Vol. 54: 71–82, 2009
doi: 10.3354/ame01260

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Printed January 2009
Published online January 27, 2009

OPEN ACCESS

Successional changes in bacterial community assemblages following anoxia in the hypolimnion of a eutrophic lake

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ABSTRACT: Dynamics of bacterial assemblages following anoxia in the hypolimnion of a eutrophic lake (Lake Aydat) were characterized. The sampling started in spring before complete anoxia and was continued weekly until complete mixing of the water column occurred in autumn. Bacterial community patterns at 3 sampled depths (10, 12 and 14 m) were investigated using temporal temperature gradient gel electrophoresis (TTGE) and terminal restriction fragment length polymorphism (T-RFLP) analyses. Results revealed changes in the structure of the bacterial communities as conditions changed from oxic to anoxic. Once anoxia had been reached, anaerobic bacterial communities continued to change and exhibited gradual successional patterns at the 3 depths. During the anoxic period, bacterial communities at 10 and 12 m were split into 2 groups corresponding to the steps 'until' and 'after' maximum stratification, which occurred on 19 August 2004. While the succession of the bacterial community in the hypolimnion was dynamic and exhibited gradual patterns at the 3 depths, a 'sensitivity gradient' to O_2 depletion was suggested: communities at 10 m appeared to be more affected by the shift in O_2 concentrations than those in deeper water layers.

KEY WORDS: Lake · Bacterial community structure · Succession · Molecular fingerprint methods

INTRODUCTION

In the water column of many eutrophic aquatic systems, 3 steps are observed during thermal stratification in regard to O_2 concentrations: (1) in spring, increasing solar radiation leads to vertical stratification, which reduces the rate of O_2 supply while aerobic respiration removes O_2; (2) in summer, the deepest water layers (hypolimnion) are anoxic; and (3) in autumn, decreasing solar radiation leads to cooling of surface waters and the deep penetration of O_2. This overturn of O_2 leads to the reoxygenation of the entire water column. Consequently, in the course of a few days, the microbial community habitat in the hypolimnion shifts from O_2 supersaturation to anoxia (or the opposite), and new microbial niches are created, filled and destroyed in rapid succession (Finlay et al. 1997).

Lake Aydat in the French Massif Central supports an extensive seasonal anoxic zone (from 6 to 14 m; Michard et al. 2001), which forms every year from May to October (Bettarel et al. 2004). This hypolimnion is populated with living and active communities of bacteria; bacterial abundance averages 5×10^6 cells ml^{-1} and bacterial production (114.2×10^6 bacteria l^{-1} h^{-1}) is greater than that in overlying oxic water (Bettarel et al. 2003, 2004). In this anoxic and aphotic water layer, fish, zooplankton and phytoplankton are absent. Grazers such as ciliates and flagellates are rare and grazing rates are low (Bettarel et al. 2003, 2004), as typically noted in anoxic waters (Weinbauer & Holfe 1998).

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Although changes in bacterial community composition are influenced by carbon and nutrient sources (i.e. bottom-up influences; e.g. Billen et al. 1990) and viral lyses (Weinbauer & Holfe 1998, Bettarel et al. 2004), this anoxic zone is an accurate and simplified (with less complex trophic interactions) system in which to analyze the responses of bacterial populations to shifting environmental conditions (e.g. anoxia).

Table 1. Primers for the PCR amplification of eubacterial 16S rDNA

Primer	Sequence 5′-3′	Source
27f	AGA GTT TGA TCC TGG CTC AG	Suzuki & Giovannoni (1996)
1492r	GGT TAC CTT GTT ACG ACT T	Suzuki & Giovannoni (1996)
27f-GC clamp	CGC CCG CCG CGC GCG GCG GGC GCG GCG GGG GCA CGG GGG GAG AGT TTG ATC CTG GCT CAG	Weisburg et al. (1991)
518r	ATT ACC GCG GCT GCT GG	Muyzer et al. (1993)

The objective of the present study was to monitor the dynamics of the dominant bacterial community members in the hypolimnion of the eutrophic Lake Aydat during the 3 periods of thermal stratification. In order to assess changes in the bacterial community, 2 cultivation-independent techniques were used on the 16S rRNA gene: terminal restriction fragment length polymorphism (T-RFLP) and temporal temperature gradient gel electrophoresis (TTGE).

MATERIALS AND METHODS

Site description and sample collection. Lake Aydat, located at 2° 59′ E, 45° 40′ N at 825 m altitude in the French Massif Central, is a small (surface area 60.3 ha, maximum depth 14.5 m) dimictic lake. It originates from a dam formed by a basaltic flow approximately 7500 yr ago.

Depth profiles of water temperature (±0.2°C) and O_2 concentration (±0.03 mg l^{-1}) were determined *in situ* using a portable multisensor probe (WTW), and values were interpolated through the water column by using Surfer 7.0 (Golden Software). For 28 wk from May to November 2004, weekly sampling was performed at 10, 12 and 14 m water depth (see Figs. 1a, b) at the deepest place in the lake using an 8 l horizontal Van Dorn bottle. The physicochemical composition of the hypolimnion has been reported in previous studies (e.g. Ogier 1999).

DNA extraction and PCR amplification. Water samples of 400 ml were filtered onto 0.2 µm pore-size polycarbonate filters (47 mm diameter, GTTP, Millipore). DNA extractions were performed as described in Jardillier et al. (2004). For T-RFLP analysis, amplification of the bacterial 16S rDNA genes was performed using a combination of the bacterial 5′-FAM-labeled 27f primer and the universal primer 1492r (Table 1). For TTGE analysis, the primer 27f, modified with a guanine-cytosine-rich sequence at the 5′ end, was used in combination with the universal primer 518r (Table 1). The reaction mixture contained 5 µl 10× buffer, 2 mM $MgCl_2$, 200 µM of each deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, dTTP; Eurobio), 1.25 U of Taq polymerase (Bioline, Abcyss), 10 pmoles of each oligonucleotide primer and 50 to 100 ng of template DNA, for a final volume of 50 µl. Amplifications were performed with a PTC-200 thermal cycler (MJ Research) using the following program: a 5 min hot start at 95°C, followed by 30 cycles consisting of denaturation (1 min at 95°C for T-RFLP; 30 s at 94°C for TTGE), annealing (1 min at 55°C for T-RFLP; 30 s for TTGE) and extension (1 min at 72°C for T-RFLP; 30 s for TTGE), with a final extension for 10 min at 72°C for T-RFLP and 7 min at 72°C for TTGE. Amplified DNA was checked by electrophoresis in 1.0% agarose in 1×Tris-Borate-EDTA (TBE) buffer.

T-RFLP procedure. Twenty-five µl of enzymatic digestion mixture containing 100 ng of labeled DNA and 20 U of *MspI* (Sigma) in the manufacturer's recommended reaction buffer were incubated for 12 h at 37°C (Lehours et al. 2005). Restriction digests were inactivated by heating to 65°C for 10 min and then purified and desalted using Micropure EZ-Microcon 30 columns (Millipore) to prevent ion interference with the uptake of DNA using electrokinetic injection (Moeseneder et al. 1999). The volume of all samples was checked after EZ column purification in order to reduce bias resulting from differences in column eluent volume, which could lead to large differences in mass loaded into the sequencer and, consequently, to large differences observed in detectable peaks (Lehours et al. 2005). Fluorescently labeled terminal restriction fragments (TRFs) were analyzed on an ABI 3700 automated sequence analyzer (Applied Biosystems) in GeneScan mode. We then mixed 2.3 µl of the restriction enzyme digest with 0.5 µl of GeneScan-1000 ROX size standard (Applied Biosystems) and 3.2 µl of deionized formamide, and then denaturated at 94°C for 3 min. Injections were performed electrokinetically at 7.7 kV for 40 s. Three replicate TRF profiles were obtained from the digested DNA by loading 3 aliquots of digested DNA onto 3 different capillaries. This replication level was performed to measure the degree of variation in TRF profiles arising solely as a result of experimental error during electrophoresis of DNA digest samples. To avoid detection of primers and

uncertainties in size determination, TRFs smaller than 50 bp and larger than 800 bp were excluded. TRFs were analyzed by aligning fragments to the size standard by using GeneScan analytical software (Applied Biosystems). Replicate profiles of each sample were compared to identify the reproducible fragments (peaks that appeared in at least 2 replicate profiles of a sample). Only reproducible TRFs were considered in the numerical analysis. TRFs that differed by less than 1 bp were considered identical (Dunbar et al. 2001) and were clustered. A program in Visual Basic for Excel was developed to automate these procedures. The TRF signal (representing height or area) was assumed to be proportional to the relative abundance of the species. TRFs were expressed as relative abundances of the total signal detected in the T-RFLP profile. TRFs below a threshold of 0.1% of the total profile signal were deleted and relative abundances of remaining TRFs were recalculated (Blackwood et al. 2007).

TTGE procedure. Three hundred ng of each amplified product were electrophoresed along an 8% (w/v) polyacrylamide gel (7 M urea, 1.25× Tris-acetate-EDTA [TAE] buffer, 0.06% Temed and 0.06% ammonium persulfate) using the DCode Universal Mutation Detection System (Bio-Rad). Runs were performed in 1.25× TAE buffer at 68 V for 17 h with a temperature range of 66 to 69.6°C and a ramp rate of 0.2°C h^{-1}. The reference patterns consisted of a mixture of amplified 16S rDNA V1 to V3 fragments of 3 bacterial strains: *Aeromonas caviae*, *Kurthia zopfii* and *Escherichia coli*. Following electrophoresis, gels were stained in a Gel Star nucleic acid gel stain bath (BMA) and the banding patterns were examined under ultraviolet light. The gels were digitally photographed (Versa Doc Imaging System, Bio-Rad), converted to TIFF files, normalized using reference patterns and analyzed with GelCompare 4.6 (Applied Maths).

Analyses of T-RFLP and TTGE patterns. From the binary data, dissimilarities among the T-RFLP and TTGE patterns were estimated using Dice's coefficient: $D = 1 - [(2N_{ab})/(2N_{ab} + N_a + N_b)]$, where N_{ab} is the number of bands or TRFs that are present in samples a and b; N_a is the number of bands or TRFs present in a; and N_b is the number of bands or TRFs present in b (Sneath & Sokal 1973). For the T-RFLP pattern, dendrograms were generated from the above matrix using Ward's method, which uses an ANOVA approach to evaluate the distances between clusters (Ward 1963). A distance of 1.1 was used to separate clusters in hierarchical classification performed from T-RFLP data. Analysis of similarity (ANOSIM; Clarke 1993) was used to test the hypothesis that communities within clusters were more similar to each other than to communities in others clusters. Correlations between T-RFLP and TTGE dissimilarity matrices were calculated using a Mantel test (Mantel 1967) with 10 000 permutations, performed with XLSTAT version 6.01 (Addinsoft). Similarity between T-RFLP patterns was calculated using correspondence analysis (CA), computed using the R software ADE4 package (cran.r-project. org/). CAs for TTGE data were performed with XLSTAT. Canonical correspondence analyses (CCAs) (Legendre & Legendre 1998) were used to determine the extent to which dissolved oxygen concentrations explained patterns of similarity within bacterial communities, and were performed with PAST 1.81 (Hammer et al. 2001). The Smith & Wilson evenness index (Smith & Wilson 1996) was calculated as previously described from T-RFLP data using the Ecological Evenness Calculator software (www.nateko.lu.se/personal/benjamin.smith/software). The number of distinct TRFs in a sample was expressed as richness (S).

RESULTS

Spatiotemporal profiles of dissolved O_2 and temperature

The changes in water temperature and O_2 contents were typical of a temperate lake, with stratification starting in spring (Fig. 1). Anoxia started to occur in deeper water in May (27 May 2004 at 14 m, T3; Fig. 1A) and progressively ascended the water column until reaching a depth of 6 m at the maximum of stratification (19 August 2004, T15; Fig. 1A). By mid-October, a deep penetration of O_2 was observed (Fig. 1A). Following the overturn (18 November 2004, T28; Fig. 1), O_2 occurred throughout the entire water column.

During the sampling effort, several different periods were observed with regard to dissolved O_2 concentrations at the 3 sampled depths (Figs. 2A to 4A): oxic, hypoxic, anoxic and re-oxidized periods (Table 2). Considering the temperature and O_2 diagrams (Fig. 1), 2 stages were distinguished during anoxia: 'until' maximum stratification and 'after' maximum stratification (Table 2).

Dynamics of bacterioplankton communities

Hierarchical cluster analyses were performed at each depth from T-RFLP binary data and results are presented in Figs. 2C & 4C. At 10 m, T-RFLP data were clustered into 5 groups considering a cut-off value of 1.1: cluster C1 grouped bacterial communities at dates T1 and T2, for which the highest concentrations of dissolved O_2 were measured (up to 7 mg ml^{-1}, Fig. 2A); cluster C2 grouped communities of the aerobic and

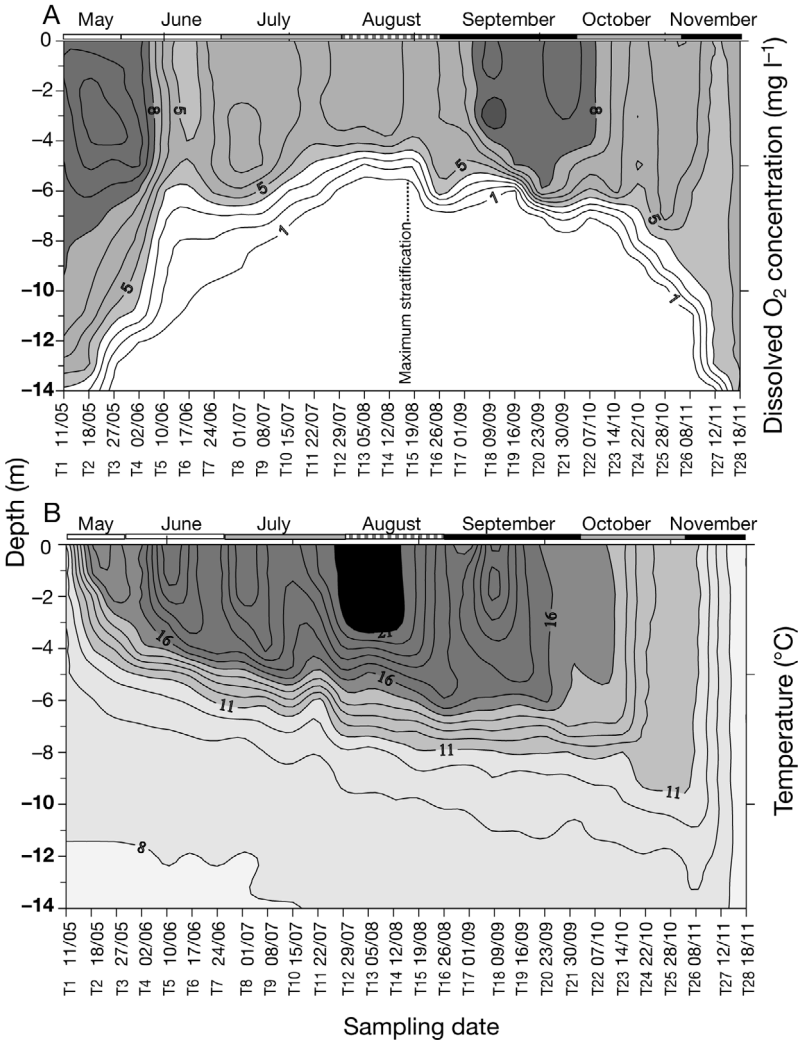

Fig. 1. Development of (A) dissolved O_2 concentrations (mg l^{-1}) and (B) temperature (°C) in the water column of Lake Aydat over the 28 weekly sampling dates from 11 May 2004 (T1) to 18 November 2004 (T28). Dates in dd/mm format. Depths sampled are indicated in **bold**. The bars on top of the panels indicate corresponding months

hypoxic periods (Fig. 2C); communities of the anoxic period were clustered into C3 and C4 according to the 2 aforementioned stages ('until' and 'after' maximum stratification, Table 2); and cluster C5 grouped the bacterial communities of the re-oxidized period. At 12 and 14 m, 3 clusters were discriminated (Figs. 3C & 4C). At 12 m, cluster C1 grouped together communities of the aerobic and hypoxic periods (T1 to T5) with those of the re-oxidized period (T27 to T28), and clusters C2 and C3 grouped together the communities of the anoxic 'until' and 'after' maximum stratification periods, respectively. Note that, at 10 and 12 m, more dissimilarity was observed between bacterial communities of the 2 anoxic stages than between communities of the oxic and anoxic periods. At 14 m, a more distinct pattern was observed. Cluster C1 grouped together communities of: the aerobic and hypoxic periods (T1 to T2), the beginning of anoxia (T3 to T9), the end of anoxia (T26 to T27) and the re-oxidized conditions (T28) (Fig. 4C). These cluster analyses from T-RFLP data were confirmed with ANOSIM, which showed that communities in each cluster were more similar to each other than to those of the other clusters (Table 3).

Similarities between TTGE patterns are illustrated by correspondence analyses (Fig. 5). At 10 m, groupings were very similar to those obtained by T-RFLP data: groups G1 and G4 grouped communities of the aerobic period before and after anoxia, respectively. At 12 m, more similarity was noted between communities at T28 and communities of the aerobic and hypoxic periods (T1 to T5) such as observed from T-RFLP data (Fig. 3C). At 14 m, group G1 clustered communities from T1 to T11, but not communities at T27, as noted from T-RFLP data (Fig. 4C). Despite some differences between T-RFLP and TTGE analyses, which were more pronounced at 14 m, significant correlations between T-RFLP and TTGE dissimilarity matrices were found at the 3 sampled depths ($r = 0.638$ at 10 m, $r = 0.683$ at 12 m, $r = 0.473$ at 14 m, $p = 0.0001$).

In the CA analysis performed from T-RFLP patterns, bacterial communities at 12 and 14 m shifted away from those at 10 m, but as the anoxia progressed, similarities between communities at the 3 depths increased (Fig. 6). More similarities were observed between the later sampling dates at 10 m (T24 to T28) and the early and intermediate sampling dates at 14 and 12 m (Fig. 6). As observed from cluster analyses (Figs. 3C & 4C), more similarities were noted between the communities of the oxic period and those of the re-oxidized conditions at 12 and 14 m.

CCA performed from relative abundance TRF data revealed that more than 30% of the variability of the bacterial community at 10, 12 and 14 m was described by dissolved O_2 and temperature variables (A.-C. Lehours et al. unpubl. data). At each depth, the distribution of the aerobic, hypoxic and anoxic ('until' and 'after' maximum stratification) communities was a response to the dissolved O_2 gradient, with communities of oxic and hypoxic period discriminated on Axis 1 from communities of the anoxic period (Fig. 7). O_2 con-

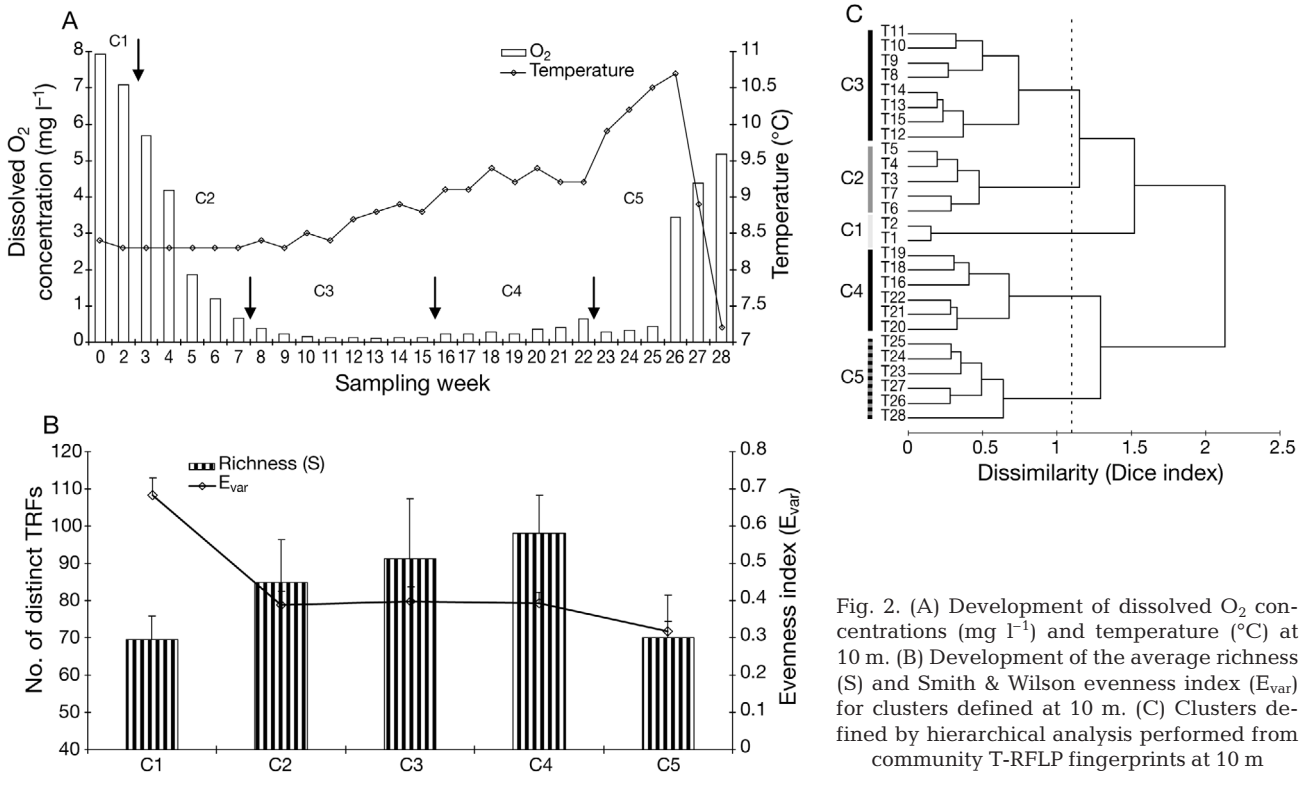

Fig. 2. (A) Development of dissolved O_2 concentrations (mg l^{-1}) and temperature (°C) at 10 m. (B) Development of the average richness (S) and Smith & Wilson evenness index (E_{var}) for clusters defined at 10 m. (C) Clusters defined by hierarchical analysis performed from community T-RFLP fingerprints at 10 m

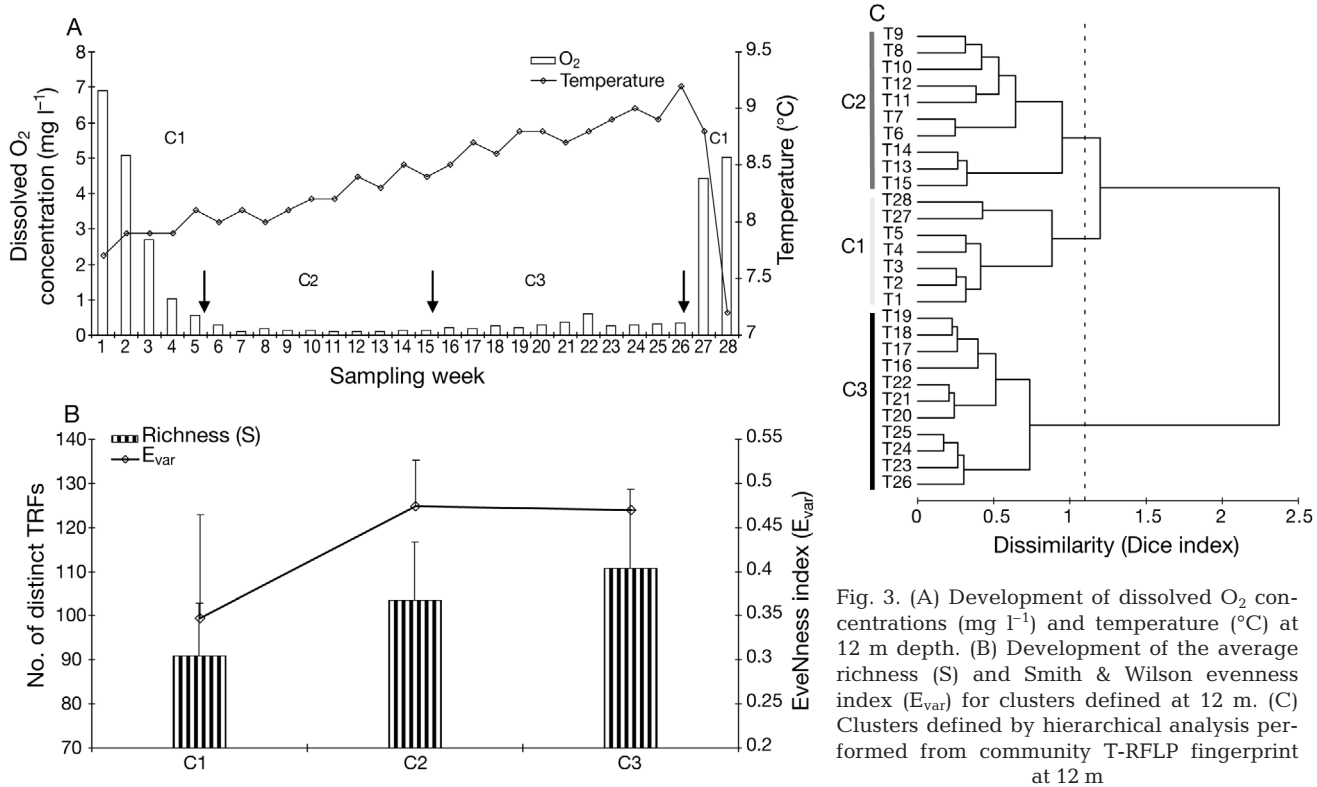

Fig. 3. (A) Development of dissolved O_2 concentrations (mg l^{-1}) and temperature (°C) at 12 m depth. (B) Development of the average richness (S) and Smith & Wilson evenness index (E_{var}) for clusters defined at 12 m. (C) Clusters defined by hierarchical analysis performed from community T-RFLP fingerprint at 12 m

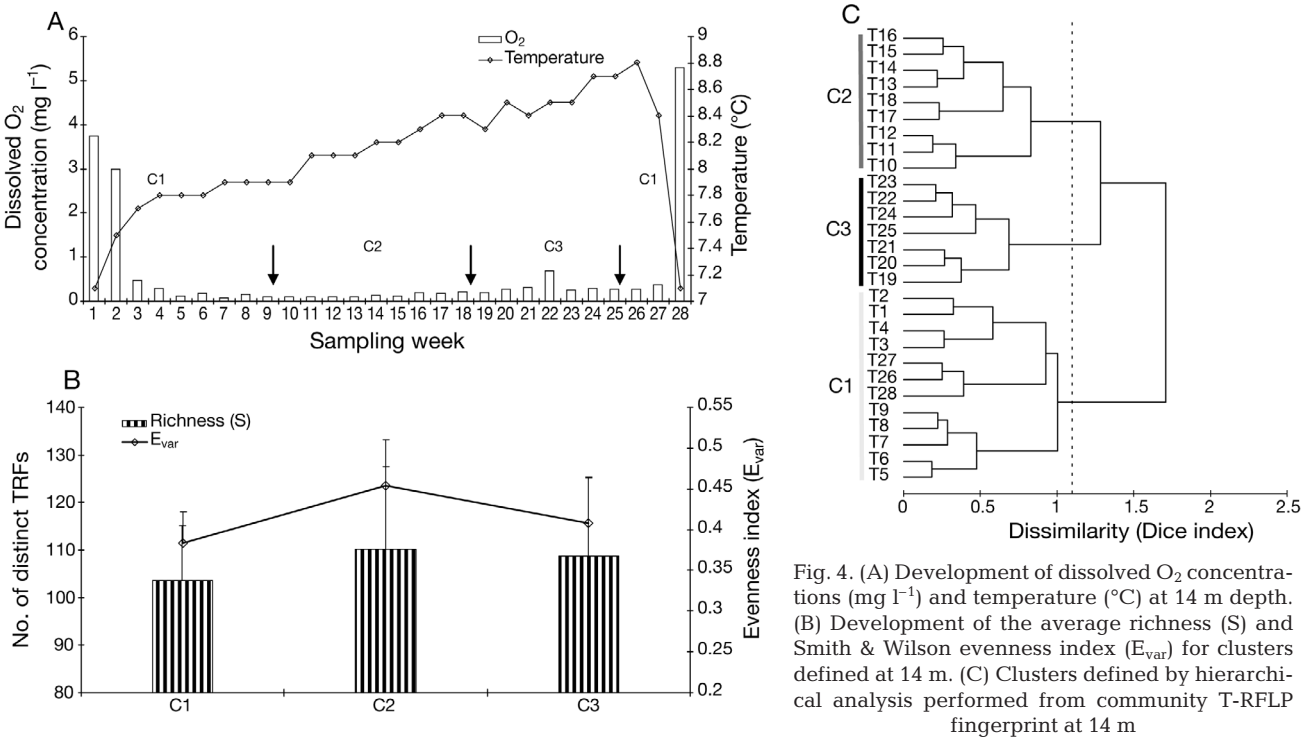

Fig. 4. (A) Development of dissolved O_2 concentrations (mg l^{-1}) and temperature (°C) at 14 m depth. (B) Development of the average richness (S) and Smith & Wilson evenness index (E_{var}) for clusters defined at 14 m. (C) Clusters defined by hierarchical analysis performed from community T-RFLP fingerprint at 14 m

Table 2. Steps observed at the 3 sampled depths in regard to O_2 concentrations during the sampling effort. T1 to T28: weekly sampling dates from 11 May 2004 (T1) to 18 November 2004 (T28)

State	Sampling period				
	Before anoxia		Anoxia (<0.75 mg l^{-1})		After anoxia
	Aerobic period	Hypoxic period (<3 mg l^{-1})	Until maximum stratification	After maximum stratification	Aerobic period
10 m	T1→T4	T5→T7	T8→T15	T16→T25	T26→T28
12 m	T1→T2	T3→T5	T6→T15	T16→T26	T27→T28
14 m	T1	T2	T3→T17	T18→T27	T28

Table 3. ANOSIM statistics for comparisons of communities between clusters C1 to C5 (for a cut-off value of 1.1) using T-RFLP similarity values derived from binary data (presence/absence). –: no data

Comparison	Sampling depth					
	10 m		12 m		14 m	
	R	p	R	p	R	p
C1 vs. C2	1	0.04	0.53	0.0001	0.49	0.0001
C1 vs. C3	1	0.02	0.92	0.0001	0.47	0.0001
C1 vs. C4	1	0.02	–	–	–	–
C1 vs. C5	1	0.05	–	–	–	–
C2 vs. C3	0.83	0.0004	0.80	0.0001	0.47	0.0002
C2 vs. C4	0.93	0.0013	–	–	–	–
C2 vs. C5	0.99	0.008	–	–	–	–
C3 vs. C4	0.80	0.0001	–	–	–	–
C3 vs. C5	1	0.0008	–	–	–	–
C4 vs. C5	0.69	0.0005	–	–	–	–

centrations explained 41, 22 and 14% of the community changes at 10 m, 12 and 14 m, respectively (Fig. 7). At 10 m, communities at T23 to T27 were also clearly discriminated by temperature on axis 2 and directly corresponded to an increase in temperature (from 9.2 at T23 to 10.7°C at T27, Fig. 2A).

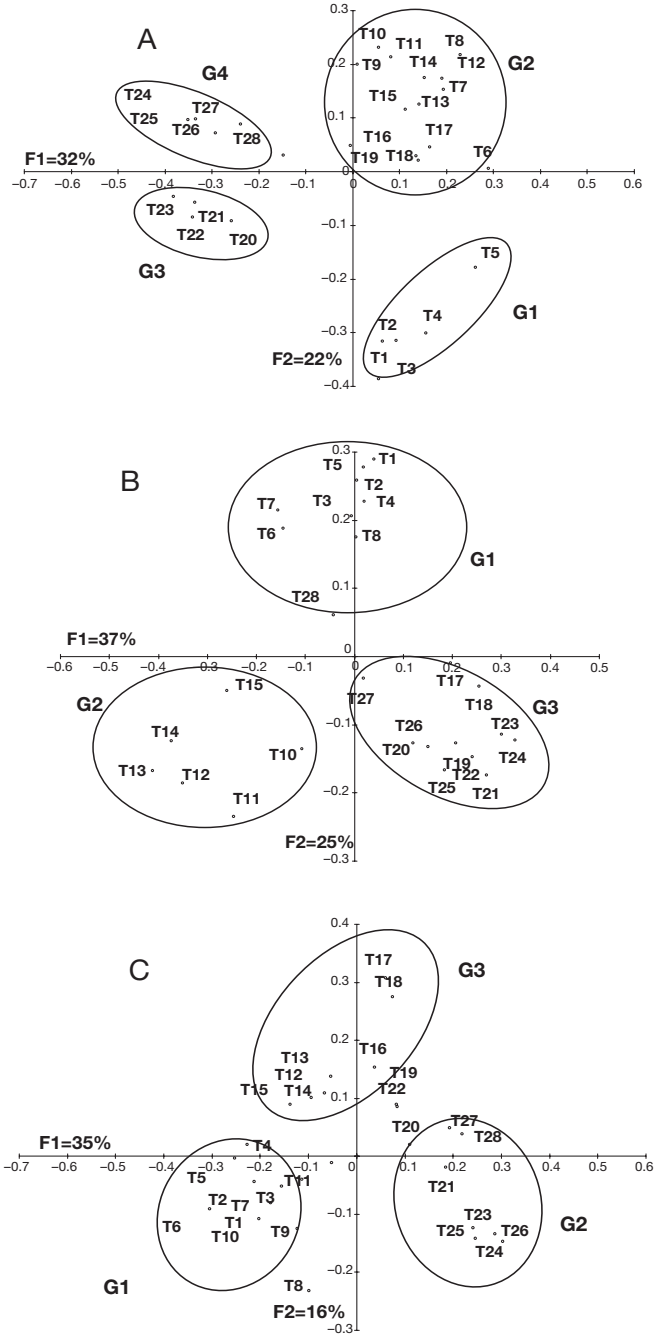

Fig. 5. Correspondence analyses (CA) performed with TTGE similarity values derived from binary data at (A) 10 m, (B) 12 m and (C) 14 m. T1 to T28: weekly sampling dates from 11 May 2004 (T1) to 18 November 2004 (T28); G1 to G4: groupings defined by CA

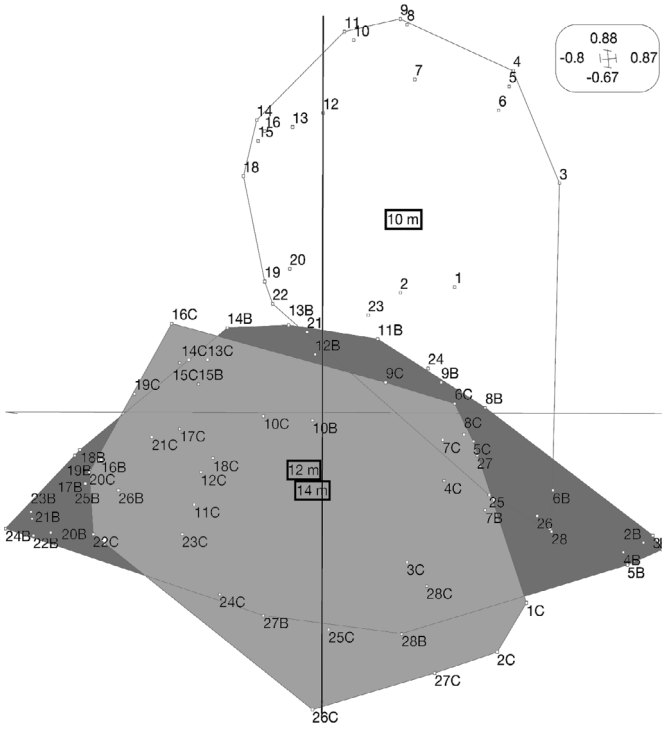

Fig. 6. Similarity among T-RFLP patterns illustrated by a correspondence analysis. Numbers alone correspond to dates at 10 m; numbers followed by B correspond to dates at 12 m; numbers followed by C correspond to dates at 14 m. Dates at (unfilled polygon) 10 m, (dark grey polygon) 12 m and (light grey polygon) 14 m, respectively. The scale of the 2-dimensional plot is indicated at the top

Diversity of bacterial communities

The average values of the Smith & Wilson evenness index (E_{var}, Figs. 2B & 4B) calculated at each depth were not significantly different between clusters defined from T-RFLP analyses (Figs. 2C & 4C). Nevertheless, we observed that the maximum values of E_{var} at 12 and 14 m occured for cluster C2, which grouped anoxic communities until maximum stratification (0.47 ± 0.05 at 12 m, 0.45 ± 0.05 at 14 m). Richness (S) was calculated from the number of different TRFs in each sample and was significantly correlated with E_{var} at the 3 depths ($r = 0.736$, $r = 0.582$, $r = 0.837$, $p < 0.0001$ at 10, 12 and 14 m, respectively). For the 3 depths, the lowest S values were noted for communities of the aerobic period (10 m: $S_{C1} = 70 \pm 5$, $S_{C5} = 70 \pm 11$; 12 m: $S_{C1} = 90 \pm 12$; 14 m: $S_{C1} = 103 \pm 11$).

DISCUSSION

Several studies have gathered routine profiles of the transition from oxic to anoxic bacterial communities

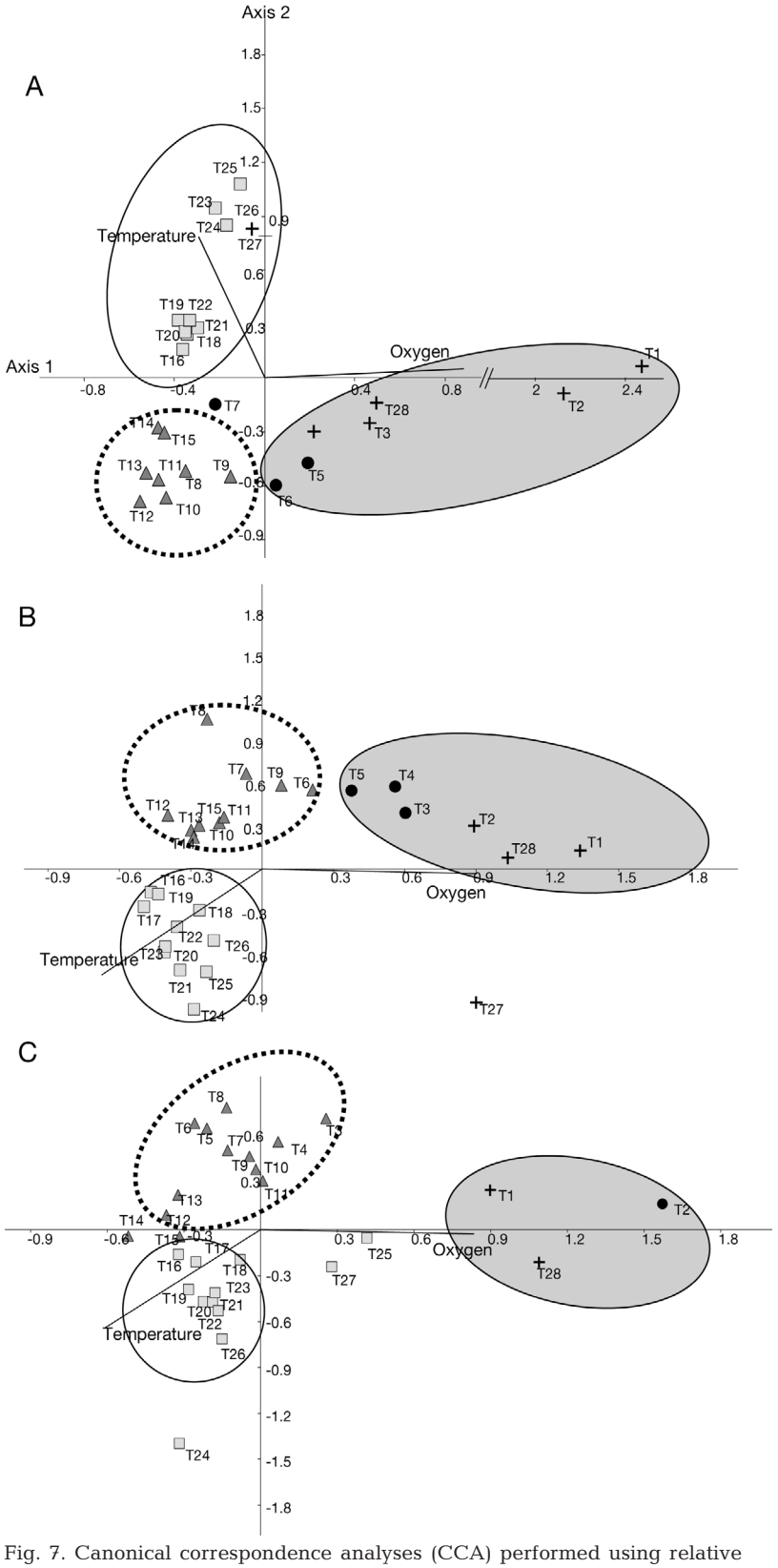

Fig. 7. Canonical correspondence analyses (CCA) performed using relative peak height of TRFs at (A) 10 m, (B) 12 m and (C) 14 m. Communities of the (+) oxic, (●) hypoxic, (▲) anoxic 'until' maximum stratification and (■) anoxic 'after' maximum stratification periods

along a persistent vertical O_2 gradient and in a permanent anoxic layer (e.g. Vetriani et al. 2003, Lüdemann et al. 2000, Lehours et al. 2005). However, to our knowledge, few studies have investigated the influence of anoxia on bacterial community structure and dynamics in the hypolimnion of a temporally stratified aquatic system. The dataset of the present study included 96 samples which were analyzed independently with 2 profiling procedures (T-RFLP and TTGE). Although these fingerprint techniques do not provide information on the functioning of bacterial communities, they are well suited to the investigation of changes in community structure over space and time. The sampling effort, performed weekly at 3 depths in the hypolimnion (10, 12 and 14 m), allowed us to investigate changes in bacterial structure following a time-lag in both the establishment of anoxic and re-oxidized conditions (Table 2, Fig. 1A).

Methodological aspects and constraints

The application of molecular methods for the description of the complexity of natural prokaryotic communities is hampered by methodological constraints; for example, more than 90% of bacteria have not yet been cultivated (Pace 1997). Whereas detailed phylogenetic information on the dominant members of microbial communities can be generated by sequencing cloned PCR products, the effort and cost involved hinders the analysis of multiple samples (Osborn et al. 2000). Rapid profiling procedures have gained in popularity when addressing questions related to the diversity, structural composition and dynamics of microbial communities (e.g. Muyzer et al. 1993, Lee et al. 1996). These methods present all the limitations common to molecular tools, including extraction of nucleic acids, biases and artifacts associated with enzymatic amplification of the nucleic acids, which have been well described in numerous publications (e.g. Head et al. 1998). We also assume that both TTGE and T-RFLP present their own pitfalls which can bias the estimation of bacterial diversity. For example, the number of TRFs observed

can be biased by the formation of pseudo-TRFs (Egert & Friedrich 2003) and by fragments of the same size originating from different taxa (Blackwood et al. 2007). The co-migrating events that occur in TTGE analysis may also lead to an underestimation of bacterial diversity (Muyzer & Smalla 1998). Additionally, molecular profiling methods such as TTGE and T-RFLP normally characterize only dominant organisms (e.g. >1% of the community) due to their detection limits (Blackwood et al. 2007). Hence, rare species, which often make up the vast majority of the diversity in microbial communities, are usually not detected (Pedrós-Alió 2006).

Considering that each enzyme presents a different level of success (fidelity) in detecting sequence variants from communities with varying richness, we selected MspI for the restriction digests, which has been shown to have increased resolving capacities (Engebretson & Moyer 2003). A previous study also revealed that consistent richness and diversity dynamics were observed in an anoxic layer with 3 independent restriction digests (MspI, HhaI and RsaI), but that MspI was the top performer in terms of the ability to identify the greatest number of TRFs (Lehours et al. 2005). The inclusion of an internal size standard in each sample in T-RFLP should provide the necessary degree of reproducibility (Osborn et al. 2000) and thus enable integrative comparison of all samples (represented by a CA, Fig. 6). The semi-quantitative T-RFLP analysis procedure again contains several sources of potential bias, but does enable a more objective comparison of fingerprint patterns than TTGE because of the automated quantification of the relative abundance of individual TRFs (Lüdemann et al. 2000). To give more relevance to the present study, the bacterial communities were also investigated by a second profiling procedure (TTGE). Both methods led to similar observations about the changes in bacterial community such as revealed by the significant correlations between TTGE and T-RFLP similarity matrices. T-RFLP and TTGE clustering (TTGE clusters not shown) were consistent, despite the fact that different primers (Table 1) were used that targeted different (but likely overlapping) portions of the 16S rRNA gene. Results suggest that shifts and patterns observed were mainly the result of changes in bacterial community structure in the hypolimnion of Lake Aydat and not of artifacts associated with the methods used.

Evenness and richness of bacterial communities

Following the conclusions of Blackwood et al. (2007), the Smith & Wilson evenness index (E_{var}) was used in the present study as a measure of diversity. At 12 and 14 m, E_{var} tended to increase from aerobic to anaerobic conditions, suggesting that the diversity of anaerobic communities is at least comparable to (if not higher than) that of aerobic communities; thus, changes in bacterial community composition were not the result of a strong decrease in bacterial evenness. While the number of TRFs (richness, S) has not been previously found to accurately predict the number of taxa in microbial communities (Loisel et al. 2006, Blackwood et al. 2007), S-values are of interest because significant correlations between S and E_{var} at the 3 depths were noted. An increase of mean (± SD) S with depth (10 m: 85 ± 14; 12 m: 103 ± 15; 14 m: 106 ± 17) was noted, suggesting that bacterial richness increased with depth in this freshwater anoxic zone as previously observed (Lehours et al. 2005).

Temporal changes in community structure

Changing environmental factors, localized gradients, stochastic processes, spatial separation and other factors could all result in random or loosely coordinated populations (Zhou et al. 2002, Martiny et al. 2003). However, during the thermal stratification of Lake Aydat, gradual successional patterns were observed at the 3 sampled depths (Figs. 2C, 3C, 4C & 6).

Shift from oxic to anoxic conditions

At 10 and 12 m, the initial consumption of O_2 by aerobic bacteria led to a separation of aerobic from anaerobic processes, and as a result, to a temporal shift in the microbial community composition such as previously observed (Risatti et al. 1994, Brune et al. 2000). Changes in both TTGE and T-RFLP community patterns directly corresponded to the depletion of O_2 (Figs. 2C & 3C). This close correlation provides strong evidence that the presence or absence of O_2 was a major factor in determining the changes in the bacterial community structure; this was also noted by Lüdemann et al. (2000) along a vertical O_2 gradient in flooded paddy soil cores. The impact of O_2 depletion on bacterial community composition was also confirmed by CCAs, which showed that oxic and anoxic communities were clearly determined by O_2 at 10 and 12 m and, to a lesser extent, at 14 m (Fig. 7).

Anoxic period

During anoxia, succession of bacterial communities from both T-RFLP and TTGE patterns continued and followed an orderly progression (Figs. 2C, 3C, 4C & 5). At 10 and 12 m during the anoxic period, the bacterial community became increasingly different from the

oxic community over the following 15 wk of sampling (Figs. 2C & 3C); this change corresponded to the date of maximum stratification (T15, Fig. 1). Crump et al. (2007) have also noted that the time available for the bacterial community composition to change depends on the amount of time that the water is anoxic. Bacterial communities within clusters 'until' (C3 at 10 m and C2 at 12 m) and 'after' (C4 at 10 m and C3 at 12 m) maximum stratification might be defined as 'early' and 'mature' anaerobic communities, respectively (Figs. 2C & 3C). These groupings probably revealed the ability of organisms to respond to disturbance (e.g. opportunists and maintenance-type organisms; Sigler & Zeyer 2004). However, we have to consider that many environmental factors may also contribute to these changes; for example, Crump et al. (2007) observed that a dramatic shift in bacterioplankton community composition in a stratified estuary occurred when sulfide concentrations were very high.

At 14 m in the present study, a special pattern was observed: after the onset of anoxia, community succession led to a mixture of aerobic and anaerobic populations (cluster C1, Fig. 4C), suggesting that some populations were able to live in both conditions (facultative anaerobic bacteria) or were maintained in anoxic micro-niches during the oxidized period. At 14 m depth (just above the sediment), low O_2 concentrations are frequently present, such as observed from T1 to T2 ($[O_2] < 4$ mg l^{-1}, Fig. 4A), raising the possibility that more communities were pre-adapted to anoxia. This may explain why aerobic communities clustered with those present at the onset of anoxia (T1 to T9). Similarities between bacterial communities from T25 to T28 (Figs. 4C & 6) and those from T1 to T5 also suggest that communities at 14 m maintained their pioneer characteristics. This is consistent with Finlay et al. (1997), who suggested that microbial species that appeared during a period of stratification returned to the sediment, where they presumably remain viable. Therefore, in Lake Aydat, the sediment constitutes a reservoir of anaerobic bacteria which could quickly colonize the anoxic niches at 14 m once the environmental conditions become favorable.

Renewal of O_2

In mid-October, O_2 concentrations started to increase in the hypolimnion (Fig. 1A) and a shift in the bacterial community at 10 m was observed (clusters C4 and G4, Figs. 2C & 5A). At 12 and 14 m, communities of the re-oxidized conditions were similar to those of the aerobic period, as noted in the correspondence and clustering analyses (Figs. 3C, 4C & 6). These results agree with the hypothesis that bacterial communities in the deepest water layers were adapted to these periodic changes in environmental conditions, and that low abundances of rare species were probably maintained throughout the stratification period.

A sensitivity gradient to O_2 depletion?

Some observations suggest that bacterial communities in the hypolimnion of Lake Aydat exhibited different sensitivities to the shift in O_2 concentrations: communities of the upper water layer (10 m) appeared to be more affected than those of the deeper water layers. For example, we noted that (1) bacterial communities of the aerobic periods 'before' and 'after' maximum stratification at 14 m, and to a lesser extent at 12 m, exhibited similarities (Figs. 3C & 4C); (2) changes in both TTGE and T-RFLP community patterns at 10 and 12 m directly corresponded to the depletion of O_2; (3) a mixture of aerobic and anaerobic populations were observed after the onset of anoxia at 14 m (cluster C1, Fig. 4C); and (4) CCA results revealed that O_2 concentrations explained more variability in the community at 10 m than at 12 and 14 m (Fig. 7). Communities at 12 m may appear as intermediate communities: they were similar to those at 14 m (Fig. 6), but exhibited responses to O_2 depletion similar to communities at 10 m. From these observations, we hypothesize that a 'sensitivity gradient' to the shift in environmental conditions (O_2 depletion or re-oxidation) may exist between communities of the upper and deeper layers of the hypolimnion. This difference in community responses may be the result of native communities composed of persistent and well-adapted populations in the deepest water layers that may ensure the resilience of the systems after the periodic shift from oxic to anoxic conditions (or vice versa).

CONCLUSION

Succession of the bacterial community in the hypolimnion of Lake Aydat followed orderly and gradual patterns with 'cyclic' trajectories at 12 and 14 m (Figs. 3C, 4C & 6). The hypolimnion of Lake Aydat is a dynamic system, periodically anoxic and subsequently refreshed with O_2. Bacterioplankton communities in this hypolimnion are probably in a constant state of succession, shifting respiratory processes and phylogenetic composition as chemical conditions change over time, such as postulated by Crump et al. (2007). In addition, bacterioplankton communities in several systems have been shown to reassemble year after year (Crump & Hobbie 2005, Fuhrman et al. 2006, Kan et al. 2006) suggesting that functional redundancy may be limited (Crump et al. 2007).

Acknowledgements. We are greatful to S. Specel (Biogema Institute, Clermont-Ferrand), who supplied capillary electrophoresis for T-RFLP analysis. Many thanks also to J. C. Romagoux for his skilled technical assistance and to J. F. Humbert for his advice.

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Editorial responsibility: Karel Šimek, Ceske Budejovice, Czech Republic

Submitted: May 19, 2008; Accepted: October 16, 2008
Proofs received from author(s): January 20, 2009