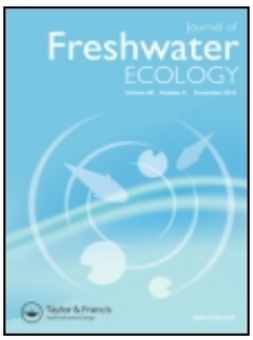

Journal of Freshwater Ecology

ISSN: 0270-5060 (Print) 2156-6941 (Online) Journal homepage: http://www.tandfonline.com/loi/tjfe20

The effect of depth on microbial communities from mesotrophic lake sediments as measured by two DNA extraction methods

Wei Xiong, Ping Xie, Shengrui Wang & Yuan Niu

To cite this article: Wei Xiong, Ping Xie, Shengrui Wang & Yuan Niu (2015) The effect of depth on microbial communities from mesotrophic lake sediments as measured by two DNA extraction methods, Journal of Freshwater Ecology, 30:2, 215-228, DOI: 10.1080/02705060.2014.915587

To link to this article: http://dx.doi.org/10.1080/02705060.2014.915587

Journal of Freshwater Ecology, 2015
Vol. 30, No. 2, 215−228, http://dx.doi.org/10.1080/02705060.2014.915587

The effect of depth on microbial communities from mesotrophic lake sediments as measured by two DNA extraction methods

Wei Xiong[a], Ping Xie[b], Shengrui Wang[c]* and Yuan Niu[c]

[a]*College of Fisheries, Huazhong Agricultural University, Wuhan 430070, P.R. China;* [b]*Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, P.R. China;* [c]*State Environmental Protection Key Laboratory for Lake Pollution Control, Research Center of Lake Eco-environment, Chinese Research Academy of Environmental Sciences, Beijing 100012, P.R. China*

(*Received 17 January 2014; accepted 10 April 2014*)

We evaluated the effect of depth on microbial communities from mesotrophic lake sediments as measured by two DNA extraction methods. DNA yields and the number of ribotypes differed significantly by depth. The mean yields at the depth interval of 0−8 cm from the two extraction methods were both higher than those within the depth range of 10−44 cm. Significant differences were also observed between the two methods in DNA yields from the deeper (10−44 cm) samples. An analysis of denaturing gradient gel electrophoresis (DGGE) profiles demonstrated that the choice of the DNA extraction method has a profound effect on the bacterial community profiles generated, which was reflected in the number of bands or ribotypes detected from samples from all depths. Higher DNA yield did not lead to a higher diversity of phylotypes. In almost all cases, extraction method 2 (M2, soil DNA test kit) resulted in a greater diversity of phylotypes compared to method 1 (M1, liquid nitrogen/cetyl trimethylammonium bromide extraction). Moreover, notable changes in bacterial diversity were detected in the uppermost layers (0−5 cm). The results of canonical correspondence analysis illustrated that the DNA extraction method did not affect the evaluation of the relationship between bacterial community structure and environmental variables in lake sediments at multiple depths, and the differences in community structure in the two extraction methods were both related to the same environmental variables (pH, total phosphorus concentration) and depth. Vertically, the community structure of sediment layers formed several separate clusters along the depth gradient. This study expands our understanding of the depth-related microbial community structure in lake sediments.

Keywords: lake sediment; DNA extraction; bacterial community structure; denaturing gradient gel electrophoresis; organic matter content; bacterial diversity

Introduction

Sediment bacteria play a significant ecological and biogeochemical role in lake ecosystems largely due to their high abundance relative to the overlying water column and their important role in mediating and regulating the transformation and speciation of major bioactive elements in these environments (Polymenakou et al. 2005). Sediment bacteria are also key players in the degradation of organic pollutants (Alexander 1994; Kieft et al. 1997; Boschker et al. 2001) and represent a major reservoir of genetic variability with a local diversity equal to that of soil systems (Torsvik et al. 2002). Despite

*Corresponding author. Email: shengruiwang@sohu.com

their importance, our knowledge of the bacteria that inhabit sediments is very limited and is based primarily on highly selective culture studies (Suzuki and DeLong 2002; Bowman et al. 2003). It is thought that less than 1% of microorganisms are culturable using conventional techniques (Amann et al. 1995; Smit et al. 2001; Schloss and Handelsman 2003), limiting our knowledge of microbial ecology. However, the application of nucleic acid based methodologies (e.g. PCR-DGGE, CARD-FISH, T-RFLP, etc.) has provided a means to overcome this limitation, allowing for the monitoring of organisms or particular genes directly from environmental samples (Olsen et al. 1986; Amann et al. 1995).

In undisturbed aquatic sediments, substrates and electron acceptors that are essential for microorganisms are gradually scavenged to greater or lesser degrees along the depth gradient. This depth-related gradient of biogeochemical properties provides niches for metabolically diverse microorganisms (Koizumi et al. 2003). Although there have been many published studies describing methods for the extraction of DNA from environmental samples, such as soil, marine sediment, and compost (Tsai and Olson 1991; Carrigg et al. 2007; Kallmeyer and Smith 2009), few studies have focused on the combined effects of depth and DNA extraction method on microbial community profiles from lake sediments.

In this study, we performed denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S rRNA genes to analyze the depth-related bacterial community profiles generated by different DNA extraction methods. Indeed, many previous studies have relied on similar techniques to evaluate DNA extraction protocols for use with environmental samples (e.g. Krsek and Wellington 1999; Griffiths et al. 2000; Robe et al. 2003). DGGE can be used to separate small variable regions of the 16S rRNA gene based on nucleotide differences (Muyzer et al. 1993). It is therefore possible to discriminate between different organisms or ribotypes within a DGGE profile of the whole community. Although this technique has several drawbacks and is only semi-quantitative at best, it remains the most efficient technique for the measurement of relative differences or temporal changes in microbial community structure in environmental samples (Carrigg et al. 2007). Additionally, the bacterial community structure can be revealed by the mobility of DGGE bands; that is, from the DGGE banding patterns, the identities of individual PCR amplicons (bands in the gel) can be resolved by sequencing and phylogenetic analysis (Koizumi et al. 2003). Thus, DGGE is an appropriate method for monitoring the successive changes in bacterial community structure along the depth gradient and for analyzing whether depth affects bacterial community profiles.

Nucleic acid based methodologies require nucleic acid extracts that are sufficiently free from inhibitory compounds but co-extraction of humic substances and other compounds inhibits downstream applications, including quantification or PCR (Zipper et al. 2003). Usage of commercial DNA extraction kits designed for use with soils/sediments is becoming more common. However, many kits produce significantly lower DNA yields (Martin-Laurent et al. 2001). Other approaches for extraction of DNA are laborious and time-consuming and not suited for processing large numbers of samples.

In the present study, two DNA extraction methods were selected for evaluating the effect of depth on microbial community structure in mesotrophic lake sediments. Each method was tested with respect to (1) DNA yield, (2) PCR amplification of isolated DNA, (3) community profiles generated by DGGE fingerprinting, and (4) relationship between environmental variables and the bacterial community structure obtained based on DGGE profiles.

Methods

Sampling

Lake Erhai is a mesotrophic lake located in Dali, Yunnan province, and is the largest fault lake in southwest China (surface area = 249.8 km^2, mean depth = 10.5 m). Three sediment cores were collected with a core sampler (HL-CN, Hengling Technology Ltd. Corp., China) at a water depth of 21 m (25° 48' 47.10" N, 100° 9' 57.01" E) in July of 2010. The cores were sectioned in 2-cm intervals between depths of 2 cm and 12 cm, in 4-cm intervals between depths of 12 and 20 cm, and in 6-cm intervals between depths of 20 and 44 cm. The corresponding sections of each of the three cores were pooled. The core samples were transported in a sealed container to the laboratory in the dark at 4 °C within 12 h for further treatment.

Chemical analysis

The pH and the oxidation−reduction potential (Eh) of sediment core sections were both measured with an ORP meter (pH/ion meter 225, Iwaki Glass, Tokyo, Japan) on location. Total nitrogen concentrations (TN), total phosphorus concentrations (TP), and total organic carbon content (TOC) were measured according to Jin and Tu (1990) after the sediment samples were freeze dried (Labconco, Cole-Parmer Instrument Co., USA).

DNA extraction

Two methods were used for the extraction of DNA from sediments. Method 1 (M1) was modified from Delong et al. (1993). Liquid nitrogen (approximately 10 mL) was mixed with 500 mg of each depth sample (wet weight) in a mortar, ground, and transferred to a micro-centrifuge tube. One milliliter of cetyl trimethylammonium bromide (CTAB) extraction buffer (Griffiths et al. 2000) was added, followed by vortexing for 30 s. After the addition of 500 μL of lysis buffer (50 μM Tris−HCl, pH 8; 40 μM ethylene diamine tetraacetic acid, pH 8; 750 μM filter-sterilized sucrose) and 20 μL of lysozyme solution (10 mg/mL; Sigma-Aldrich, Germany), mixtures were briefly vortexed (30 s) and incubated at 37 °C for 30 min. Sodium dodecyl sulfate was added to a final concentration of 2%, then the samples were again vortexed and incubated at 70 °C for 1 h. Next, 6 μL of proteinase K (Sigma-Aldrich) was added. Samples were then vortexed and incubated at 50 °C for a further 30 min followed by centrifugation for 15 min (10,000 × g). The supernatants were transferred to fresh micro-centrifuge tubes, and the aqueous phase was extracted by mixing an equal volume of chloroform−isoamyl alcohol (24:1) followed by centrifugation (10,000 × g) for 10 min. Total nucleic acids were then precipitated from the extracted aqueous layer with 0.6 vol. of isopropanol overnight, at room temperature, followed by centrifugation (10,000 × g) for 15 min. The pelleted nucleic acids were washed in 70% (v/v) ice-cold ethanol and air dried before re-suspension in 50 μL diethylpyrocarbonate (DEPC) treated water.

Method 2 (M2) employed the E.Z.N.A.® soil DNA kit (Omega Bio-Tek, USA). DNA was extracted from 500 mg of sediment from each depth following the manufacturer's protocol. To inspect the quality of extracted DNA, 5-μL portions from three replicate extractions were pooled, digested with RNAase, and electrophoresed on a 0.8% (w/v) agarose gel, with λ-DNA/HindIII molecular size marker (Promega, USA). Gel images were captured using a UV transillumination table and the AlphaDigiDoc 1201 system (Alpha Innotech, USA).

Quantification of DNA yield

The quantity of extracted DNA was estimated using the PicoGreen® dsDNA quantitation kit (Molecular Probes, USA; Sandaa et al. 1998). Briefly, samples were diluted 400-fold in 1 × TE (10 mM Tris−HCl, 1 mM EDTA, pH 8.0) and 100 μL of these dilutions was added to 100 μL of a 200-fold dilution of PicoGreen dye and allowed to incubate for 5 min in the dark at room temperature. Fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. TE buffer was used as the blank sample, and DNA standards were generated using bacteriophage λ DNA stocks.

Nucleic acid purification

Crude DNA extracts were purified to facilitate successful PCR amplification. DNA samples extracted with M1 were purified using 2% (w/v) low-melting-point agarose and the GELase™ Agarose Gel-Digesting Preparation (Epicentre Biotechnologies, USA) according to the manufacturer's instructions. DNA samples isolated with M2 were diluted by 1/10th using sterile deionized water.

PCR amplification

The extracted DNA was used as a template to amplify the 16S rRNA gene fragment with the forward primer 341f (5'-CCTACGGGAGGCAGCAG-3') with a GC clamp (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGC-3'); 907r (5'-CCGTCAATTCCTTTGAGTTT-3') was used as the reverse primer (Teske et al. 1996). These primers were capable of amplifying most bacteria (excluding Archaea) from the sediment samples. PCR mixtures (50 μL) contained 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 mM each of dNTP, 0.2 mM of each primer, 2.5 U of Taq DNA polymerase (Takara, Shiga, Japan), and 20 ng of template DNA. A 5-min initial denaturation at 94 °C was followed by a thermal cycling program as follows: 20 cycles of denaturation (1 min at 94 °C), annealing (1 min at an initial temperature of 65 °C, decreasing 0.5 °C every cycle to a final temperature of 55 °C), and extension (3 min at 72 °C); and 10 cycles of denaturation (1 min at 94 °C), annealing (1 min at 55 °C), and extension (3 min at 72 °C), followed by a final 8-min extension at 72 °C. A negative control, in which the template was replaced by an equivalent volume of sterile deionized water, was included. PCR products were confirmed by 1.5% agarose gel electrophoresis.

Denaturating gradient gel electrophoresis (DGGE)

PCR products were loaded on to a 6% (w/v) polyacrylamide gel (37.5:1 acrylamide:bisacrylamide) with a denaturing gradient that ranged from 45% to 60%, where 100% denaturant is defined as 7 M urea and 40% deionized formamide. DGGE was performed with a Dcode system (Bio-Rad Laboratories, USA) using 1 × TAE running buffer (20 mM Tris, 10 mM acetic acid, 0.5 mM EDTA, pH 8.0) at 60 °C for 11 h at 100 V. The gel was stained with the GelRed (Biotium, USA) nucleic acid staining solution (diluted 1:10,000) for 30 min and photographed using a bioimaging system (Syngene, Maryland, USA) under UV light.

Cluster analysis of DGGE profiles and statistical analysis

DGGE profiles and statistics were analyzed according to Niu et al. (2011). Briefly, cluster analysis of DGGE profiles was performed with the NTSYS program version 2.10e (Exeter

Software, Setauket, NY, USA). Gel-Pro Analyzer (version 4.5) was used to analyze the gel images. A densitometric curve was calculated for each lane and the relative intensities of all bands were obtained. The Shannon−Weiner index (H') was calculated to estimate changes in bacteria community composition using the formula

$$H' = -\sum_{i=1}^{n}(p_i^* \ln p_i),$$

where p_i is the relative intensity of each band and n is the total number of bands in each lane. Canonical correspondence analysis (CCA) was carried out using CANOCO 4.5 software. Statistical analysis of the physical-chemical parameters was conducted using SPSS 17.0 software, and one-factor analysis of variance (ANOVA) was used to calculate the effects the extraction method.

Results

Sediment analysis

The physicochemical properties (pH, Eh, TN, TP, TOC) of the samples are shown in Figure 1. An increase in the concentrations of TN and TOC was observed from the 8−10 cm depth upward to the surface sediment sample at all sampling sites with the highest concentrations of TN and TOC found at the 0−2 cm depth range. The TN concentration ranged from 2.4 mg/g (44 cm) to 4.2 mg/g (2 cm), the TP concentration ranged from 0.8 mg/g (44 cm) to 1.13 mg/g (2 cm), and the TOC ranged from 1.6 mg/g (44 cm) to 4.6 mg/g (2 cm). In addition, the lower layers of sediment were more acidic than the upper layers.

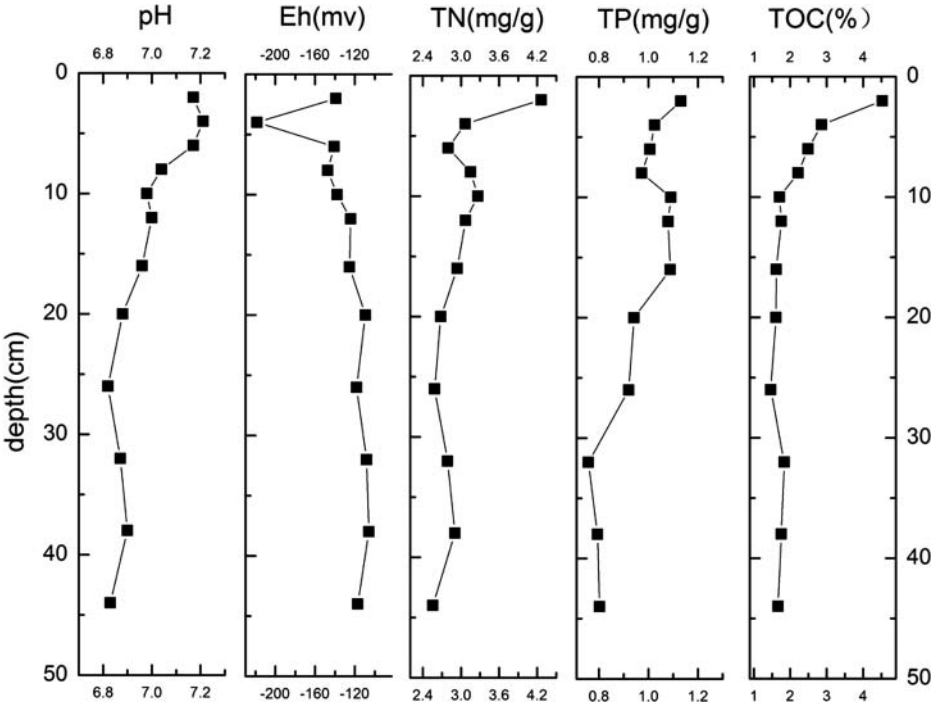

Figure 1. Vertical profiles of pH, Eh, TN, TP, and TOC of the sediment samples.

DNA yield and fragmentation

DNA was successfully extracted from samples at different depths using the two methods and this DNA was visible on agarose gels (Figure 2). In almost all cases in M1, the size of the DNA was at least 23 kb, whereas in M2 the DNA was sheared into smaller fragments. The crude extracts of the sediment samples from M1 were contaminated with humic acids (dark-brown appearance). Conversely, very little co-extracted humic substance was observed when the DNA was extracted from the samples using M2.

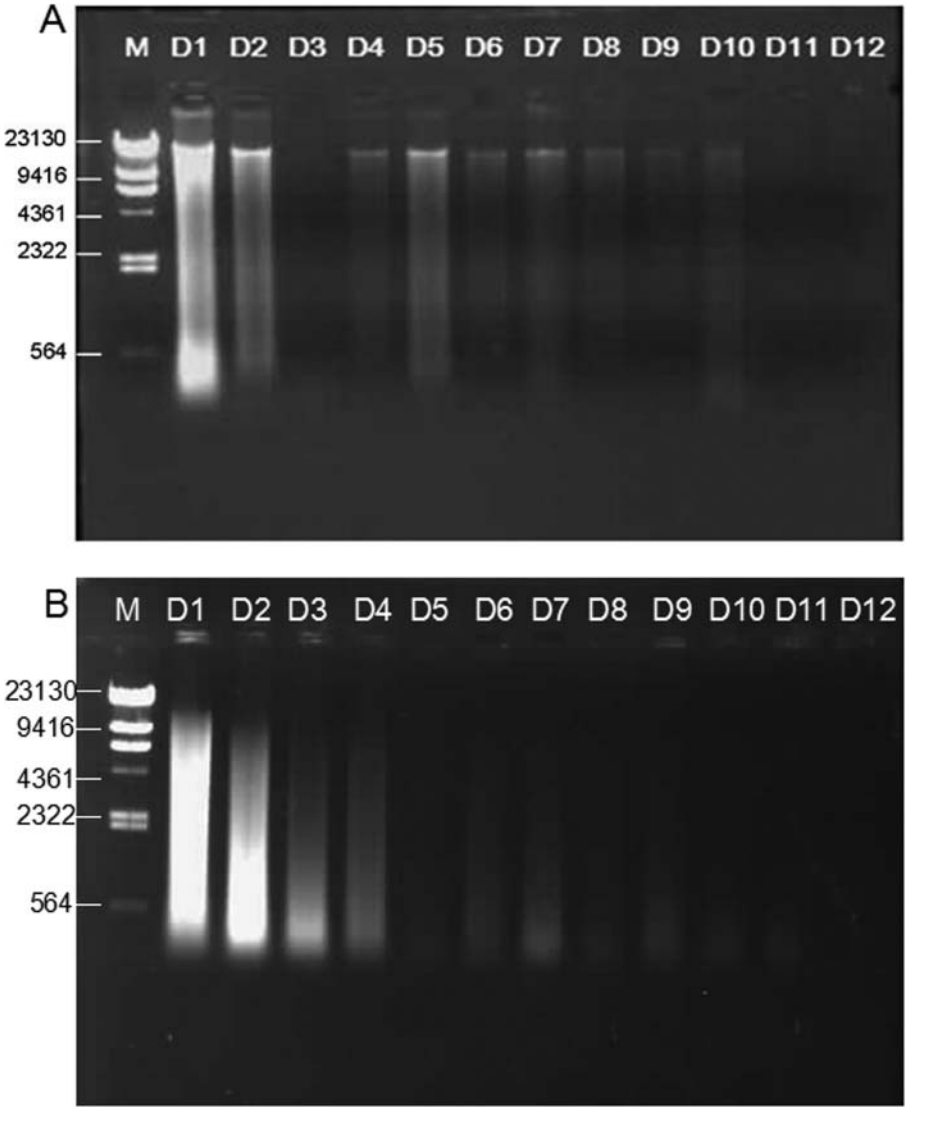

Figure 2. Ethidium bromide stained 0.8% agarose gel displaying genomic DNA run with λ DNA cut with HindIII. (A): M1; (B): M2. D1, 0−2 cm; D2, 2−4 cm; D3, 4−6 cm; D4, 6−8 cm; D5, 8−10 cm; D6, 10−12 cm; D7, 12−16 cm; D8, 16−20 cm; D9, 20−26 cm; D10, 26−32 cm; D11, 32−38 cm; D12, 38−44 cm.

DNA yields were determined before and after DNA purification using the PicoGreen assay (Sandaa et al. 1998). The two methods were very similar with regard to crude DNA yield (Table 1); mean yields of 3.3 and 3.6 μg/g were achieved with M1 and M2, respectively. However, the mean yields at the depth interval of 0−8 cm (M1 = 7.1 μg/g; M2 = 10.4 μg/g) were higher than those within the depth range of 10−44 cm (M1 = 2.5 μg/g; M2 = 2.3 μg/g).

When one-factor ANOVA was carried out on the DNA yields, significant differences were observed among depths ($p < 0.05$); however, significant differences were also observed between the two methods with respect to DNA yields from the deeper (10−44 cm) samples ($p < 0.05$). Also, there was a significant correlation between the crude DNA yield and TOC content (Pearson, $p < 0.01$). Although generally suitable for

Table 1. Crude and final DNA yields and band numbers (±1 standard deviation [SD]) with respect to each DNA extraction method tested.

Sediment depth	Extraction method	Crude DNA yield (μg/g)[a] (±SD)	Final DNA yield (μg/g)[a] (±SD)	Crude DNA recovery (mean %)	Number of bands
2 cm	M1	9.9 (±2.5)	4.8 (±1.1)	49	16.0 (±1.0)
4 cm	M1	4.4 (±1.2)	2.4 (±0.8)	56	15.3 (±1.2)
6 cm	M1	1.3 (±0.4)	1.0 (±0.3)	69	6.7 (±0.6)
8 cm	M1	2.4 (±0.5)	1.1 (±0.4)	47	11.0 (±1.0)
10 cm	M1	3.8 (±0.9)	1.8 (±0.4)	46	14.7 (±1.2)
12 cm	M1	1.9 (±0.4)	1.2 (±0.5)	63	13.3 (±0.6)
16 cm	M1	2.4 (±0.3)	1.3 (±0.4)	56	12.7 (±0.6)
20 cm	M1	2.5 (±1.0)	1.4 (±0.4)	59	7.7 (±1.2)
26 cm	M1	3.1 (±0.8)	1.9 (±0.7)	63	10.7 (±0.6)
32 cm	M1	3.6 (±1.4)	1.5 (±0.9)	50	2.7 (±1.2)
38 cm	M1	2.3 (±0.8)	1.3 (±1.0)	52	6.3 (±1.5)
44 cm	M1	2 (±1.2)	1.3 (±0.6)	66	7.0 (±1.0)
2 cm	M2	10.3 (±1.7)	5.2 (±1.3)	n/a	27.3 (±1.5)
4 cm	M2	10.5 (±1.5)	5.7 (±1.1)	n/a	27.0 (±1.0)
6 cm	M2	5.3 (±0.7)	3.4 (±0.2)	n/a	26.3 (±1.5)
8 cm	M2	4.3 (±0.9)	2.4 (±0.3)	n/a	34.0 (±1.7)
10 cm	M2	0.8 (±0.4)	0.5 (±0.2)	n/a	34.3 (±0.6)
12 cm	M2	1.1 (±0.5)	0.6 (±0.3)	n/a	34.7 (±1.2)
16 cm	M2	2.7 (±1.0)	1.3 (±0.5)	n/a	34.0 (±1.0)
20 cm	M2	1.0 (±0.4)	0.6 (±0.2)	n/a	34.0 (±0.0)
26 cm	M2	2.1 (±0.4)	1.2 (±0.3)	n/a	32.3 (±0.6)
32 cm	M2	1.8 (±0.4)	1.0 (±0.2)	n/a	22.0 (±0.0)
38 cm	M2	2.4 (±0.3)	1.2 (±0.1)	n/a	22.7 (±1.5)
44 cm	M2	0.9 (±0.3)	0.4 (±0.06)	n/a	6.7 (±0.6)
Marine	1	1.1 (±0.1)	0.7 (±0.1)	n/a	10.0 (±3.0)
Sediment[b] (0−1 cm)	Ultraclean™	0.1 (±0.02)	0.1 (±0.02)	n/a	8.3 (±1.5)
Stream sediment[c]	Three methods	0.35−1.74	n/a	n/a	n/a

[a]Wet weight.
[b]Carrigg et al. (2007)
[c]Leff et al. (1995)

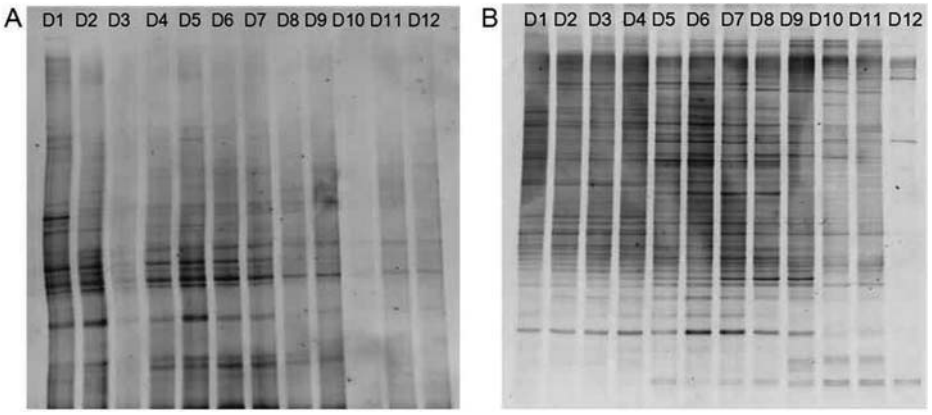

Figure 3. DGGE profiles (negative image) of 16S rRNA gene fragments. (A): M1; (B): M2. D1, 0−2 cm; D2, 2−4 cm; D3, 4-6 cm; D4, 6-8 cm; D5, 8−10 cm; D6, 10−12 cm; D7, 12−16 cm; D8, 16-20 cm; D9, 20−26 cm; D10, 26-32 cm; D11, 32−38 cm; D12, 38−44 cm.

direct PCR, the crude extracts obtained with M2 were further purified by 1/10th dilution in DEPC-treated water and the crude extracts obtained with M1 were purified using GELase enzyme digestion. The gel digestion approach successfully removed any contaminating substances and brown color from the crude DNA extracts.

Community structure

Visible changes were noted in the relative brightness and position of the DGGE banding patterns of the 16S rRNA gene fragments in various segments (Figure 3). An analysis of the DGGE profiles generated from M1 and M2 extracts demonstrated that the choice of the DNA extraction method significantly influenced the bacterial community profiles generated which was reflected in the number of bands or ribotypes detected in each sample (Figure 3; Table 1). For example, one-factor ANOVA revealed significant differences in the apparent number of ribotypes present in the DGGE profiles of samples from all depths ($p < 0.001$; Table 1). All sediment samples extracted by M2 contained more bands than those extracted by M1.

The cluster analysis (UPGMA) dendrograms of bacterial community composition (BCC) revealed remarkable spatial differences (Figure 4). The bacterial communities in

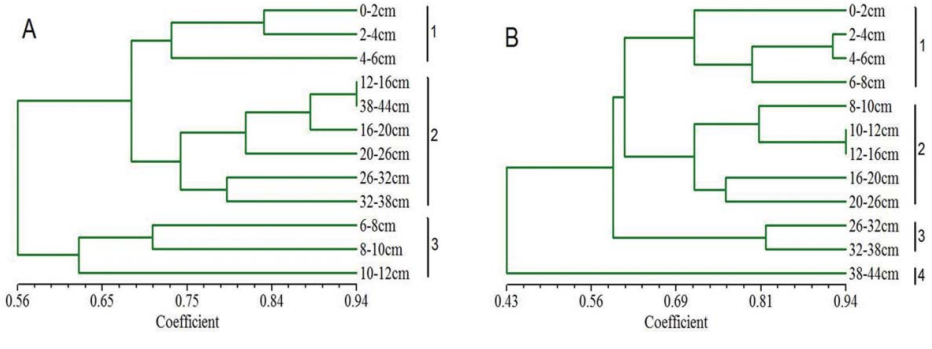

Figure 4. Cluster analysis of BCC based on DGGE profiles. (A): M1; (B): M2.

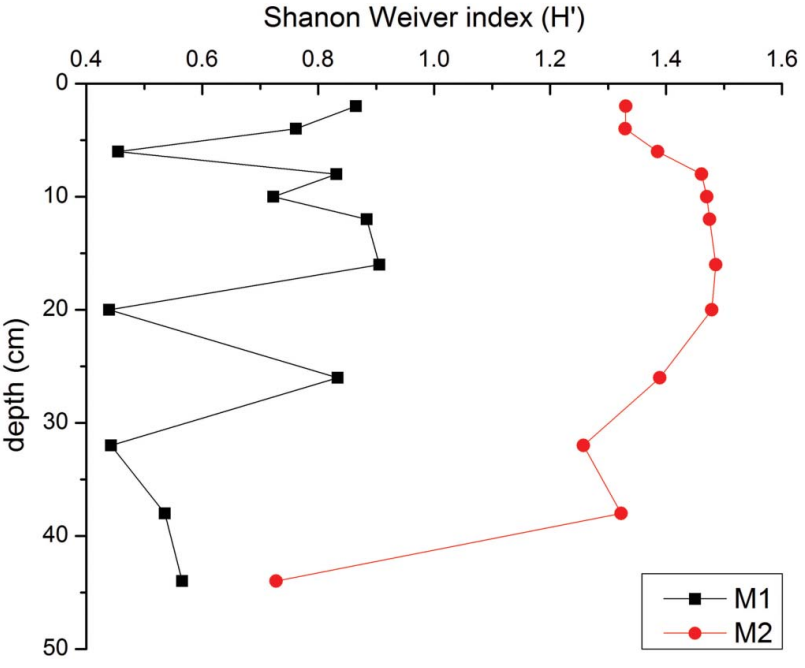

Figure 5. Vertical changes in H' of bacteria.

various layers extracted by M1 (Figure 4(A)) were grouped into three defined clusters, and samples extracted by M2 formed four separate clusters (Figure 4(B)).

The spatial variations in H' of the bacterial community using both methods are shown in Figure 5. A mean H' of 0.69 was achieved with M1, which was much lower than the H' of M2, which was 1.34. When one-factor ANOVA was carried out on the diversity index (H'), significant differences were found between the two methods ($p < 0.001$).

CCA based on DGGE data and environmental variables was carried out separately for the two methods. The results of CCA (Figure 6) suggest that the differences in BCC were related to the three most important environmental variables (pH, TP, and depth; $p < 0.05$). The three variables and the two axes explained 78% and 28% of the observed variation in BCC with M1; the first axis was positively related to depth ($r = 0.97$) and negatively related to TP ($r = -0.89$), and the second axis was positively related to pH ($r = 0.51$). The three variables and the two axes explained 81% and 41% of the observed variation in BCC with M2; the first axis was positively related to depth ($r = 0.98$) and negatively related to TP ($r = -0.93$); the second axis was positively related to pH ($r = 0.63$).

The CCA biplot revealed that samples extracted with M1 formed four clusters, whereas samples extracted with M2 were grouped into three clusters (Figure 6). Samples extracted with M1 from 2 to 6 cm formed one cluster, samples from 6 to 16 cm formed the second cluster, and samples from 16 to 26 cm formed the third cluster, whereas the remaining samples (26−44 cm) formed the fourth cluster. Samples extracted with M2 were grouped into three clusters; cluster 1 consisted of samples from 0 to 8 cm, cluster 2 contained samples from 8 to 26 cm, and cluster 3 was composed of samples from 26 to 44 cm. These results are similar to those obtained via cluster analysis of BCC based on the DGGE profiles (Figure 4(B)).

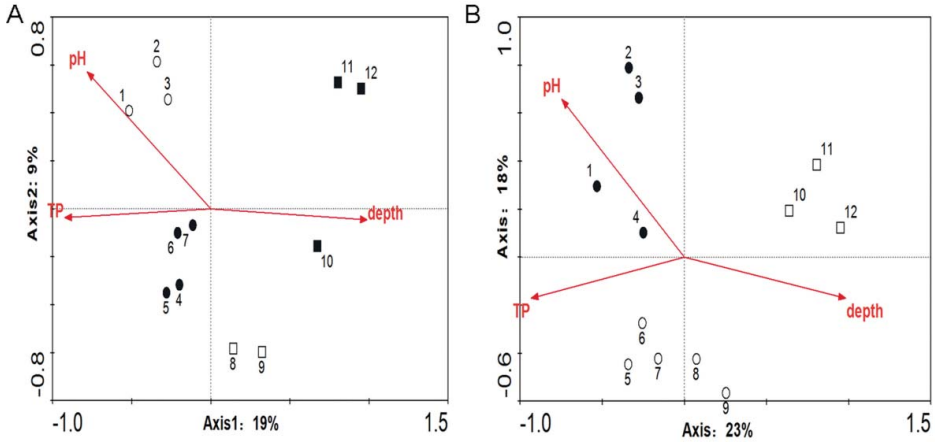

Figure 6. CCA biplot based on DGGE data and environmental variables. (A): M1; (B): M2. D1, 0−2 cm; D2, 2−4 cm; D3, 4−6 cm; D4, 6-8 cm; D5, 8-10 cm; D6, 10−12 cm; D7, 12-16 cm; D8, 16−20 cm; D9, 20−26 cm; D10, 26-32 cm; D11, 32−38 cm; D12, 38−44 cm.

Discussion

Several environmental DNA isolation and purification strategies have previously been investigated with variable rates of success (Cullen and Hirsch 1998; Miller et al. 1999; Tien et al. 1999). In this study, extraction techniques that are compatible with sediments obtained from multiple depths were evaluated based on DNA yield, ability to detect microbial community members by PCR-DGGE, and ability to determine the relationships between the microbial community and environmental variables.

In the present study, the crude DNA yield and organic matter content in samples obtained from 0 to 8 cm depths were higher than those in samples obtained from 10 to 44 cm depths (Table 1; Figure 1). There was a significant positive correlation between the crude DNA yield and organic matter content ($p < 0.01$). These results agree with a previous report that showed a significant correlation between crude DNA yield and soil organic carbon content ($r = 0.73$, $p = 0.01$, Zhou et al. 1996). It has been documented that the efficiency of soil microbial DNA extraction depends on soil quality, particularly on its clay and organic matter contents, because microorganisms can interact with soil colloids, such as clay−organic aggregates (Roose-Amsaleg et al. 2001). Higher cell counts are directly proportional to high levels of organic carbon and nitrogen, clay, and humic acid; high levels of organic carbon and nitrogen also indicate greater microbial activity (Lakay et al. 2007). Moreover, pH, Eh, TN, TOC, and TP varied significantly with depth ($p < 0.05$) and there were also significant positive correlations between TOC and both pH and TN ($p < 0.01$). It is possible that trends in both DNA yield and TOC might be primarily defined by integrative conditions rather than a single factor.

In our study, significant differences were found among depths ($p < 0.05$) with respect to crude DNA yields. Significant differences were also observed between the two methods with respect to DNA yields from the lower sediment layers (10−44 cm); however, no significant difference was noted between the two methods in DNA yields from the upper sediment layers (0−10 cm, Table 1). As mentioned above, crude DNA yields were positively correlated with organic matter content. Furthermore, the organic matter content in the upper sediment layers was higher than that in the lower sediment layers (Figure 1). These results disagree with a previous report indicating that the relationship between

organic matter content and DNA yield was dependent upon the extraction method (LaMontagne et al. 2002). Our results indicate that in the case of sediment with low organic matter content, DNA yields were significantly influenced by the DNA extraction method. We speculate that low levels of organic carbon in the deeper sediment layers are directly proportional to lower cell counts; consequently, deeper sediment layers have low DNA content and are more sensitive to the DNA extraction method.

The two extraction and purification methods yielded 1.0−4.8 μg DNA/g and 0.4−5.7 μg DNA/g of wet sediment (Table 1). A comparison of other DNA yields from sediment samples that have been reported in the literature is shown in Table 1. Although direct comparisons are difficult to make due to the diverse nature of the sediment samples being tested, these data provide several reference points for the current study. The final DNA yields obtained from lake sediments extracted with M1 and M2 were both higher than those from marine (0.7−1.7 μg DNA/g) and stream (0.35−1.74 μg DNA/g) sediments (Table 1). We inferred that the specific type of habitat was the major factor contributing to these discrepancies. The marine environment, from which a sediment sample was taken at a depth of 4800 m, and the stream biotope were not favorable for sedimentation.

It has been documented that humic substances and DNA fluoresce at similar wavelengths (Sandaa et al. 1998; Zipper et al. 2003) and humic acids are thought to sequester ethidium bromide, thereby reducing the amount available for DNA intercalation (Rochelle et al. 1992). These factors may have resulted in artificially high estimates of initial DNA yields based on PicoGreen analysis and exaggerated DNA losses upon purification (Carrigg et al. 2007). Although humic acids are known to interfere with PicoGreen at high concentrations (Bachoon et al. 2001), PicoGreen can be used on dilute samples in which small amounts of DNA are still present and humic acid interference is negligible (Sandaa et al. 1998; Stark et al. 2000). Our results indicated that DNA recovery was little affected by humic acid co-extraction; DNA recovery with M1 was found to be 46%−69% (Table 1), which is within the expected range based on the literature values (Robe et al. 2003). This result is an improvement over that reported by Miller et al. (1999), who achieved a DNA recovery of 29% $\pm$ 17% with a similar gel-based purification method.

An analysis of the DGGE profiles revealed that in almost all cases, the use of M2 resulted in a greater diversity of phylotypes compared with M1, although the same volume of sediment was used in each method (Table 1; Figures 3 and 5). Several studies have evaluated extraction and purification protocols and these studies generally conclude that the total yield of DNA and the observed diversity strongly depend on the techniques used (Martin-Laurent et al. 2001; Luna et al. 2006; Lakay et al. 2007). Our results were inconsistent with previous reports indicating that a higher DNA yield leads to a higher diversity of phylotypes in some cases (e.g. Martin-Laurent et al. 2001; Luna et al. 2006); however, other studies did not identify such a relationship (e.g. Gabor et al. 2003).

According to a previous study in a large shallow lake, bacterial diversity decreased with sediment depth, while the bacterial communities in the upper sediment layers (0−5 cm) were almost homogeneous. However, in our field study, notable changes of bacterial diversity were detected in the uppermost layers. It is possible that the upper sediment layer of the shallow lake undergoes frequent mixing by climate factors, e.g. wind-induced resuspension.

The CCA results generated based on the two DNA extraction methods revealed that differences in BCC were related to the same environmental variables (pH, TP) and depth ($p < 0.05$, Figure 6). A comprehensive evaluation of the DNA extraction methods suggested that M1 and M2 were both suitable for use in studies analyzing the relationships

between bacterial communities and environmental variables of sediments obtained from multiple depths. Vertically, the BCC in each sediment layer formed several separate clusters along the depth gradient, which was attributed to the effect of depth gradients in redox potential and oxygen concentration.

In conclusion, significant differences were observed among depths with respect to crude DNA yields. The mean yields at the depth interval of 0−8 cm based on the two extraction methods were both higher than those within the depth range of 10−44 cm. Significant differences were also observed between the two methods with respect to DNA yields from the deeper (10−44 cm) samples. An analysis of DGGE profiles demonstrated that the choice of the DNA extraction method has a profound effect on the bacterial community profiles generated, which was reflected in the number of bands or ribotypes detected from samples from all depths, and revealed that in almost all cases, the use of M2 resulted in a greater diversity of phylotypes compared with M1. Moreover, notable changes of bacterial diversity were detected in the uppermost layers (0−5 cm). The results of CCA illustrate that the DNA extraction method did not affect the evaluation of the relationship between bacterial community structure and environmental variables in lake sediments at multiple depths, and the differences in BCC based on the two extraction methods were both related to the same environmental variables (pH, TP) and depth. Vertically, the BCC in each sediment layer formed several separate clusters along the depth gradient.

Acknowledgements

We are grateful to Dr Yuanying Shen and Haoxiang Luo for allowing us to use their laboratory, and Wenbin Liu, Bin Hu, Haiping Hu, Tai Zhang and Chaonan Cui for their help with sample collection.

Funding

This study was supported by the National Water Pollution Control and Management Technology Major Projects of China [grant number 2008ZX07105-005].

References

Alexander M. 1994. Biodegradation and bioremediation. San Diego (CA): Academic Press.

Amann RI, Ludwig W, Schleifer KH. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev. 59:143−169.

Bachoon DS, Otero E, Hodson RE. 2001. Effects of humic substances on fluorometric DNA quantification and DNA hybridization. J Microbiol Methods. 47:73−82.

Boschker HTS, Graaf W, Köster M, Meyer-Reil LA, Cappenberg TE. 2001. Bacterial populations and processes involved in acetate and propionate consumption in anoxic brackish sediment. FEMS Microbiol Ecol. 35:97−103.

Bowman JP, McCammon SA, Gibson JAE, Robertson L, Nichols PD. 2003. Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. Appl Environ Microbiol. 69:2448−2462.

Carrigg C, Rice O, Kavanagh S, Collins G, O'Flaherty V. 2007. DNA extraction method affects microbial community profiles from soils and sediment. Appl Microbiol Biotechnol. 77:955−964.

Cullen DW, Hirsch PR. 1998. Simple and rapid method for direct extraction of microbial DNA from soil for PCR. Soil Biol Biochem. 30:983−993.

DeLong EF, Franks DG, Alldredge AL. 1993. Phylogenetic diversity of aggregate-attached vs free-living marine bacterial assemblages. Limnol Oceanogr. 38:924−934.

Gabor EM, de Vries EJ, Janssen DB. 2003. Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. FEMS Microbiol Ecol. 44:153−163.
Griffiths RI, Whitely AS, O'Donnell AG, Bailey MJ. 2000. Rapid method for co-extraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol. 66:5488−5491.
Jin XC, Tu QY. 1990. The standard methods for observation and analysis of lake eutrophication. 2nd ed. Beijing: China Environmental Science Press. (In Chinese).
Kallmeyer J, Smith DC. 2009. An improved electroelution method for separation of DNA from humic substances in marine sediment DNA extracts. FEMS Microbiol Ecol. 69:125−131.
Kieft TL, Wilch E, O'connor K, Ringelberg DB, White DC. 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. Appl Environ Microbiol. 63:1531−1542.
Koizumi Y, Kojima H, Fukui M. 2003. Characterization of depth-related microbial community structure in lake sediment by denaturing gradient gel electrophoresis of amplified 16S rDNA and reversely transcribed 16S rRNA fragments. FEMS Microbiol Ecol. 46:147−157.
Krsek M, Wellington EMH. 1999. Comparison of different methods for the isolation and purification of total community DNA from soil. J Microbiol Methods. 39:1−16.
Lakay FM, Botha A, Prior BA. 2007. Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. J Appl Microbiol. 102:265−273.
LaMontagne MG, Michel FC, Jr, Holden PA, Reddy CA. 2002. Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. J Microbiol Methods. 49:255−264.
Leff G, Dana JR, McArthur JV, Shimkets LJ. 1995. Comparison of methods of DNA extraction from stream sediments. Appl Environ Microbiol. 61:1141−1143.
Luna GM, Dell'Anno A, Danovaro R. 2006. DNA extraction procedure: a critical issue for bacterial diversity assessment in marine sediments. Environ Microbiol. 8:308−320.
Martin-Laurent F, Philippot L, Hallet S, Chaussod R, Germon JC, Soulas G, Catroux G. 2001. DNA extraction from soils: old bias for new microbial diversity analysis methods. Appl Environ Microbiol. 67:2354−2359.
Miller DN, Bryant JE, Madsen EL, Ghiorse WC. 1999. Evaluation and optimization of DNA purification procedures for soil and sediment samples. Appl Environ Microbiol. 65:4715−424.
Muyzer G, Dewaal EC, Uitterlinden AG. 1993. Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal-RNA. Appl Environ Microbiol. 59:695−700.
Niu Y, Shen H, Chen J, Xie P, Yang X, Tao M, Qi M. 2011. Phytoplankton community succession shaping bacterioplankton community composition in Lake Taihu, China. Water Res. 45:4169−4182.
Olsen GJ, Lane DJ, Giovannoni J, Pace NR, Stahl DA. 1986. Microbial ecology and evolution: a ribosomal RNA approach. Ann Rev Microbiol. 40:337−365.
Polymenakou PN, Bertilsson S, Tselepides A, Stephanou EG. 2005. Links between geographic location, environmental factors, and microbial community composition in sediments of the Eastern Mediterranean Sea. Microb Ecol. 49:367−378.
Robe P, Nalin R, Capellano C, Vogel TM, Simonet P. 2003. Extraction of DNA from soil. Eur J Soil Biol. 39:183−190.
Rochelle PA, Fry JC, Parkes RJ, Weightman AJ. 1992. DNA extraction for 16S rRNA gene analysis to determine genetic diversity in deep sea sediment communities. FEMS Microbiol Lett. 100:59−66.
Roose-Amsaleg CL, Garnier-Sillam E, Harry M. 2001. Extraction and purification of microbial DNA from soil and sediment samples. Applied Soil Ecology. 18:47−60.
Sandaa RA, Enger Ø, Torsvik V. 1998. Rapid method for fluorometric quantification of DNA in soil. Soil Biol Biochem. 30:265−268.
Schloss PD, Handelsman J. 2003. Biotechnological prospects from metagenomics. Curr Opin Biotechnol. 14:303−310.
Smit E, Leeflang P, Gommans S, van den Broek J, van Mil S, Wernars K. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. Appl Environ Microbiol. 67: 2284−2291.

Stark PC, Mullen KI, Banton K, Russotti R, Soran D, Kuske CR. 2000. Pre-PCR DNA quantitation of soil and sediment samples: method development and instrument design. Soil Biol. Biochem. 32:1101–1110.

Suzuki MT, DeLong EF. 2002. Marine prokaryote diversity. In: Staley JT, Reysenbach A-L, editors. Biodiversity of microbial life. New York: Wiley; p. 209–234.

Teske A, Wawer C, Muyzer G, Ramsing NB. 1996. Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. Appl Environ Microbiol. 62:1405–1415.

Tien CC, Chao CC, Chao WL. 1999. Methods for DNA extraction from various soils: a comparison. J Appl Microbiol. 86:937–943.

Torsvik V, Ovreas L, Thingstad TF. 2002. Prokaryotic diversity – magnitude, dynamics, and controlling factors. Sci Signal. 296:1064.

Tsai YL, Olson BH. 1991. Rapid method for direct extraction of DNA from soil and sediments. Appl Environ Microbiol. 57:1070–1074.

Zhou J, Bruns MA, Tiedje JM. 1996. DNA recovery from soils of diverse composition. Appl Environ Microbiol. 62:316–322.

Zipper H, Buta C, Lämmle K, Brunner H, Bernhagen J, Vitzthum F. 2003. Mechanisms underlying the impact of humic acids on DNA quantification by SYBR Green I and consequences for the analysis of soils and aquatic sediments. Nucleic Acids Res. 31:e39–e39.