GC. Methane production rates were determined from the linear increase of CH₄ with incubation time and expressed in nmol CH₄ h^{–1} and cm^{–3} sediment. To analyze organic acids dissolved in the pore water of the different sediment depths after 6 d of incubation, liquid samples were recovered and analyzed by high-performance liquid chromatography (HPLC) (Nüsslein et al. 2001).

Sediment slurry experiment—Sediment slurries were prepared from 10 mL of profundal sediment (0–10 cm; July 2004) and 10 mL of anoxic sterile distilled water in 60-mL serum bottles as described by Nüsslein et al. (2001). To one set of slurries, unlabeled *P. gatunense* cells were added at a final concentration of about 8 mg dry weight per slurry (corresponding amount of the annual sedimentation event of *P. gatunense*). The influence of the added algal biomass on CH₄ production and on active microbial populations was followed by a control without addition of algal biomass. All slurry setups were incubated as triplicates at in situ temperature (15°C) in the dark. Gas samples (0.1 mL) were repeatedly taken with a gastight syringe and analyzed by gas chromatography for CH₄, CO₂, and H₂ as described by Nüsslein et al. (2001). Concentrations of CH₄ and CO₂ were calculated as the sum from the gaseous and liquid phase of the slurries and expressed in μmol CH₄ and CO₂ per cm³ slurry, respectively. Liquid samples for analysis of organic acids dissolved in the pore water were recovered and analyzed by HPLC (Nüsslein et al. 2001). For the analysis of microbial communities, slurry incubations were opened after 0, 1, and 6 d, and slurry samples were stored frozen (–75°C) for nucleic acid extraction.

Community analysis by 16S rRNA-based T-RFLP analysis—At time point zero (day 0) and after 1 and 6 d of incubation, total ribosomal nucleic acids were extracted in triplicate from 2 ml of slurry samples of the control incubation and of the incubation with addition of algal biomass as described previously (Schwarz et al. 2007b). RNA for reverse-transcription polymerase chain reaction (RT-PCR) was obtained by removal of coextracted DNA as described by Scheid et al. (2003). The integrity of the received small-subunit ribosomal RNA (16S rRNA) was checked by standard agarose gel electrophoresis on a 1% agarose gel and ethidium bromide staining.

Terminal restriction fragment length polymorphism (T-RFLP) analysis of active bacterial and archaeal communities was performed with primers Ba27f-FAM/Ba907r and Ar109f/Ar912rt-FAM by RT-PCR using a one-step RT-PCR system (Access Quick1 RT-PCR-System, Promega) as described previously by Schwarz et al. (2007b). Amplicons were digested by *Hae*III and *Taq*I for *Bacteria* and *Archaea*, respectively, separated on an ABI 310 genetic analyzer (Applied Biosystems) and analyzed according to Schwarz et al. (2007b). The relative abundance of T-RFs obtained from T-RFLP analysis of triplicate sediment slurries exhibited in most cases a standard error of <1%.

Sequence data, phylogenetic analysis, and assignment of T-RFs—Bacterial amplicons for sequence analysis were generated from 16S rRNA with the primers Ba27f/Ba907r and RT-PCR conditions as described before (Schwarz et al. 2007b). Fragments were cloned by using the TOPO TA cloning kit (Invitrogen), and randomly selected clones were sequenced by the ADIS DNA core facility (Max Planck Institute for Plant Breeding Research) on an ABI Prism 3700 sequencer (Applied Biosystems). Raw sequence data were assembled and checked with the Lasergene software package DNASTAR. All cloned bacterial 16S rRNA sequences were compared to sequences of public databases by using NCBI megaBlast (<http://www.ncbi.nlm.nih.gov/blast/megablast.html>). Sequences that showed the highest matches in the Blast search were obtained from the GenBank database (<http://www.ncbi.nlm.nih.gov/Genbank>). Chimeric sequences were identified as described before (Schwarz et al. 2007b). Phylogenetic analyses of retrieved clonal 16S rRNA sequences were conducted by phylogenetic treeing using the ARB software package (version Linux Beta 030822; <http://www.arb-home.de>) according to Schwarz et al. (2007a). Besides using an ARB-integrated software tool for in silico T-RFLP analysis (Ricke et al. 2005) of all clones, randomly selected clones were also analyzed for their in vitro T-RF in order to support the assignment of in silico detected T-RFs to phylogenetic groups. Sequence data have been submitted to the EMBL database under accession numbers AM409800 to AM410040.

Assignment of archaeal T-RFs to phylogenetic groups refers to earlier studies on Lake Kinneret profundal sediment, in particular to recent analyses of archaeal 16S rRNA gene and 16S rRNA clone libraries (Nüsslein et al. 2001; Schwarz et al. 2007b).

Results

Effect of algal biomass addition on sediment biogeochemistry—Concentrations of fatty acids and CH₄ production rates were measured in different-depth layers of the profundal sediment (sampled in October 2004) after 6 d of incubation of intact sediment cores with and without (control) addition of *P. gatunense* cells (Fig. 1A,B). In the control core, acetate was the only fatty acid detectable, occurring at a low concentration of about 30 μmol L⁻¹ within the upper sediment layers (Fig. 1A). Addition of algal biomass to the sediment core resulted in increased concentrations of acetate. Propionate was also detected with highest concentrations in the upper sediment layers (Fig. 1A). Methane production rates were also highest in the upper sediment layers and decreased with depth (Fig. 1B). Addition of algal biomass resulted in doubling of the CH₄ production rate from within the upper sediment layers (Fig. 1B).

Addition of algal biomass to sediment slurries (0–10-cm depth; sampled in July 2004) resulted in increased CH₄ and CO₂ accumulation compared to the control incubation without algae (Fig. 2A,B). Sulfate was not detected (<10 μmol L⁻¹) after 1 d of incubation. The highest production rates of CH₄ (0.40 ± 0.01 μmol CH₄ d⁻¹ cm⁻³) and CO₂

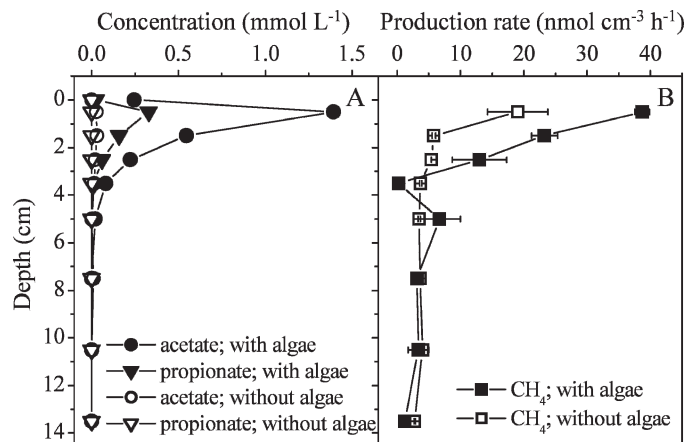


Fig. 1. (A) Total concentration of fatty acids (mmol L⁻¹) and (B) CH₄ production rates (nmol cm⁻³ sediment h⁻¹) in different-depth intervals of sediment cores obtained from Lake Kinneret in October 2004 after addition of algal biomass and without addition of algal biomass (control) after 6 d of incubation at 15°C; means ± SE; *n* = 3.

(0.26 ± 0.01 μmol CO₂ d⁻¹ cm⁻³) were observed between day 2 and day 10. After 20 d of incubation, CH₄ and CO₂ production rates decreased and then equaled those in the control incubation, which were constant at 0.02 μmol CH₄ d⁻¹ cm⁻³ and 0.01 μmol CO₂ d⁻¹ cm⁻³. However, since Lake Kinneret sediment has a high CaCO₃ content of about 50% (Serruya 1971) and therefore is well buffered, the measured increase of CO₂ (Fig. 2B) did not reflect total CO₂ production. Partial pressures of H₂ increased transiently up to 4.8 Pa in slurries with algal biomass during the first 10 d of incubation, whereas it remained constant at low H₂ partial pressures (0.2 Pa) in the control incubation (Fig. 2C). After 30 d of incubation, H₂ partial pressures decreased to about 0.2 Pa also in the incubations with algae. Acetate and propionate were the only two fatty acids detectable in the incubations with algae, while no fatty acids were detected in the control (Fig. 3). Accumulation of acetate started immediately and reached the maximum (420 μmol L⁻¹) at day 3–6. Accumulation of propionate started after a lag and reached the maximum (300 μmol L⁻¹) at day 6–10. These maxima coincided with the accumulation of H₂ during the first 10 d of incubation (Fig. 2C). After 15 d of incubation, the acetate concentration dropped below the detection limit, and the propionate concentration decreased to about 80 μmol L⁻¹. After 30 d of incubation, propionate also could no longer be detected.

T-RFLP analysis of the active microbial populations—Bacterial T-RFLP profiles of RNA extracted from the control (incubated without algae) showed highly similar patterns after 0, 1, and 6 d of incubation (Fig. 4). In algae-amended incubations, by contrast, the composition of bacterial T-RFLP profiles changed after 1 and 6 d (Fig. 4). Immediately before the start of the incubation (day 0), a total of 26 distinct bacterial T-RFs were identified. These T-RFs all showed a relative abundance of >1%, and those of 40, 68/70, 183, 204, 206, 210, 222/223, and 876 base pairs (bp) in length even exhibited a relative abundance of >4%.

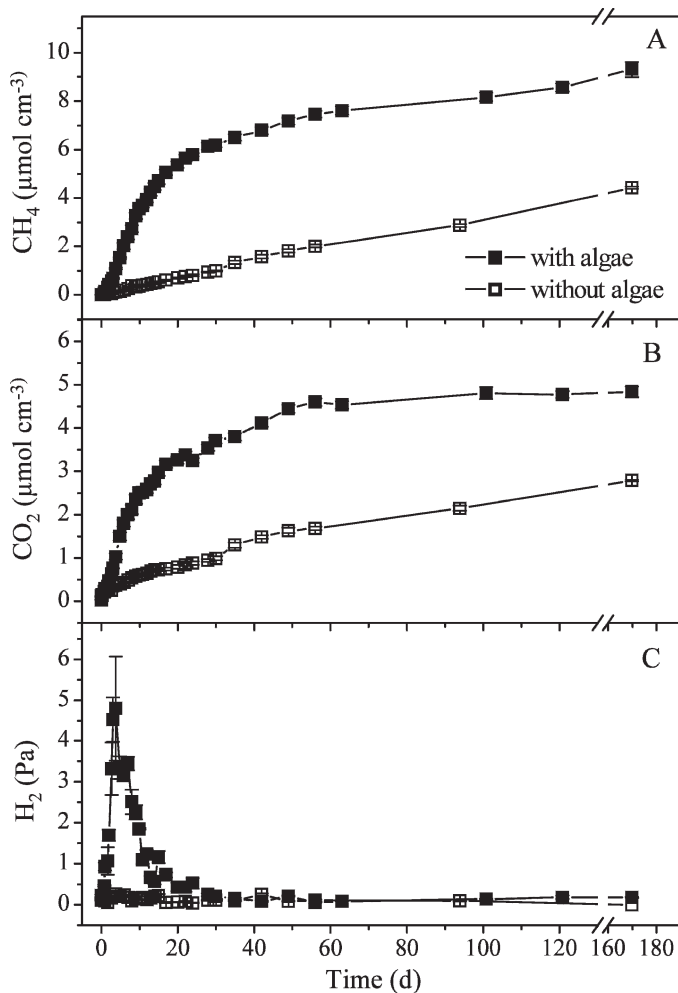


Fig. 2. (A) CH_4 and (B) CO_2 production ($\mu\text{mol cm}^{-3}$ slurry) and (C) H_2 partial pressure (Pa) during incubation of sediment slurries after addition of algal biomass and without addition of algal biomass (control); means \pm SE; $n = 3$; the sediment samples (0–10-cm depth) were obtained from Lake Kinneret in July 2004.

After 1 d, the relative abundance of most of these dominant T-RFs had changed only little, so that these T-RFs were still among the most abundant ones within the bacterial T-RFLP profile. However, a new T-RF at 278 bp appeared and became the most abundant one (relative abundance $>13\%$; Fig. 4). Furthermore, another characteristic T-RF of 297/299 bp (relative abundance of 3%) was detected for the first time. After 6 d of incubation, the structure of the active bacterial community had changed again. Now the 40-bp T-RF dominated the active bacterial populations with a relative abundance of about 26% (Fig. 4). In addition, seven new T-RFs (118, 215, 237, 251, 393, 410, and 412 bp) were detected for the first time.

Archaeal T-RFLP profiles in both the control and the incubation with algae showed three major T-RFs (284, 392, and >700 bp) throughout the incubation (Fig. 5). The 284-bp T-RF was the most abundant one. The relative abundance of the different archaeal T-RFs did not change much in the control during 6 d of incubation. However, in the algal-amended incubations the relative abundance of

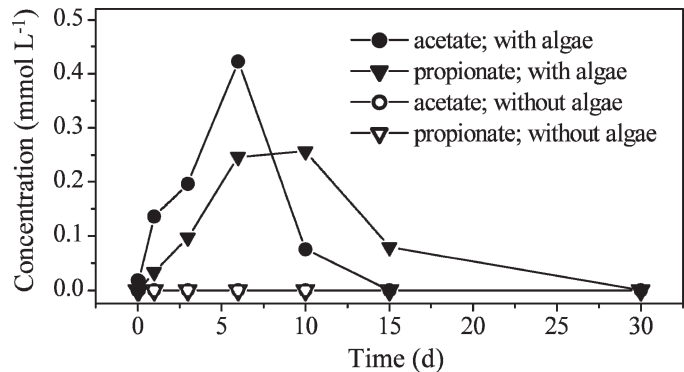


Fig. 3. Total concentration of fatty acids (mmol L^{-1}) during incubation of sediment slurries after addition of algal biomass; means \pm SE; $n = 3$; the sediment samples (0–10-cm depth) were obtained from Lake Kinneret in July 2004.

the 284-bp T-RF increased from 49% to 62%, while the relative abundance of the T-RFs of 392-bp and >700 -bp length decreased from 32% to 27% and 19% to 12%, respectively.

Phylogenetic analysis and assignment to T-RFs—The archaeal community of Lake Kinneret profundal sediment has been studied before using T-RFLP analysis and comparative sequence analysis (Nüsslein et al. 2001; Schwarz et al. 2007a). Therefore, all T-RFs detected after 0, 1, and 6 d of incubation could be assigned to archaeal taxa. The T-RF of 392 bp was assigned to the hydrogenotrophic *Methanomicrobiaceae* and *Methanospirillaceae* and the nonmethanogenic euryarchaeotal group III (*Thermoplasma* relatives). The T-RF of >700 -bp length was also assigned to euryarchaeotal group III. The predominant T-RF of 284-bp length was assigned to *Methanosaetaceae* with acetoclastic *Methanosaeta concilii* as closest relative (Schwarz et al. 2007a).

In order to identify *Bacteria*, three clone libraries were generated from bacterial 16S rRNA templates retrieved from the sediment after 0 (d0 [$n = 89$]), 1 (d1 [$n = 85$]), and 6 d (d6 [$n = 74$]) of incubation with algae (Table 1). The phylogenetic affiliation of the various clone sequences was determined by calculating phylogenetic trees using the ARB database (Schwarz et al. 2007b). Phylogenetic analysis of the clone sequences revealed highly diverse bacterial populations involved in decomposition of the added algal biomass. At day 0, 16S rRNA sequences were dominated by *Deltaproteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, *Betaproteobacteria*, and *Gammaproteobacteria* (Table 1). After 1 d of incubation, the active bacterial populations were dominated by *Deltaproteobacteria* (T-RF: 278 bp; Fig. 4; Table 1). Within the *Deltaproteobacteria*, the sequences were most closely related to sulfate reducers of the *Desulfobacteraceae*, to an environmental sequence clone cws275 (AY799892), and to *Desulfococcus multivorans* DSM 2059 (AF418173). Furthermore, the T-RFs of 204, 206, and 210 bp, which exhibited a high relative abundance at day 0 and stayed abundant (14%) after 1 d of incubation, were affiliated to the *Desulfobacterales*, *Syntrophobacterales*, and *Myxococ-*

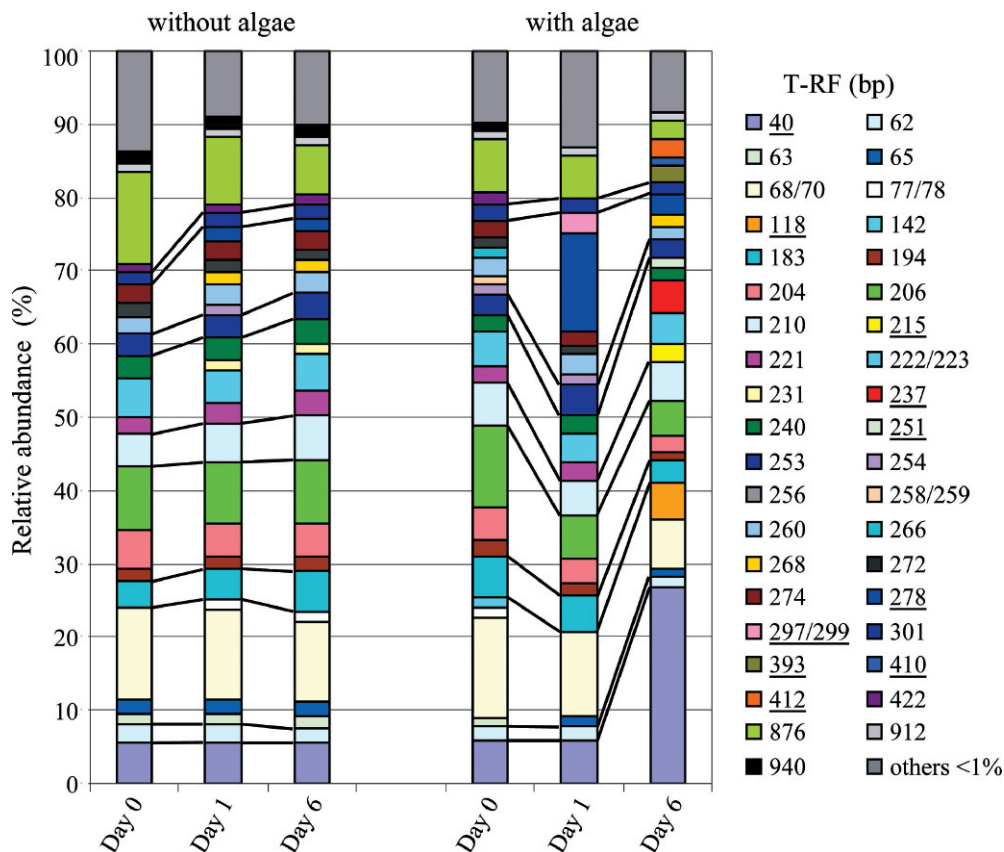


Fig. 4. Relative abundance of bacterial 16S rRNA amplicons recovered from profundal sediment after incubation without and with addition of algal biomass based on terminal restriction fragment polymorphism (T-RFLP) analysis after restriction with *Hae*III; means of triplicate incubations; standard error was in most cases <1%. Numbers in the legend indicate the length of T-RFs in base pairs (bp). T-RFs characteristic for one time point and T-RFs with enhanced increase in their relative abundance are underlined, respectively.

cales (Table 1). Another T-RF (T-RF of 297/299 bp), also characteristic for day 1, was assigned to *Clostridiales*. The most closely related sequences (99% sequence identity) were to clone sequence LrhB59 (AM159365) from the rice rhizosphere and M10Ba34 (AY360624) from rice field soil. *Clostridium tunisiense* (AY187622, 95% sequence identity) and *Clostridium aff. estertheticum* (AJ297442, 97% sequence identity) were the most closely related isolates, both from within Cluster I of the *Clostridia*.

After 6 d of incubation, the active bacterial community was dominated by the 40-bp T-RF (Fig. 4). Most of the clone sequences (71%) characteristic for this T-RF were allocated to the *Bacteroidetes* phylum (Table 1). The clone sequences were most closely related to environmental sequences, that is, clone O33T7 (DQ110029, 96% sequence identity) and clone BS049 (AB240238, 97% sequence identity). *Cytophaga fermentans* (M58766) and *Alkaliflexus imshenetskii* Z-7010^T (AJ784993) were the most closely related isolates. The T-RFs of 118, 215, 251, 410, and 412 bp in length, which were detected after 6 d of incubation for the first time, were also assigned to members of the *Bacteroidetes* phylum (Table 1). Furthermore, *A. imshenetskii* Z-7010^T (AJ784993) was the most closely related isolate to the clone sequences with the characteristic T-RF

of 118 and 215 bp. The T-RF of 237 bp was assigned to *Bacteriovorax* within the *Deltaproteobacteria*. The T-RF of 393 bp was not represented by any of the clone sequences and therefore could not be assigned to any phylogenetic group.

Discussion

The profundal sediment of deep lakes is characterized by constant temperature throughout the year. Increased metabolic activity in summer thus can be caused only by change in chemistry, for example, input of fresh organic matter by sedimentation of algal biomass. Our simulated sedimentation of *P. gatunense* cells onto intact sediment cores indeed resulted in an enhanced CH₄ production rate and in the formation of products of organic matter fermentation (propionate, acetate), especially within the upper sediment layers (Fig. 1), confirming similar experiments done with sediment of Lake Constance (Schulz and Conrad 1995). The stimulation was also observed when sediment slurries were amended with algae. The immediate response might be attributed to a high fraction of dissolved organic matter (DOM) within the freeze-dried algae cells. Earlier studies on the degradation of *P. gatunense* in Lake

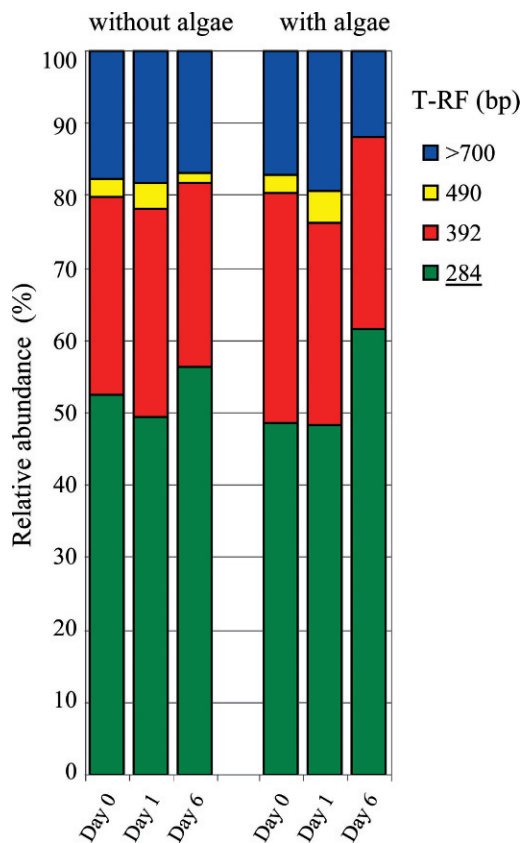


Fig. 5. Relative abundance of archaeal 16S rRNA amplicons recovered from profundal sediment after incubation without and with addition of algal biomass based on terminal restriction fragment polymorphism (T-RFLP) analysis after restriction with *TaqI*; means of triplicate incubations; standard error was in most cases <1%. Numbers in the legend indicate the length of T-RFs in base pairs (bp). T-RFs characteristic for one time point and T-RFs with enhanced increase in their relative abundance are underlined, respectively.

Kinneret showed that about 50% of added algal biomass was degraded within the first 3 d of incubation (Zohary et al. 2000a). Furthermore, enzyme activity assays (cellulase and β -glucosidase) showed that the labile dissolved parts (DOM) of *P. gatunense* cells were consumed first, while degradation of particular organic matter (POM) occurred later (Zohary et al. 2000b). According to our observations DOM was probably converted by the concerted activity of fermenting bacteria, syntrophic H_2 -producing bacteria, and methanogenic archaea, resulting in increase of acetate, propionate, H_2 , CO_2 , and CH_4 concentrations immediately after addition of algal biomass.

Besides the progress of activity, we also analyzed the change in composition of the sediment microbial community on addition of algal biomass. In marine sediment, addition of organic matter resulted in significant change in the composition of the microbial community (Rosselló-Mora et al. 1999; Luna et al. 2004). Analysis of the microbial community by fluorescence in situ hybridization and denaturing gradient gel electrophoresis showed that addition of algal biomass resulted in a fast and selective

increase of distinct bacterial groups (Rosselló-Mora et al. 1999). Furthermore, the quantity as well as quality of available organic material caused changes in the structure of the bacterial community (Luna et al. 2004). In marine sediments, respiratory processes, sulfate reduction in particular, dominate the decomposition of organic matter. In freshwater systems, where CH_4 production dominates, the effect of algal biomass on the composition of the microbial community has not yet been studied.

In previous studies, we already analyzed the microbial community composition by T-RFLP, cloning and sequencing of microbial genes coding for 16S rRNA using sediment sampled in January 2003, July 2003, and October 2004 (Schwarz et al. 2007a). We also analyzed reverse-transcribed 16S rRNA on addition of acetate to sediment sampled in July 2003 (Schwarz et al. 2007b). Here, we analyzed reverse-transcribed 16S rRNA on addition of algal biomass to sediment sampled in July 2004. Although the data cannot be directly compared because of different sampling times and targeting rRNA versus rDNA, they are consistent with respect to the main features of the archaeal and bacterial communities. In particular, we always observed a relatively high abundance of members of the *Deltaproteobacteria* and *Bacteroidetes* phylum (compare below) but also of *Chloroflexi*, *Planctomycetes*, *Gammaproteobacteria*, and *Epsilonproteobacteria* (Schwarz et al. 2007a; Table 1). In the following, we focus on changes in the active microbial community on addition of algal biomass.

Already after 1 d of incubation, the bacterial T-RFLP profile had changed. Changes both in the abundance of single T-RFs and in composition of the entire T-RFLP profile were detected in the algal-amended incubation (Fig. 4). After 1 d of incubation, T-RFs allocated to *Desulfobacterales*, *Syntrophobacterales*, and *Myxococcales* dominated the bacterial T-RFLP profile of the algal-amended slurries. Since sulfate was not detectable after 1 d of incubation, the high relative abundance of *Desulfobacterales* must be due to metabolism other than sulfate reduction. For example, many sulfate-reducing bacteria are able to gain energy by the fermentation of organic substrates (Rabus et al. 2006). The detection of *Syntrophobacterales* is reasonable since members of this order, such as *Syntrophobacter wolinii* (X70906), may have contributed to the syntrophic degradation of propionate that transiently accumulated on addition of algae (Boone and Bryant 1980). Members of the detected *Myxococcales* (i.e., most closely related with *Polyangiaceae*) are known as proteolytic-bacteriolytic bacteria and as cellulose degraders, respectively, which can grow on sugars (Reichenbach 2001). Thus, these bacteria may have been involved in the degradation of polysaccharides of *P. gatunense* thecae.

Clostridiales of Cluster I (Collins et al. 1994) were also detected after 1 d of incubation in the presence of algal biomass. Members of this clostridial cluster are known as hydrolytic (saccharolytic and proteolytic) and fermenting bacteria, which may have caused the accumulation of acetate and H_2 at the onset of the algal-amended incubation. Members of *Clostridiales* are assumed to be important during the anaerobic degradation of organic material in freshwater sediments (Mallet et al. 2004).

Table 1. Phylogenetic affiliations and numbers of 16S rRNA sequences retrieved in clone libraries generated from sediment slurries at time point zero, after 1 and 6 d of algal-amended incubations. Characteristic T-RFs for different clone groups are given; T-RFs with relative abundance of more than 4% are indicated in bold; T-RFs detected in more than one phylogenetic group are marked with an asterisk (*); T-RFs characteristic for one time point and T-RFs with enhanced increase in their relative abundance are underlined, respectively.

Phylogenetic group	Day 0		Day 1		Day 6	
	No. of clones	T-RF (bp)	No. of clones	T-RF (bp)	No. of clones	T-RF (bp)
<i>Acidobacteria</i>	1	255	1	204*		
<i>Actinobacteria</i>	2	237	1	92	1	212
<i>Bacteroidetes</i>						
<i>Flavobacteriales</i>						
Unclassified	4	40* , 173, 410	8	40* , 370, 410	115	613 40* , 118 , 215 , 251 , 253, <u>412</u> , 910
<i>Chloroflexi</i>	7	68* , 218, 223 , 225, 228, 253, 263	14	68* , 185, 198, 215, 219, 221, 222 , 223* , 256, 279	9	64, 75, 221, 222* , 223* , 230, 259, 263, 265
<i>Cyanobacteria</i>	4	139, 183 , 295	2	183	1	318
<i>Deferribacteres</i>	4	81, 294, 302	2	268, 302		
<i>Fibrobacteres</i>	1	255	1	324		
<i>Firmicutes</i>						
<i>Clostridiales</i>			5	221, 255, <u>297</u> , <u>299</u>		
<i>Fusobacteria</i>	1	154	1	280		
<i>Planctomycetes</i>	10	70* , 81, 146, 147, 207, 215, 227, 236, 276	4	172, 219, 268	3	172, 238, 252
<i>Spirochaetes</i>	1	244	1	196	4	183, 208, 220
<i>Verrucomicrobia</i>	3	142, 181, 204*	8	68* , 258, 223* , 179, 190	2	223* , 422
<i>Alphaproteobacteria</i>						
<i>Rhizobiales</i>			2	40* , 196		
<i>Betaproteobacteria</i>						
<i>Burkholderiales</i>	1	218				
<i>Hydrogenophilales</i>	1	70*	1	220		
<i>Nitrosomonadales</i>	1	222			1	222*
<i>Rhodocyclales</i>			1	220	4	199, 222*
<i>Gammaproteobacteria</i>						
<i>Alteromonadales</i>	1	40*				
<i>Chromatiales</i>	2	77, 178			2	187
<i>Methylococcales</i>	10	70* , 78, 252	6	70 , 244, 252	9	70 , 252
Unclassified	1	198			3	40* , 199
<i>Deltaproteobacteria</i>						
<i>Bdellovibrionales</i>					2	237
<i>Desulfobacterales</i>	12	79, 210 , 212, 242, 274, 940	16	191, 194, 204* , 210 , 211, 217, 242, 276, 278 , 427, 940	8	<u>42</u> , 191, 206 , 210 , 212, 258, 242
<i>Desulfuromonales</i>	2	210 , 217	1	217	1	207
<i>Myxococcales</i>	3	68* , 194, 224	3	204* , 206 , 236		
<i>Syntrophobacterales</i>	8	78, 204* , 206	4	78, 204 , 240	2	204, 205
Unclassified					2	245
<i>Epsilonproteobacteria</i>						
<i>Campylobacterales</i>			1	545		
Candidate division OP3	1	245			2	88, 253
C. "Endomicrobia"	2	266, 286	1	216		
Chloroplast	5	314, 876	1	876	1	876
Unknown affiliation	1	69			1	339

After 6 d of incubation, the structure of the active bacterial population changed again with members of the *Bacteroidetes* phylum (formerly known as *Cytophaga-Flavobacteria-Bacteroides* group (Kirchman 2002) becoming dominant. The *Bacteroidetes* phylum has already been

described as one of the most abundant bacterial groups in aquatic habitats including freshwater lakes sediments (Kirchman 2002). Most of the members of this phylum are able to degrade biopolymers, in particular all kinds of polysaccharides, many also under anaerobic conditions.

Therefore, we assume that *Bacteroidetes*-related bacteria were involved in the degradation of *P. gatunense* thecae, which consist mainly of polysaccharides. *Bacteroidetes*-related bacteria (designated as “Cytophaga”) became also abundant in algal-amended marine sediments (Rosselló-Mora et al. 1999). Our data are also consistent with those from an earlier study on degradation of *P. gatunense* in Lake Kinneret (Zohary et al. 2000b) in which the predominant bacterial populations changed from free-living rod-shaped to attached gliding filamentous bacteria, designated as “Cytophaga,” after about 4–7 d of incubation. Although the next relatives of the clone sequences retrieved gave no clue to a particular phenotype, we assume that populations of *Bacteroidetes*-related bacteria were primarily involved in the degradation of POM and became abundant as soon as the more labile DOM had been consumed by *Clostridiales* and *Deltaproteobacteria*.

Another bacterial group detected for the first time after 6 d of incubation was affiliated with the genus *Bacteriovorax*. *Bacteriovorax* are predatory bacteria possessing a biphasic life cycle. They are often found as intracellular parasites of Gram-negative bacteria but can also live as saprophytes on nutrient-rich medium (Baer et al. 2000). The ecological niche of these bacteria in the algae-amended sediment is unclear. Maybe they contribute to predation of attached bacteria, such as “Cytophaga,” which are less suitable prey for heterotrophic nanoflagellates (Zohary et al. 2000b).

Addition of algal biomass also resulted in a change in the composition of the community of *Archaea* (Fig. 5). Initially, acetate accumulated in the sediment slurries during the first days of incubation (Fig. 3). At this time acetate was probably produced by fermentation of easily accessible DOM, and production was not yet balanced by consumption; that is, acetate was formed more rapidly than it was consumed by methanogenic archaea. Similar results were observed during the initial stage of anaerobic degradation of rice straw in a flooded rice field soil (Chidthaisong et al. 1999). Actually, only minor amounts of CH₄ had been produced until day 2 (Fig. 3). However, after 6 d of incubation, CH₄ production rate increased and acetate concentration decreased, indicating that acetoclastic methanogens became active. Indeed, T-RFLP analysis showed that the composition of the active archaeal community did not change much until day 1 but that 16S rRNA of acetoclastic *Methanosaeta* spp. increased in relative abundance between days 2 and 6. Previous experiments showed that *Methanosaetaceae* were activated on addition of acetate (Schwarz et al. 2007b). Hence, we assume that algae affected *Methanosaeta* spp. via the increased production of acetate by fermenting bacteria (Fig. 3).

In summary, our study has shown that the addition of algae to the profundal sediment of a freshwater lake resulted in a systematic sequential change in both activity and composition of the active microbial community, which eventually led to methanogenic decomposition of the added algal organic matter. Future research will aim at determining which of the detected active microbial taxa are

involved in which of the different metabolic reactions that sequentially degrade polysaccharides via sugars, fatty acids, and acetate to CH₄.

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