CHAPTER 5:

ANALYSIS OF THE MICROBIAL COMMUNITY DIVERSITY IN PHOSLOCK® TREATED AND CONTROL AREAS OF HARTBEESPOORT DAM USING PCR-DGGE

1. Introduction

Many fresh water lakes and dams worldwide have been affected by eutrophication, largely as a result of high external nutrient loading with nitrogen and phosphorus compounds (Van der Gught *et al*., 2005). Eutrophication can result in visible cyanobacterial blooms which are often toxic and present severe health risks (Codd *et al*., 2005). The significance of phosphorus in eutrophication has resulted in the development of many remediation plans based on the management of the phosphorus concentration. It is accepted that phosphorus control is more achievable than that of nitrogen, because, unlike nitrogen, there is no atmospheric source of phosphorus that is bio-available. In addition, the general equation for photosynthesis shows that only one gram of phosphorus is required for every seven grams of nitrogen for the formation of the organic matter created in the process (Hereve, 2000). This indicates that a small degree of phosphorus reduction can achieve a much greater degree of growth reduction of cyanobacteria than a reduction of a similar magnitude in the nitrogen level.

Traditional classification systems for cyanobacteria- the bacteriological approach (Rippka *et al*., 1979) and the botanical approach (Anagnostidis & Komárek, 1985)- rely mainly on the morphology of cells and colonies and do not always lead to the identification of phylogenetically coherent taxa (Castenholz, 1992; Wilmotte & Golubic, 1991). At all taxonomic levels, especially above species level, the sequence analysis of genes encoding small-subunit ribosomal RNA (16S RNA) is currently the most promising approach for the phylogenetic classification of cyanobacteria (Nübel *et al*., 1997).

16S rDNA PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) is one of the most frequently used techniques to assess the genetic diversity of microbial communities (Muyzer, 1999). Sequences of 16S rRNA genes are independent from cultivation or growth conditions and can be retrieved by PCR of small amounts of DNA extracted from natural environments. Currently, 16S rDNA sequences constitute the largest gene-specific data set, and the number of entries in generally accessible databases is continually increasing, making 16S rDNA-based identification of unknown bacterial isolates more likely (von Wintzingerode *et al*., 2002).

Several approaches to 16S rRNA analysis in cyanobacteria have been suggested, all of which focused on extending the analysis of the cyanobacterial 16S rRNA beyond axenic cultures. Wilmotte *et al*. (1992) used antibiotics inhibiting peptidoglycan synthesis to suppress the growth of contaminating heterotrophic bacteria in non-axenic cultures of cyanobacteria in order to extract workable amounts of RNA. Garcia-Pichel *et al*. (1996) used micromanipulation to isolate representative samples of field populations of the cyanobacterium *Microcoleus chthonoplastes* from their environment, and thus obtained seven corresponding cultured strains. Mat samples of *M. chthonoplastes* were cleaned by being dragged through agarose gel which removed other cyanobacteria, diatoms and heterotrophic bacteria, and DNA was extracted directly from the cleansed bundles and amplified by PCR to obtain the 16S rRNA gene. Weller *et al*. (1991) used random priming of the 16S rRNA to allow cDNA synthesis anywhere along the molecule. Fragments were cloned and screened for plasmid inserts of interest by sequencing.

However, it was Nübel *et al*. (1997) who developed a set of oligonucleotide primers for the specific amplification of 16S rRNA gene segments from cyanobacteria and plastids by PCR, namely CYA359F (forward), CYA781R(a) and CYA781R(b) (reverse). CYA781R(a) and CYA781R(b) differ by two polymorphic bases situated at positions 7 and 23 (5'to 3'), and were designed to be used in combination as an equimolar mixture. These primers produced a PCR product corresponding to variable regions V3 and V4, which contain significant information for phylogenetic assignments (Yu & Morrison, 2004). PCR products were obtained from all cultures of cyanobacteria and diatoms that were tested, but not from other bacteria and archaea. Gene segments retrieved from cyanobacteria in unialgal but non-axenic cultures could be directly sequenced. The use of this specific PCR in combination with DGGE to probe cyanobacterial diversity in complex microbial communities was also demonstrated (Nübel *et al*., 1997).

The primers designed by Nübel *et al*. (1997) have been used in numerous studies investigating cyanobacterial diversity in environmental samples. Geiß *et al*. (2004) used CYA359F and CYA781R, an equimolar mixture of CYA781R(a) and CYA781R(b), to amplify cyanobacterial 16S rDNA fragments in order to investigate the cyanobacterial diversity of a shallow estuary at the Southern Baltic Sea. The cyanobacterial component of the microbial assemblages of Lake Cisó and Lake Vilar in Spain were analysed by performing PCR-DGGE and sequence analysis of 16S rRNA gene fragments using

CYA359F and CYA781R, with the addition of a GC clamp to the 5' end of primer CYA359F for DGGE purposes (Casamayor *et al*., 2000). Zwart *et al*. (2005) specifically amplified cyanobacterial rDNA for DGGE, but adapted the protocol of Nübel *et al*. (1997) to enable direct comparison of cyanobacterial community profiles with overall bacterial profiles in Lake Loosdrecht in the Netherlands. A single step amplification procedure was used for the bacteria, and a nested PCR for the cyanobacteria. The first round of the nested procedure was performed with cyanobacterial specific primers, and the general bacterial primers were used in the second round. Cyanobacterial bands that were not detectable in the general bacterial pattern were identified in the cyanobacterial specific DGGE. Boutte *et al*. (2006) investigated the variation in banding profiles caused by the position of the GC clamp on the forward or reverse primer, and the combination of the primers designed by Nübel *et al*. (1997) which allowed an optimum investigation of the cyanobacterial community diversity. They found that, irrespective of the position of the GC clamp, the diversity of the bands obtained was lower when both reverse primers were used together than the sum of the bands obtained separately with the primers (a) and (b). This indicates that, when used together, the reverse primers compete for template hybridization, making the genetic fingerprint less complete. In addition, sequence results showed that when the (a) reverse primer was used, filamentous cyanobacterial species were preferentially amplified, whereas the (b) reverse primer targeted unicellular cyanobacteria. This is because the polymorphism at position 23 is situated in the region critical for the specificity of annealing during PCR; the reverse primer (a) amplifies preferentially the filamentous cyanobacteria, whereas the reverse primer (b) targets mainly the unicellular cyanobacteria. It was recommended that the reverse primers CYA781R(a) and CYA781R(b) be used separately with CYA359F in order to give a more complete view of the cyanobacterial community composition, rather than in an equimolar mixture as was originally described by Nübel *et al*. (1997).

This study aims to compare the changes in the cyanobacterial and general bacterial community diversities of two areas of Hartbeespoort Dam over time using DGGE, one area that received a Phoslock® treatment and one that remained untreated as a control. The treated area had a phosphorus concentration significantly lower than that of the control area (Chapter 4). Samples were taken from mid-winter until the end of summer

in order to observe the effect of phosphorus limitation on both the cyanobacterial community and directly or indirectly, on the heterotrophic bacterial community.

2. Materials and Methods

2.1. Sampling and DNA extraction

Water samples were taken from both the Phoslock® treated area and the untreated control area monthly from July 2006 to February 2007. 100ml of water from each sample was ultrasonicated at 50Hz for 30s to break apart cyanobacterial colonies and reduce buoyancy, after which the samples were centrifuged at 10 000g for 15min to obtain a cell pellet. The pellets were resuspended in 567µl of 10mM Tris-1mM EDTA, pH 8, and treated with 30µl of 10% sodium dodecyl sulphate and 3µl Proteinase K (Sigma-Aldrich) (20mg.ml^{-1}) for 60min at 37°. 100µl of 5M NaCl and 80µl of 10% CTAB in 0.7M NaCl was added to each tube and the solutions incubated at 65°C for 10min. Following addition of an equal volume of chloroform-isoamyl alcohol (24:1) was the tubes were centrifuged for 5min at 10 000g. The supernatants were transferred to new tubes and mixed with an equal volume of phenol-chloroform isoamyl alcohol (25:24:1) and centrifuged for 5min at 10 000g. The DNA in the supernatant was precipitated with 0.6vol isopropanol and collected by centrifugation for 15min (10 000g). Finally, DNA was cleaned by washing with 500µl of 70% ethanol and the pellets recovered by centrifugation for 5min. The supernatant was removed and the pellets dried under vacuum at room temperature. DNA was resuspended in 20µl of DNase/RNase free water and maintained at -20°C.

2.2. Polymerase chain reactions

2.2.1. General bacterial PCR

A portion of the 16S eubacterial gene was amplified by means of PCR from the total extracted DNA using the primers PRUN518r (K) and pA8f-GC (M) (Table 1). pA8f-GC was designed specifically for DGGE and thus a GC clamp is included at the 5' end. A reaction with no template DNA was included as a negative control. 0.5µl of DNA

(~25ng.µl^{-1}) was added to 19.5µl of amplification mixture containing 12.8µl sterile MilliQ water, 2.5µl PCR buffer with $MgCl_2$ (10x) (Fermentas), 2µl dNTPs (2.5µM), 1µl PRUN518r (10pM), 1µl pA8f-GC (10pM), 0.2µl Taq DNA polymerase (Super Therm) (5U.µl^{-1}) to give a final volume of 20µl.

DNA amplification was performed in a PCR thermal cycler (Biorad) using the following program: 10min at 95°C, 35 cycles of 30s at 94°C, 30s at 58°C and 1min at 72°C, followed by 10min at 72°C then held at 4°C. The PCR product was analysed on a 1% TAE (40mM Tris, 20mM acetic acid, 1nM EDTA (pH 8.3)) agarose gel.

Table 1 Oligonucleotide primers used in this study

Primer	Sequence (5'- 3')	Reference
PRUN518r (K)	5'ATTACCGCGGCTGCTGG3'	(Muyzer *et al*., 1993)
pA8f-GC (M):	5'CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGAGAGTTTGATCCTGGCTCAG3'	(Fjellbirkeland *et al.* 2001)
CYA359F	5'CGCCCGCCGCGCCCCGCGCCGGTCCCGCCGCCCCCGCCCGGGGGAATYTTCCGCAATGGG3'[a]	(Nübel *et al*., 1997)
CYA781R(a)	5'GACTACTGGGGTATCTAATCCCATT3'	(Nübel *et al*., 1997)
CYA781R(b)	5'GAC TAC AGG GGT ATC TAA TCC CTT T3'	(Nübel *et al*., 1997)

[a] Y, a C/T nucleotide degeneracy (Liébecq, 1992).

2.2.2. Cyanobacterial specific PCR

A portion of the conserved region of the cyanobacterial 16S gene was specifically amplified using the primers CYA359F, CYA781R(a) and CYA781R(b) as recommended by Boutte *et al*. (2006) (Table 1). CYA359F, the forward primer, has a 40-nucleotide GC-clamp attached at the 5' end for better resolution during DGGE. A reaction with no template DNA was included as a negative control. 1µl of DNA (~25ng.µl^{-1}) was added to 19µl of amplification mixture containing 12.3µl sterile distilled MilliQ water, 2.5µl PCR buffer with $MgCl_2$ (10x) (Fermentas), 2µl dNTPs (2.5µM), 1µl CYA359F (10pM), 1µl CYA781R(a) (10pM) or 1µl CYA781R(b) (10pM), 0.2µl Taq DNA polymerase (Super Therm) (5U.µl^{-1}) to give a final volume of 20µl.

DNA amplification was performed in a PCR thermal cycler (Biorad) using the following program, modified from Nübel *et al*. (1997): 5min at 94°C, 35 cycles of 1min at 94°C, 1min at 60°C and 1min at 72°C, followed by 5min at 72°C then held at 4°C. The PCR product was analysed on a 1% TAE (40mM Tris, 20mM acetic acid, 1nM EDTA (pH 8.3)) agarose gel.

2.3. DGGE

16S PCR products from the general bacterial and cyanobacterial specific (using (a) and (b) reverse primers separately) reactions were analysed by DGGE according to the method described by Muyzer *et al*. (1993). 10µl each of the general bacterial and cyanobacterial specific PCR products containing approximately 250ng of 16S rDNA were loaded per lane on three separate denaturing gradient gels. A standard DNA was not added as each DGGE gel was treated as a separate data set. The gel for general bacteria contained a 35-55% formamide/urea denaturing gradient, whereas the gels for the cyanobacterial specific (a) and (b) PCRs had a 40-50% denaturing gradient (Table 2). Gels were run at 70V for 17h at a constant temperature of 60°C. From the gels graphic cluster representations of the banding patterns were drawn using Gel2K (Norland, 2004). The program estimates band peak intensity along the lane. Peaks can be manipulated to ensure that, should more than one peak be registered per band, they can be grouped together. Dominant species per lane are indicated as dark prominent bands across the lane. CLUST (Norland, 2004) was used to compile a dendrogram of each banding pattern drawn in order to analyse species diversity. CLUST is based on Shannon index algorithms and groups the species profiles in each sample according to how similar in community composition the samples are. Dominant bands were picked from the gels under blue light, placed into 30µl sterile MilliQ and allowed to stand over night at 4°C to dissolve, before being used for sequencing.

Table 2: Denaturing gradient table showing volumes in millilitres of denaturing stock solution A (DSSA): 8% acrylamide in 0.5x TAE (40nM Tris, 20mM acetic acid, 1nM EDTA (pH 8.3) buffer) and denaturing stock solution B (DSSB): 8% acrylamide, 7M urea, 40% formamide in 0.5x TAE buffer, mixed to form a gradient within the gel.

Denaturing percentage	DSSA	DSSB	Total volume
15	12.3	2.2	14.5
20	11.6	2.9	14.5
25	10.9	3.6	14.5
30	10.2	4.4	14.5
35	9.4	5.1	14.5
40	8.7	5.8	14.5
45	8.0	6.5	14.5
50	7.3	7.3	14.5
55	6.5	8.0	14.5
60	5.8	8.7	14.5
65	5.1	9.4	14.5
70	4.4	10.2	14.5
75	3.6	10.9	14.5

2.4. Sequencing and phylogenetic analysis

DNA from each dominant DGGE band was first amplified in an up-PCR (as described above) using the K and M primers for DNA picked from the general bacterial gel, and CYA359F, CYA781R(a) and CYA781R(b) for the DNA from the cyanobacterial gels. Up-PCR product was cleaned by transferring the entire volume to a 0.5ml Eppendorf tube, adding 2µl of 3M sodium acetate (pH 4.6) and 50µl 95% ethanol, and allowing it to stand on ice for 10min. The tubes were then centrifuged at 10 000rpm for 30min. The ethanol solution was removed, the pellet rinsed in 150µl 70% ethanol and centrifuged at 10 000rpm for 5min. The ethanol was aspirated and the pellet dried under vacuum for approximately 10min. The pellet was then re-suspended in 20µl sterile water. Each amplified PCR was then sequenced in an Eppendorf tube containing 1µl clean PCR product, 2µl Big Dye sequencing mix (Roche), 0.32µl primer and 1.68µl deionised filter-sterilised water. For the bands from the general bacterial DGGE gel, partial sequences of the 16S bacterial gene were obtained using the K primer above, and nucleotide sequence order was confirmed by comparing it to the sequence obtained

using the M primer. Similarly, the CYA359F and CYA781R(a) primers were used for the DNA from the cyanobacterial gel that targeted filamentous cyanobacteria, and CYA359F and CYA781R(b) for the gel targeting unicellular cyanobacteria. Sequence PCR products were cleaned in the same manner as the amplification PCR, except that 15µl of sterile water was added to the PCR before transferring it to a 0.5ml tube, and the dried pellet obtained at the end was not re-suspended in water. Tubes were transferred on ice to the sequencer, and DNA sequences were determined using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase Applied Biosystems, UK). Sequences were deposited in GenBank, and the accession numbers are presented in Tables 3 and 4.

Each sequence was subjected to a BLAST analysis on the GenBank database, and by determining the sequences with the highest percentage match and coverage, tentative species identification was possible. A phylogenetic analysis was performed on the cyanobacterial sequences from the DGGE gels. Separate trees were drawn, one for each cyanobacterial DGGE gel and one that combined the sequences from both gels. Closely related sequences for each cyanobacterial sequence were selected from GenBank for alignment and inclusion in the trees. Sequence orientation was checked using Vector NTI (Invitrogen), and where necessary the orientation was changed. Sequences were then aligned with Clustal X (Thompson et al., 1994) and inserted gaps were treated as missing data. Ambiguously aligned regions were excluded from the data set before analysis. Phylogenetic analysis was based on parsimony using PAUP 4.0b8 Phylogenetic Analysis Using Parsimony (Swofford 2000). Heuristic searches were done with random addition of sequences (1000 replicates), tree bisection-reconnection (TBR), branch swapping, MULPAR-effective and MaxTrees set to auto-increase. Phylogenetic signal in the data sets was assessed by evaluating tree length distributions over 100 randomly generated trees. The consistency (CI) and retention indices (RI) were determined for all data sets. The phylogenetic tree of sequences from the DGGE gel targeting filamentous cyanobacteria was rooted with *Calothrix*, and that of the unicellular cyanobacteria was rooted with *Thermatoga maritima.* The tree combining sequences from both gels was rooted with *T. maritima* and a *Pseudomonas* species. Bootstrap analyses were conducted, retaining groups with 70% consistency, to determine confidence in branching points (1000 replicates) for the most parsimonious trees generated.

3. Results

3.1. DGGE targeting filamentous cyanobacteria

The DGGE gel that targeted the filamentous cyanobacteria in the monthly samples is illustrated in Figure 1. The species diversity between the months is compared in the dendrogram in Figure 3. The months of July, August, October and November fall in the same dominant clade (III) for both the treated and control areas, whereas September, December, January and February group together (II). This means that the species diversity was similar in these months in the control and treated areas. October and November therefore showed a species diversity in both the treated and control areas that was similar to the winter months, but September had a diversity comparable to the summer months. The diversity of the control and treated areas for most months appears to be similar, as they are grouped together at the lowest level in most cases. One exception is the control area in January, which had a very low diversity. In contrast, the diversity of the treated area in January was much higher.

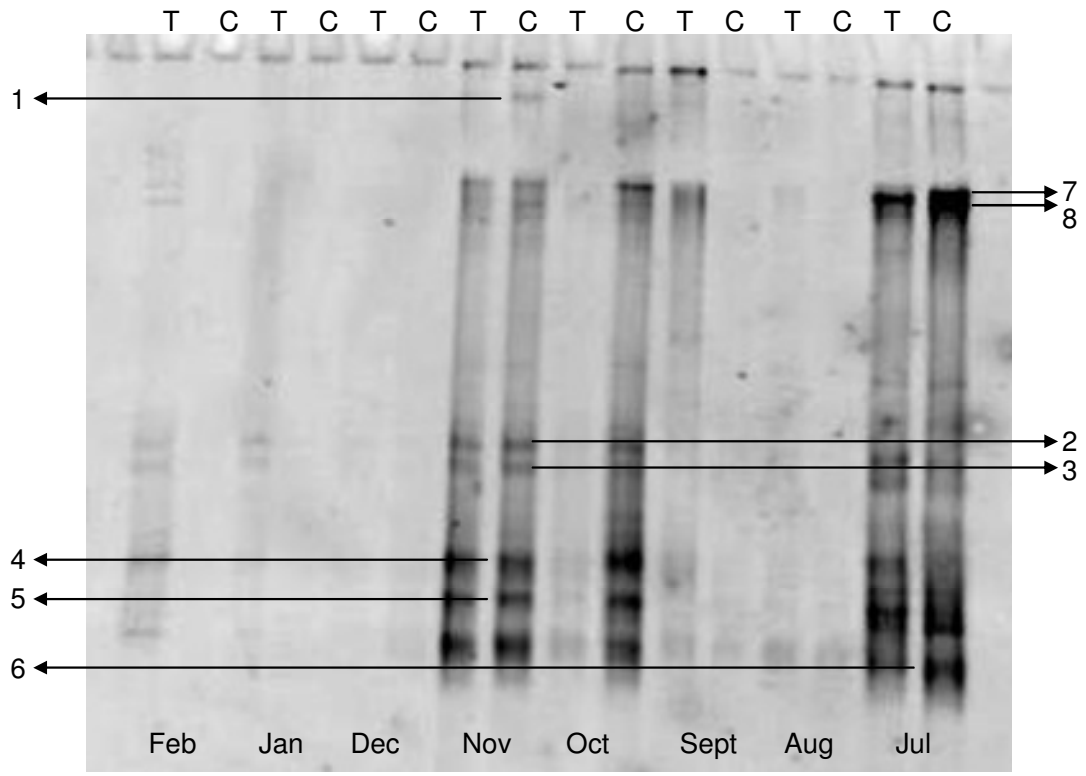


Figure 1: DGGE gel of filamentous cyanobacteria ('a' reverse primer) showing the banding patterns for each month. C= control area T= area treated with Phoslock®

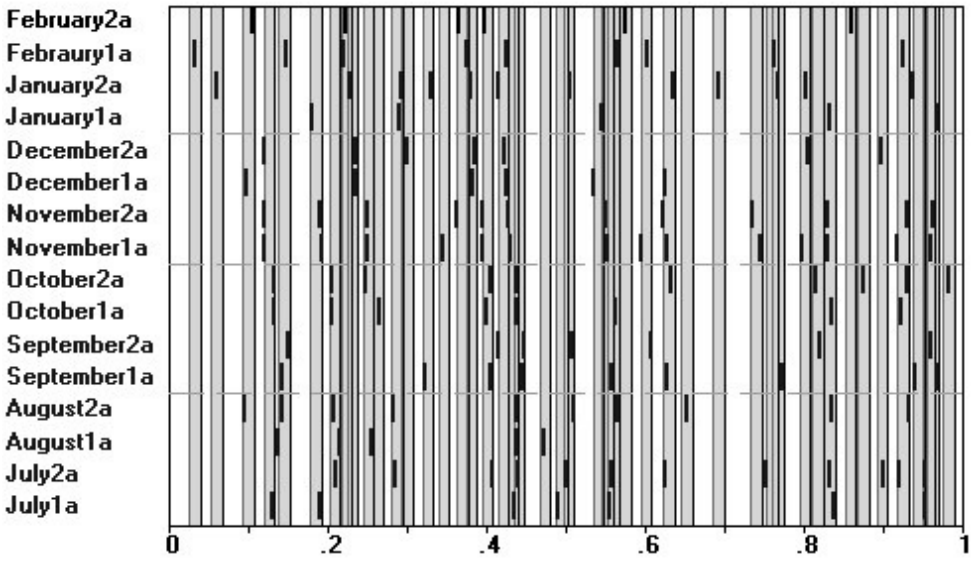


Figure 2: Schematic representation of the banding pattern of the DGGE gel targeting filamentous cyanobacteria (a). Black bars represent dominant species in each sample 1a= control area, 2a= treated area

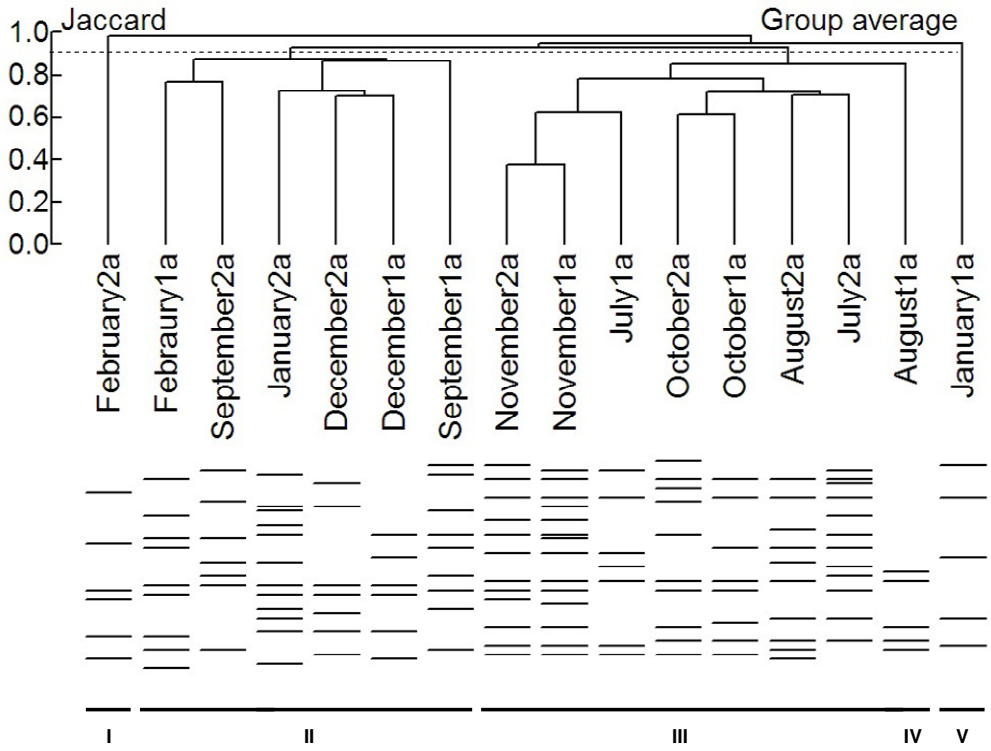
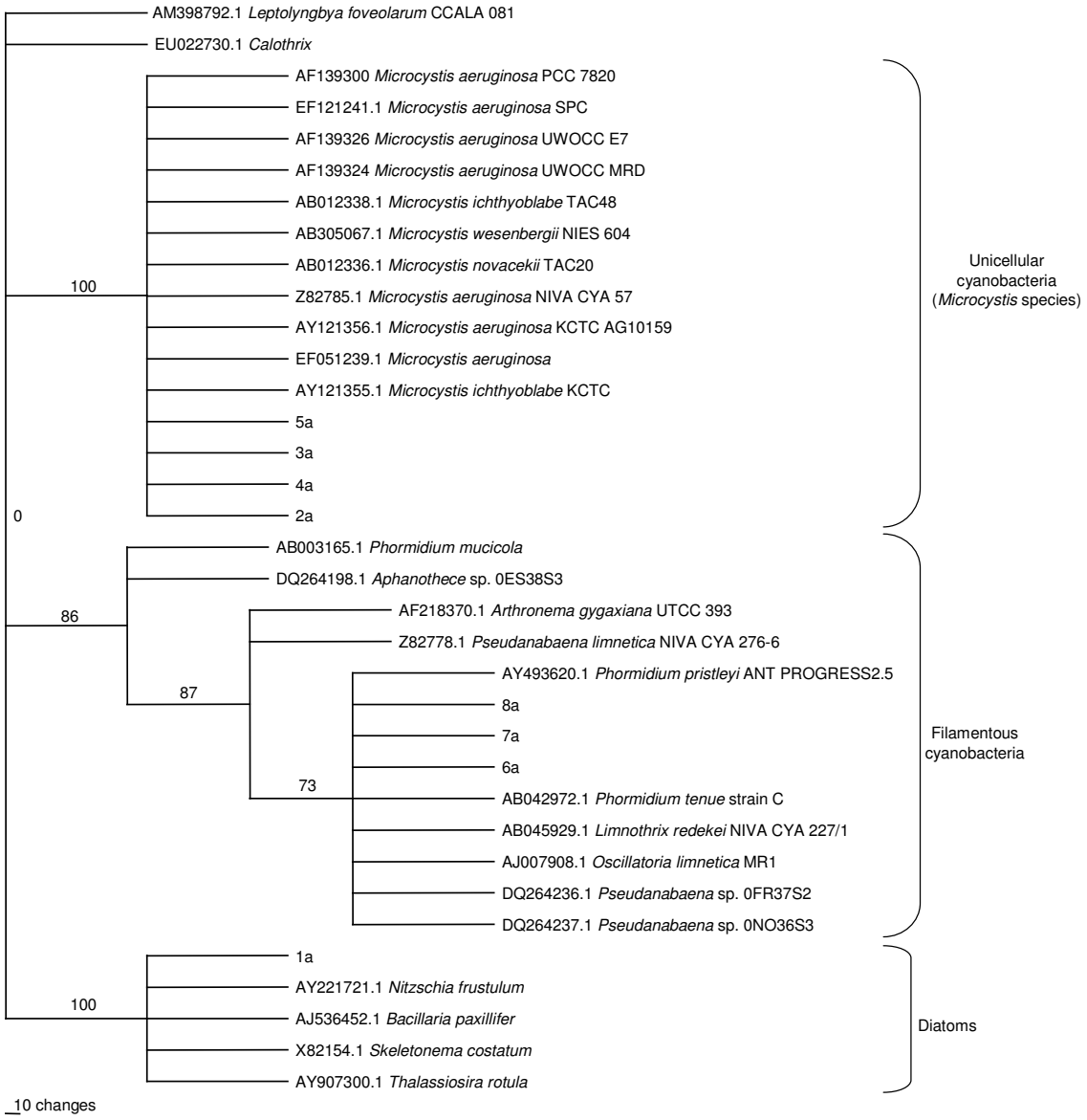


Figure 3: Dendrogram to show the differences in the species diversity of monthly samples with targeted filamentous cyanobacteria (a) using a group average, Jaccard setting. 1a= control area, 2a= treated area

In order to determine the species composition, dominant bands were picked from the gel (1-8 on Figure 1) and sequenced (Appendix A). The closest matching species are presented in Table 3. The sequence in band 1 matched closely to the chloroplast 16s rRNA gene of *Nitzschia frustulum*, a diatom. Sequences in bands 2-5 were close matches to various *Microcystis* species (unicellular cyanobacteria), and bands 6-8 matched with sequences of filamentous cyanobacteria such as *Pseudanabaena* sp., *Limnothrix redekei* and *Oscillatoria limnetica.* These are non-heterocystis species (not capable of nitrogen fixation). The primer combination of CYA359F and CYA781R(a) therefore picked up both unicellular and filamentous species of cyanobacteria, and not just the filamentous species as was expected. Band 1 was only visible until November, indicating that the diatom *Nitzschia frustulum* was not present during the summer months of December through to February. Interestingly, the *Microcystis* species (bands 2-5) were prominent during July, but disappeared in August and September. During October, *Microcystis* was dominant in the control area, whereas in the treated area bands 2-5 were very faint. During January and February, *Microcystis* species, with the

exception of band 5, were present in the treated area but not the control, which was unexpected. In terms of the filamentous cyanobacterial species, band 6, most likely a *Pseudanabaena* sp., was prominent in both the treated and control areas from July until November. However, in January and February it was present only in the treated area. Bands 7 and 8 were present in both the treated and control areas in July, October and November, but only in the treated area in September. The bands disappeared until January, when they were only present in the treated area. According to the gel, the control area in January and February had no cyanobacterial species, as no bands are visible. However, the Gel2K software picked up 5 bands in January and 6 in February (Figure 2). The species diversity in the treated area was higher during January and February than in the control area.

The phylogenetic tree of the sequences obtained from the gel in Figure 1 (1-8) and their closely related sequences obtained from BLAST is presented in Figure 4. The sequences from the gel grouped with the expected sequences: sequence 1 grouped with the diatoms, sequences 2-5 grouped with the unicellular *Microcystis* species, and sequences 6-8 grouped with the filamentous cynobacteria. Therefore the tentative identifications presented in Table 3 appear to be correct, at least up to species level. The high retention index of 0.8483 indicated that the data set was significant.



Tree length = 103.28666
Consistency index (CI) = 0.5338
Retention index (RI) = 0.8483

Figure 4: Phylogeny of cyanobacterial 16s rRNA gene amplicons recovered from the DGGE gel in Fig. 1 and closely related sequences obtained from Genbank (Distance values are indicated above branches)

3.2. DGGE targeting unicellular cyanobacteria

The DGGE gel which targeted the unicellular cyanobacteria in the monthly samples is illustrated in Figure 7. The diversity between the months is compared in the dendrogram in Figure 6. The treated and control areas in July group together (clade I), as they have a similar low diversity, as is expected for the winter months. In clade II, November, January and February of the treated area group closely with August and September of the control area. The treated area in December had a similar diversity to the control area in October (clade III). This indicates that the diversity of the control area in spring is comparable with that of the treated area in summer. An exception to this was the treated area in September, which grouped with the control area in November (clade IV). In terms of the diversity, therefore, the reduced phosphorus in the water due to the Phoslock® treatment appeared to have a greater effect on the unicellular cyanobacteria than was obvious in the gel that targeted filamentous cyanobacteria.

In order to determine the species composition, dominant bands were picked from the gel (9-16 on Figure 5) and sequenced (Appendix A). The closest matching sequences obtained from BLAST are presented in Table 3. Band 9 closely matched the chloroplast 16S rDNA of the diatoms *Aulacoseira ambigua* and *Haslea wawrikae*. Bands 10-16 were all close matches to species of *Microcystis*, predominantly *M. aeruginosa*, *M. viridis*, *M. botrys* and *M. wesenbergii*. For each sequence that was run on BLAST, the closest matching sequences had the same percentage match as well as coverage, so it was not possible to identify the sequences up to species level. The combination of CYA359F and CYA781R(b) primers only amplified unicellular cyanobacteria, no filamentous cyanobacterial sequences were detected. Band 9 (diatom chloroplast 16S rDNA) was present from July until December, but appeared to be more dominant in the treated area from September. Bands 13, 14 and 15 (near the top of the gel) were present in both the treated and control areas for all the months sampled. Bands 10, 11, 12 and 16 (near the bottom of the gel) were only predominant until November. It is possible that the *Microcystis* species in bands 13-15 were able to out-compete those present in bands 10-12 and 16 when bloom conditions were experienced.

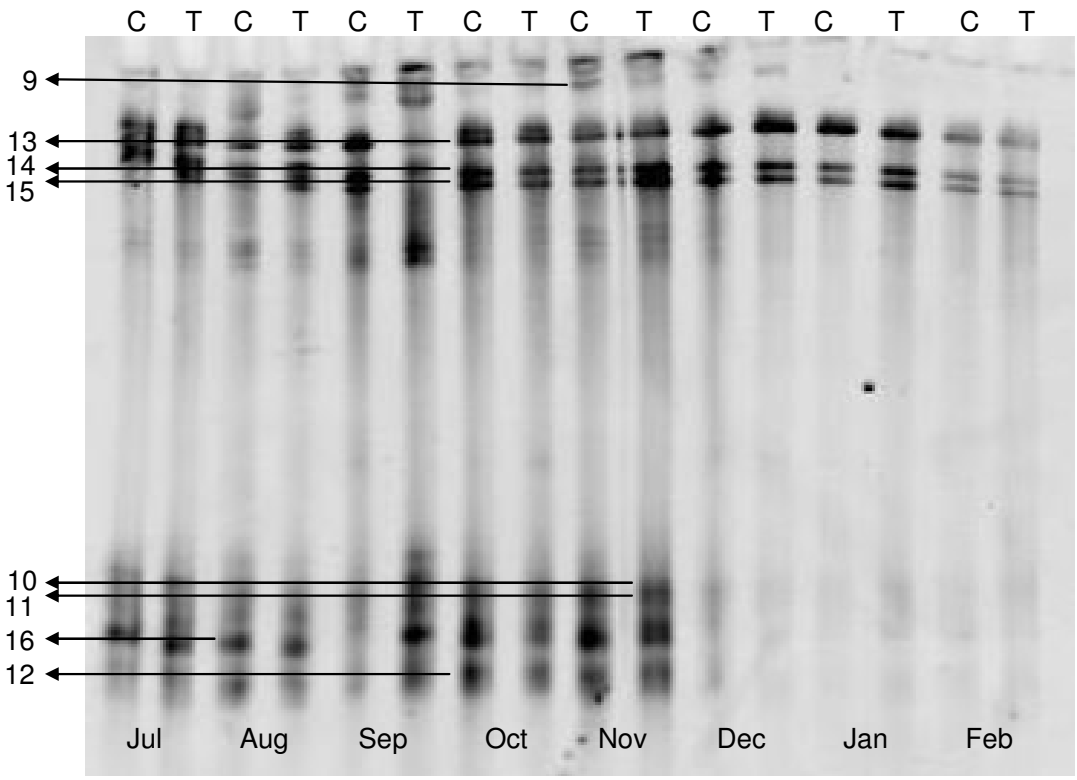


Figure 5: DGGE gel of unicellular cyanobacteria ('b' reverse primer) showing the banding patterns for each month. C= control area T= Area treated with Phoslock®

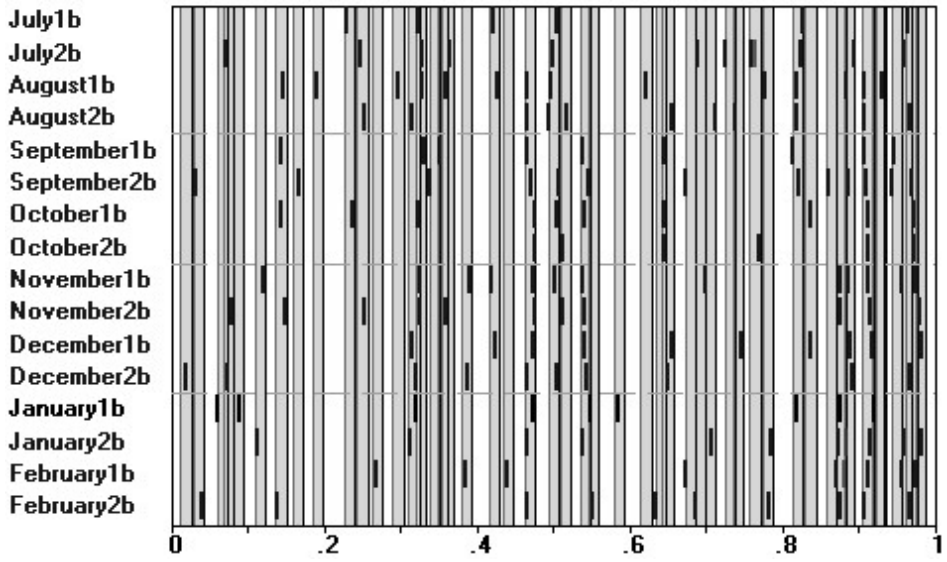


Figure 6: Schematic representation of the banding pattern of the DGGE gel which targeted unicellular cyanobacteria (b). 1b= control area, 2b= treated area

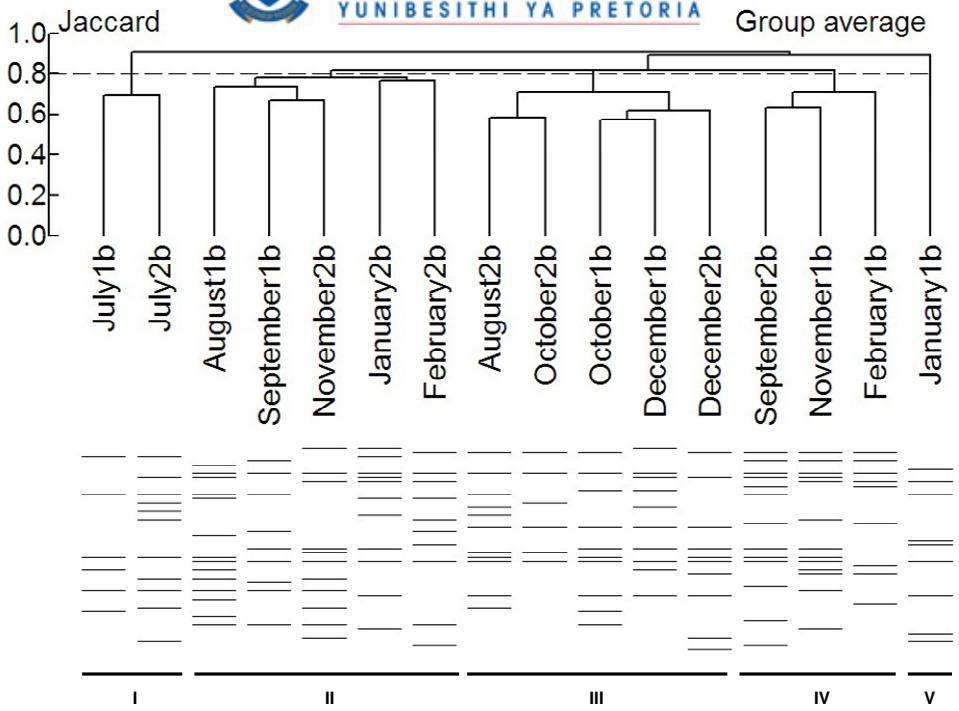
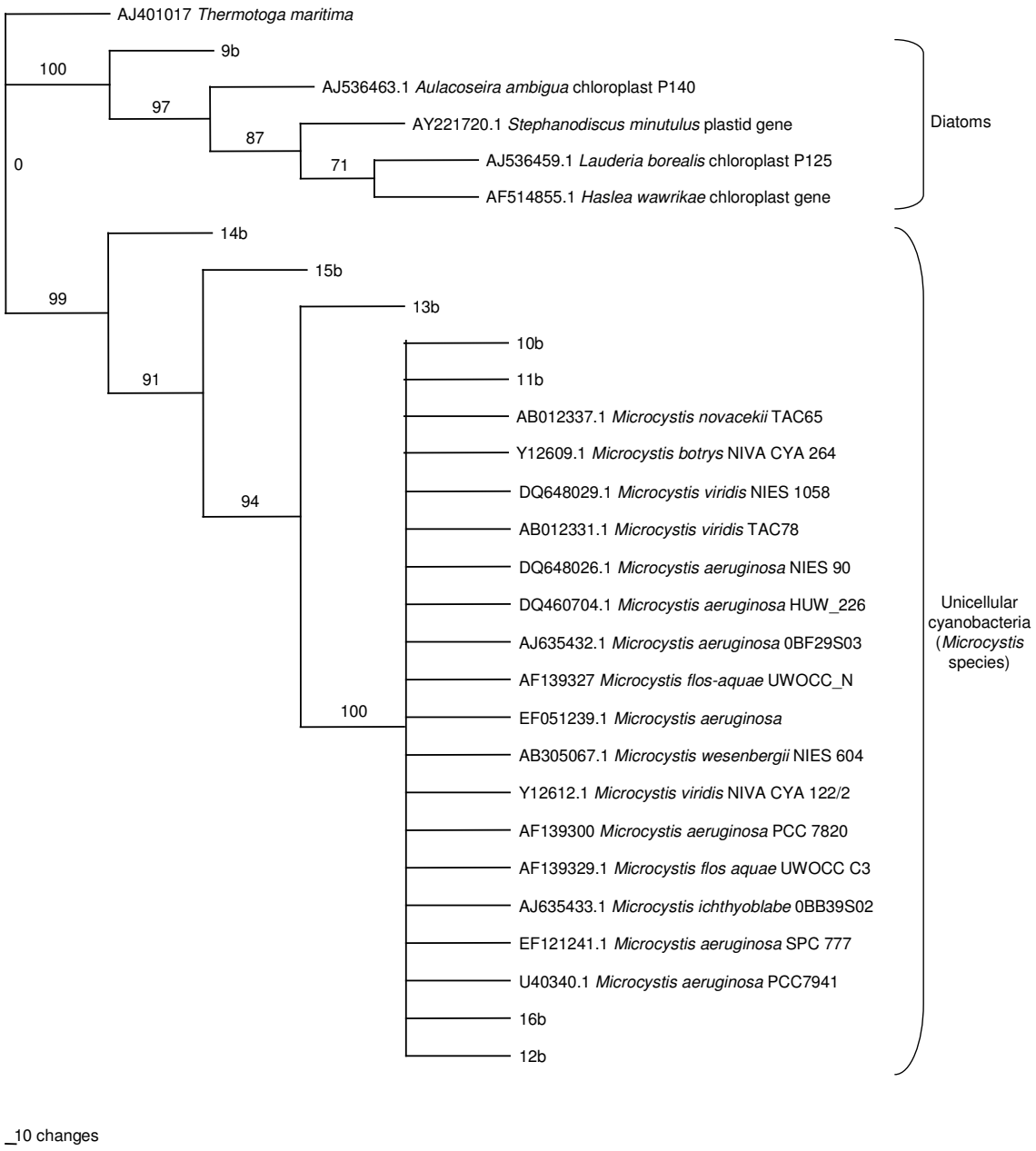


Figure 7: Dendrogram to show the differences in the species diversity of monthly samples with targeted unicellular cyanobacteria (b) using a group average, Jaccard setting 1b= control area, 2b= treated area

The phylogenetic tree of the sequences obtained from the gel in Figure 4 (9-16) and their closely related sequences obtained from BLAST is presented in Figure 8. The high retention index of 0.9352 indicated that signal within the data set was significant. The sequences from the gel grouped with the expected sequences. Sequence 9 grouped with the diatoms, and sequences 10-16 grouped with the unicellular *Microcystis* species, although 13, 14 and 15 were basal to the main clade containing the related sequences and 10, 11, 12 and 16. This difference in grouping corresponds to the banding pattern described above, with the sequences from the bands near the top of the gel grouping with the related *Microcystis* species, and the sequences from the bands near the bottom of the gel falling basal.

Figure 9 presents a phylogenetic tree combining the sequences from the gels in figures 1 and 5. Sequences 2-5 group with the unicellular *Microcystis* species along with 10, 11, 12 and 16, but once again 13, 14 and 15 were basal to this clade, although they did not group with the filamentous cyanobacteria or the diatoms. The filamentous cyanobacterial sequences appeared to be more closely related to the diatom chloroplast 16S rDNA that the unicellular cyanobacteria.



Tree length = 111.21071
Consistency index (CI) = 0.8360
Retention index (RI) = 0.9352

Figure 8: Phylogeny of cyanobacterial 16s rRNA gene amplicons recovered from the DGGE gel in Figure 5 and closely related sequences obtained from Genbank (Distance values are indicated above branches)

Tree length = 148.33752
Consistency index (CI) = 0.6739
Retention index (RI) = 0.9037

Figure 9: Phylogeny of cyanobacterial 16s rRNA gene amplicons recovered from the DGGE gels in Figure 1 and Figure 5 and closely related sequences obtained from Genbank (Distance values are indicated above branches)

3.3. DGGE targeting all bacteria, including cyanobacteria

The DGGE gel that was run with the DNA amplified with K and M primers capable of amplifying all bacterial (including cyanobacterial) 16S rDNA is presented in Figure 10, as well as the bands (1-18) that were picked for sequencing. The sequences from BLAST that were the closest match to the sequences obtained from the gel (Appendix A) are presented in Table 4. Bands 7, 8, 10-13 and 18 were close matches to cyanobacteria. Band 7 (most likely *Microcystis aeruginosa*) was not present in the control area until October, and only appeared in November in the treated area. This band was also less bright in the treated area for January than the control area. Bands 10 and 11, which were also close matches to *Microcystis* species, were present in all the months sampled, but band 10 was only present in the control area in January and February. Band 13, most likely *Anabaena flos-aquae*, a filamentous cyanobacterium, was present in the treated and control areas until October, after which it was more dominant in the treated areas until February. This heterocystous species was not present in the DGGE gel which targeted filamentous cyanobacteria, but followed the same pattern. The sequences from bands 1-6, 9, and 14-17 correspond to bacterial 16S rDNA sequences, mainly uncultured α-, β- and δ-proteobacteria, as well as uncultured actinobacteria. Bands 3, 5, 6, 9 and 14-17 were more dominant through the winter months. Bands 3 and 5 disappeared after September, and bands 9 and 14-17 were only present until October. Band 6 was only present until October, but was dominant in the treated area through winter. Bands 1 and 4 (β- and δ-proteobacteria) were not present during the winter months, and only appeared in October in both the treated and control areas. Band 2 was present throughout the sampled months, in both the treated and control areas.

The diversity between the months is compared in the dendrogram in Figure 12. The winter and spring months of July, August, and September grouped together (clades III and IV), and the summer months (October to February) grouped together in clades I and II. On the whole, the Phoslock® treatment did not appear to affect the species diversity, as the treated and control areas for each month were grouped according to season.

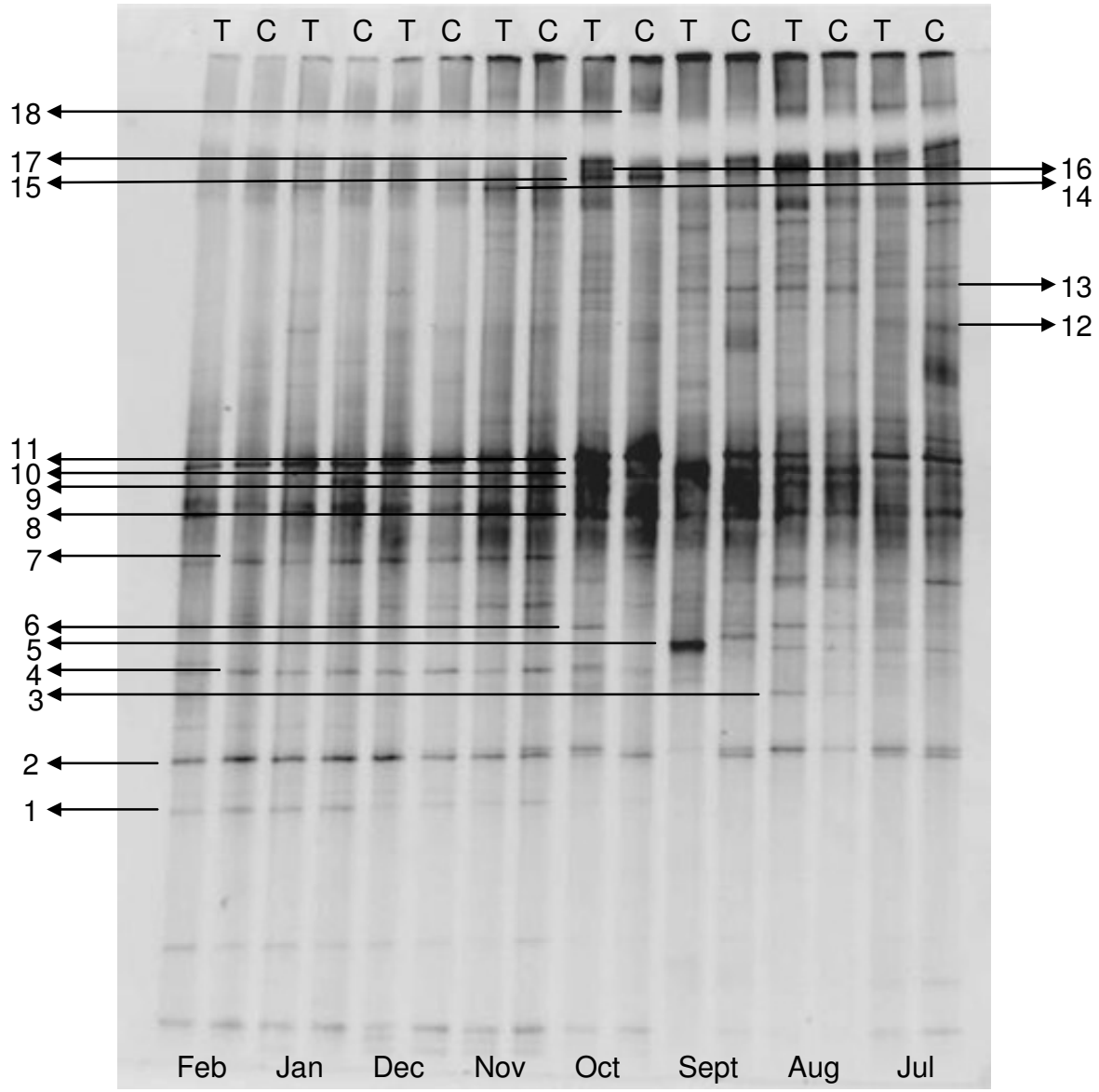


Figure 10: General bacterial DGGE gel showing the banding patterns for each month. C= control area T= Area treated with Phoslock®

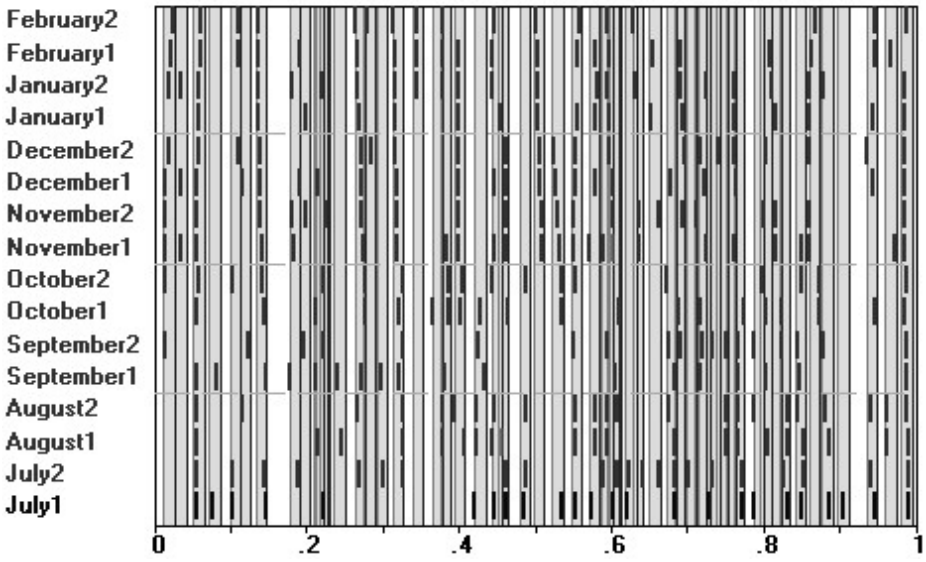


Figure 11: Schematic representation of the banding pattern of the bacterial DGGE gel. 1= control area, 2= treated area

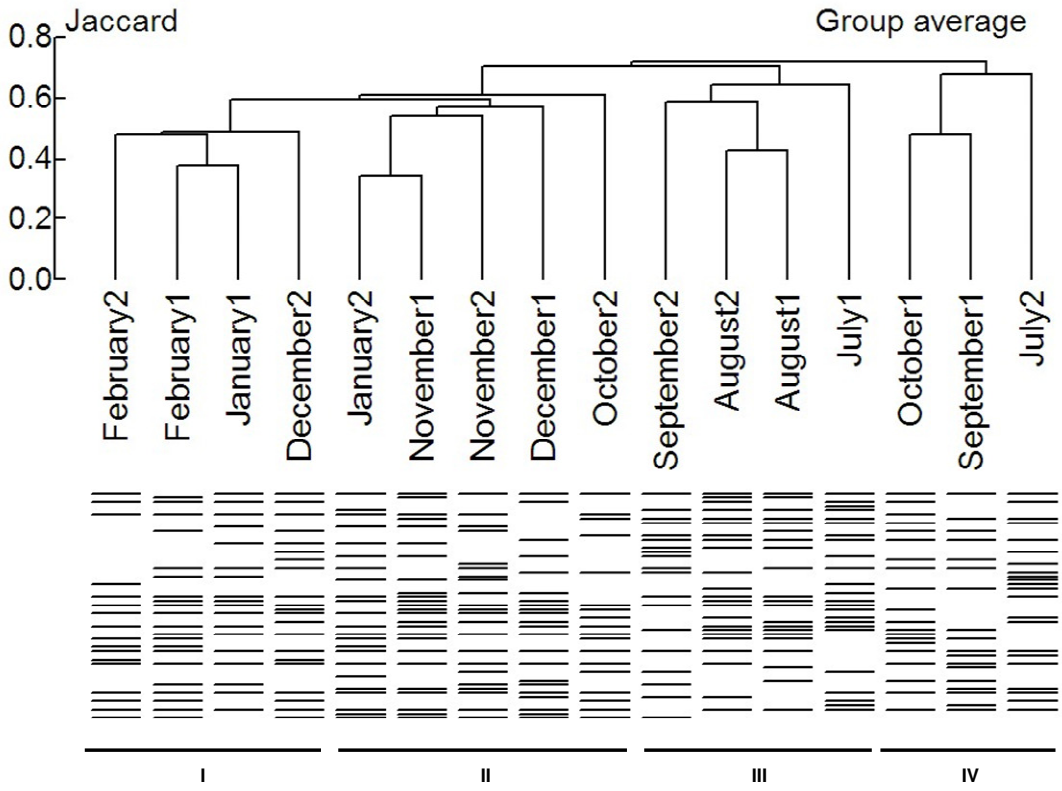


Figure 12: Dendrogram to show the differences in the species diversity of bacteria in the monthly samples using a group average, Jaccard setting 1= control area, 2= treated area

Table 3: Cyanobacterial 16s rDNA sequences from bands picked from DGGE gels in Figures 1 and 2. Species that had the highest percentage match with the highest coverage and their descriptions are presented

Band number (Accession number)	Matching GenBank accession numbers	Closest species identification	Percentage match (Query coverage)	Description
1 (EU94509)	AY221721.1	*Nitzschia frustulum*	91% (82%)	16S ribosomal RNA gene, partial sequence; plastid gene for plastid product
	AJ536452.1	*Bacillaria paxillifer*	89% (88%)	Chloroplast 16S rRNA gene
2 (EU94510)	EF051239.1	*Microcystis aeruginosa*	92% (86%)	16S ribosomal RNA gene, partial sequence
	EF121241.1	*Microcystis aeruginosa*	93% (84%)	Strain SPC 777, 16S ribosomal RNA gene, partial sequence
3 (EU94511)	Z82785.1	*Microcystis aeruginosa*	92% (88%)	Strain NIVA-CYA 57, 16S rRNA gene
	AB012337.1	*Microcystis novacekii*	92% (88%)	Isolate TAC65, gene for 16S rRNA, partial sequence
	AB305067.1	*Microcystis wesenbergii*	92% (88%)	Strain NIES-604, gene for 16S ribosomal RNA, partial sequence
4 (EU94512)	EF051239.1	*Microcystis aeruginosa*	88% (93%)	16S ribosomal RNA gene, partial sequence
	AY121356.1	*Microcystis aeruginosa*	88% (89%)	Strain KCTC AG10159, 16S ribosomal RNA gene partial sequence
	AY121355.1	*Microcystis ichthyoblabe*	88% (89%)	Strain KCTC AG10160, 16S ribosomal RNA gene partial sequence
	AY074802.1	*Microcystis novacekii*	88% (89%)	Strain NIER-10029, 16S ribosomal RNA gene partial sequence
5 (EU94513)	EF051239.1	*Microcystis aeruginosa*	96% (92%)	16S ribosomal RNA gene, partial sequence
	DQ786006.1	*Microcystis aeruginosa*	96% (89%)	Strain PCC 7820, 16S ribosomal RNA gene, partial sequence
	DQ264219.1	*Microcystis ichthyoblabe*	96% (89%)	Strain 9EH38S1, 16S ribosomal RNA gene, partial sequence
6 (EU94514)	DQ264237.1	*Pseudanabaena* sp.	91% (86%)	0NO36S3, 16S ribosomal RNA gene, partial sequence
	AB045929.1	*Limnothrix redekei*	90% (86%)	Strain NIVA-CYA 227/1, 16S ribosomal RNA gene, partial sequence
	AF218370.1	*Arthronema gygaxiana*	90% (86%)	Strain UTCC 393, 16S ribosomal RNA gene, partial sequence
7 (EU94515)	DQ264236.1	*Pseudanabaena* sp.	94% (90%)	0NO36S3, 16S ribosomal RNA gene, partial sequence
	AB045929.1	*Limnothrix redekei*	93% (90%)	Strain NIVA-CYA 227/1, 16S ribosomal RNA gene, partial sequence
8 (EU94516)	AJ007908.1	*Oscillatoria limnetica*	89% (88%)	strain MR1, 16S rRNA gene, partial

	AB045929.1 AB003165.1	*Limnothrix redekei* *Phormidium mucicola*	88% (88%) 83% (84%)	Strain NIVA-CYA 227/1, 16S ribosomal RNA gene, partial sequence gene for 16S ribosomal RNA
9 (EU94517)	AJ536463.1 AF514855.1 AJ536459.1	*Aulacoseira ambigua* *Haslea wawrikae* *Lauderia borealis*	96% (90%) 96% (90%) 95% (90%)	Strain P140, chloroplast 16S rRNA gene, 16S ribosomal RNA gene partial sequence; chloroplast gene for chloroplast product Strain P125, chloroplast 16S rRNA gene
10 (EU94518)	EF121241.1 AF139329.1 Y12612.1	*Microcystis aeruginosa* *Microcystis flos-aquae* *Microcystis viridis*	98% (100%) 98% (100%) 98% (100%)	Strain SPC 777, 16S ribosomal RNA gene, partial sequence Strain UWOCC C3, 16S ribosomal RNA gene, partial sequence Strain NIVA-CYA 122/2, partial16S ribosomal RNA gene sequence
11 (EU94519)	DQ460704.1 DQ648029.1 Y12609.1	*Microcystis aeruginosa* *Microcystis viridis* *Microcystis botrys*	98% (97%) 98% (97%) 98% (97%)	Strain HUW 226 16S ribosomal RNA gene, partial sequence Strain NIES-1058 16S ribosomal RNA gene, partial sequence Strain NIVA-CYA 264 16S ribosomal RNA gene, partial sequence
12 (EU94520)	DQ648028.1 AJ635432.1 AB012336.1	*Microcystis wesenbergii* *Microcystis aeruginosa* *Microcystis novacekii*	95% (100%) 95% (100%) 95% (100%)	Strain NIES-107 16S ribosomal RNA gene, partial sequence Strain 0BF29S03 partial 16S rRNA gene Isolate TAC20, 16S rRNA gene, partial sequence
13 (EU94521)	AB012331.1 AF139327 DQ460704.1	*Microcystis viridis* *Microcystis flos-aquae* *Microcystis aeruginosa*	92% (99%) 92% (99%) 92% (99%)	Isolate TAC78, 16S rRNA gene, partial sequence Strain UWOCC N 16S ribosomal RNA gene, partial sequence Strain HUW 226 16S ribosomal RNA gene, partial sequence
14 (EU94522)	DQ460704.1 Y12609.1	*Microcystis aeruginosa* *Microcystis botrys*	91% (94%) 91% (94%)	Strain HUW 226 16S ribosomal RNA gene, partial sequence Strain NIVA-CYA 264 16S ribosomal RNA gene, partial sequence
15 (EU94523)	DQ460704.1 DQ648028.1 DQ648029.1	*Microcystis aeruginosa* *Microcystis wesenbergii* *Microcystis viridis*	90% (99%) 90% (99%) 90% (99%)	Strain HUW 226 16S ribosomal RNA gene, partial sequence Strain NIES-107 16S ribosomal RNA gene, partial sequence Strain NIES-1058 16S ribosomal RNA gene, partial sequence
16 (EU94524)	EF051239.1 AJ133174.1	*Microcystis aeruginosa* *Microcystis wesenbergii*	99% (99%) 99% (97%)	16S ribosomal RNA gene, partial sequence Strain NIES 104 partial 16S rRNA gene

Table 4: Bacterial 16s rDNA sequences from bands picked from DGGE gel in figure 7. Species that had the highest percentage match with the highest coverage and their descriptions are presented

DGGE gel band sequence number	Matching GenBank accession numbers	Closest species identification	Percentage match (Query coverage)	Description
1 (EU94525)	EF665917.1	Uncultured δ-proteobacterium	91% (47%)	Clone GASP-MB3W2 C12, 16S ribosomal RNA gene, partial sequence
2 (EU94526)	AY509417.1	Uncultured α-proteobacterium	97% (100%)	Clone LiUU-3-194, 16S ribosomal RNA gene, partial sequence
	AF538712.1	*Roseomonas mucosa*	94% (100%)	Strain MDA5527, 16S ribosomal RNA gene, partial sequence
3 (EU94527)	DQ628961.1	Uncultured Microbacteriaceae bacterium	93% (96%)	Clone SOC1 6H, 16S ribosomal RNA gene, partial sequence
	DQ316367.1	Uncultured Actinobacterium	93% (96%)	Clone ST11-6, 16S ribosomal RNA gene, partial sequence
4 (EU94528)	AY824332.1	Uncultured β-proteobacterium	79% (83%)	Clone cloRDC+39, 16S ribosomal RNA gene, partial sequence
	AB211233.1	*Ideonella* sp.	79% (83%)	Strain 0-0013, gene for 16S rRNA, partial sequence
	AF244133.1	*Burkholderia cepacia*	68% (98%)	16S ribosomal RNA gene, partial sequence
5 (EU94529)	EF428988.1	*Aeromonas veronii*	98% (100%)	16S ribosomal RNA gene, partial sequence
6 (EU94530)	EF520353.1	Uncultured Actinobacterium	76% (64%)	Clone ADK-GRe02-60, 16S ribosomal RNA gene, partial sequence
	AY337957.1	Uncultured Microbacteriaceae bacterium	76% (64%)	Clone M13-99, 16S ribosomal RNA gene, partial sequence
7 (EU94531)	DQ887510.1	*Microcystis aeruginosa*	80% (100%)	FC-070, 16S ribosomal RNA gene, partial sequence

	AF139328.1 DQ648028.1	*Microcystis flos-aquae* *Microcystis wesenbergii*	80% (100%) 79% (100%)	Strain UWOCC C2, 16S ribosomal RNA gene, partial sequence Strain NIES-107, 16S ribosomal RNA gene, partial sequence
8 (EU94532)	AJ133171.1 DQ648029.1	*Microcystis aeruginosa* *Microcystis viridis*	85% (50%) 84% (50%)	Strain PCC 7941, partial 16S rRNA gene Strain NIES-1058, 16S ribosomal RNA gene, partial sequence
9 (EU94533)	AJ518316.1 AY371926.1	Unidentified bacterium Bacteroidetes bacterium	96% (24%) 91% (27%)	Clone Neu2P1-29, partial 16S rRNA gene JS5 16S ribosomal RNA gene, partial sequence
10 (EU94534)	AB193613.1 DQ648029.1 AJ635429.1	Uncultured Clostridiales bacterium *Microcystis viridis* *Microcystis aeruginosa*	89% (54%) 86% (54%) 86% (54%)	Clone RsC01-042, gene for 16S rRNA, partial sequence Strain NIES-1058, 16S ribosomal RNA gene, partial sequence Strain 1BB38S07, partial 16S rRNA gene
11 (EU94535)	DQ887510.1	*Microcystis aeruginosa*	84% (70%)	Strain FC-070, 16S ribosomal RNA gene, partial sequence
12 (EU94536)	AF139295.1 AB012331.1	*Microcystis aeruginosa* *Microcystis viridis*	95% (77%) 94% (77%)	Strain UWOCC 019, 16S ribosomal RNA gene, partial sequence Isolate TAC78, 16S rRNA gene, partial sequence
13 (EU94537)	AY887021.1	*Anabaena flos-aquae*	80% (26%)	Strain CCAP, 1403/13F 16S ribosomal RNA gene, partial sequence
14 (EU94538)	AJ853587.1	Uncultured bacterium	72% (86%)	Clone GZKB93, partial 16S rRNA gene,
15 (EU94539)	AF107335.1 DQ316386.1	Uncultured freshwater bacterium Uncultured Actinobacterium	71% (75%) 71% (75%)	LCK-79, 16S ribosomal RNA gene, partial sequence Clone STH5-5, 16S ribosomal RNA gene, partial sequence
16 (EU94540)	EF516194.1	Uncultured bacterium	94% (21%)	Clone FCPT473, 16S ribosomal RNA gene, complete sequence
17 (EU94541)	AM690823.1	Uncultured α-proteobacterium	88% (96%)	Clone TH1-19, partial 16S rRNA gene
18 (EU94542)	DQ887510.1 AB035553.1	*Microcystis aeruginosa* *Microcystis wesenbergii*	90% (100%) 89% (100%)	Strain FC-070, 16S ribosomal RNA gene, partial sequence Gene for 16S rRNA, partial sequence

4. Discussion

Both cyanobacterial primer combinations amplified diatom chloroplast 16S rDNA. Plastids are believed to be of origin early in the cyanobacterial evolutionary line (Nelissen *et al*., 1995). After what was probably a single primary endosymbiotic event, a nearly simultaneous radiation of the ancestors of recent cyanelles, rhodoplasts and chloroplasts occurred, and other plastids evolved from secondary endosymbioses (Bhattacharya & Medlin, 1995). The primers have one or more mismatches to a large amount of chloroplast sequences. For most mismatching sequences however, the mismatches are few and only rarely at the 3' end (Zwart *et al*., 2005). Therefore it can be expected that most chloroplast 16S rDNA sequences will be amplified. In the gel targeting filamentous cyanobacteria, the band corresponding to the chloroplast 16S rDNA of the diatom *Nitzschia frustulum* was only present until November, and in the unicellular specific gel, the diatom band (corresponding to the diatoms *Aulacoseira ambigua* and *Haslea wawrikae*) was only present until December, but was more dominant in the treated area from September. These results indicate that the diatoms were out-competed by the cyanobacteria in both the control and treated areas after December, despite the fact that the N:P ratio of the treated area was higher than that of the control area. However, because the diatoms were more prevalent in the treated area than the control area between September and December, it would appear that the lower phosphorus level did favour diatom growth.

The DGGE gel which targeted filamentous cyanobacteria also contained bands that closely matched *Microcystis* species, which was confirmed by their grouping in the phylogenetic tree. This was unexpected, as Boutte *et al*. (2006) tested 381 sequences from unicellular strains, and found that 92.6% matched with the primer CYA781R(b), but only 5.0% matched with primer CYA781R(a), none being *Microcystis* species. During October, the bands corresponding to *Microcystis* were dominant in the control area, whereas in the treated area they were very faint. It seems that the *Microcystis* bloom occurred earlier in the control area, which may have been a result of the low phosphorus concentration in the treated area. During January and February, bands corresponding to the filamentous cyanobacterial species were only present in the treated area. The low relative phosphorus concentration may have allowed for greater

cyanobacterial species diversity in the treated area by preventing dominance by one species, although it was not able to prevent the occurrence of the bloom.

The DGGE gel targeting unicellular cyanobacteria showed that the diversity of the control area in spring was comparable with that of the treated area in summer. This indicated that the reduced phosphorus in the water due to the Phoslock® treatment had an effect on the diversity of the treated area. The combination of CYA359F and CYA781R(b) primers only amplified unicellular cyanobacteria, which was expected. When the sequences were run on BLAST they were close matches to species of *Microcystis*, however it was not possible to identify the sequences up to species level. The *Microcystis* species represented by the bands near the top of the gel (13-15) were present in the treated and control areas for all the months sampled, but the four species represented by bands at the bottom of the gel (10-12 and 16) were only present until November. The sequences in these bands also grouped differently in the phylogenetic tree, indicating that they were not the same species. It is possible that, once bloom conditions were experienced, certain *Microcystis* species were able to out-compete others for dominance within the bloom.

Better species resolution (at or below species level) is possible through cyanobacterial specific amplification of other regions of the DNA apart from the 16S rDNA region. The *rpoC1* gene, which encodes the γ subunit of cyanobacterial RNA polymerase that is absent in other bacteria, has been used to analyse cyanobacterial phylogeny (Bergsland & Haselkorn, 1991) and community structure (Palenik, 1994) However, sequence data for this gene is limited (Nübel *et al*., 1997). DGGE of *hetR*, a gene involved in heterocyst differentiation, has been used to study isolated strains of the cyanobacterial genera *Trichodesmium* and *Nostoc* (Rasmussen & Svenning, 2001; Orcutt *et al*., 2002). *nifH*, a gene encoding nitrogenase reductase in many organisms including cyanobacteria was used by Lovell *et al*. (2001) in the DGGE analysis of nitrogen fixing cyanobacterial species. Phylogeny based on *nifH* is generally in agreement with the phylogeny inferred by 16S rRNA gene sequences (Ueda *et al*., 1995) and is currently one of the largest non-ribosomal datasets (Zehr *et al*., 2003). More recently, Roeselers, *et al*., (2007) used *nifD*, a gene encoding the dinitrogenase enzyme, as a phylogenetic marker, and found it to give more resolution than *nifH* among closely related diazotrophic cyanobacteria, although compared to *nifH* there are relatively few *nifD* sequences available for

phylogenetic analysis. An important drawback of these protein-encoding genes is that they are present in only a limited number of cyanobacterial genera. Janse *et al*. (2003) focused their research on the rRNA 16S to 23S internal transcribed spacer (rRNA-ITS), which allowed high-resolution discrimination of a variety of cyanobacteria, including *Microcystis* spp. The difference in resolution with 16S and ITS DGGE in *Microcystis* can be explained by the fact that the average sequence diversity of rRNA 16S is less than 1%, (Boyer *et al*., 2001), whereas that of rRNA-ITS is up to 7% (Otsuka *et al*., 1999). It may therefore be possible to gain more information on the specific *Microcystis* species in each sample by performing an rRNA-ITS DGGE.

Some of the apparently different *Microcystis* species in the two cyanobacterial DGGE gels may in fact be multiple bands of one species. Nikolausz *et al*. (2005) observed that dominant amplicons could be distributed at different positions in the same pattern. If several domains have similar melting properties, stochastic effects may cause one to denature before the other in a fraction of the amplicon population and could also explain the presence of different bands with the same sequence in one lane (Boutte *et al*., 2006). Thus, cyanobacterial sequences from bands 10, 11, 12 and 16 in the unicellular specific gel may in fact be one species of *Microcystis*, as they all group together in the phylogenetic tree.

The general bacterial 16S rDNA DGGE gel provided further information on the cyanobacterial species in the treated and control areas. Generally, the *Microcystis* species became dominant earlier in summer in the control area than in the treated area, and in some cases were absent from the treated area during January and February. Band 13 was a close match to the heterocystous filamentous cyanobacterium *Anabaena flos-aquae*, and was the only filamentous cyanobacterial species to be detected on the gel. Interestingly, it was not present on the filamentous specific cyanobacterial gel. This band was present in the treated and control areas until October, after which it was more dominant in the treated areas until February. It thus followed the same pattern as the filamentous cyanobacteria in the filamentous specific gel. The general bacterial DGGE gel therefore provided a confirmation of the information already gained from the cyanobacterial specific gels, but did not provide as much detail. The use of cyanobacterial specific primers prevents the amplification of the abundant DNA of non-cyanobacterial microbes in field samples. The resulting DGGE profiles are less complex

than those generated with general bacterial primers, making detection of cyanobacteria that are less abundant or have lower amplification efficiencies more feasible (Janse *et al*., 2003). This is clear when the DGGE gels in this study are compared, as the profile generated from general bacterial primers contained less bands corresponding to cyanobacteria than in the cyanobacterial specific gels for each sample, especially the filamentous species.

The bacterial species composition represented in the general DGGE gel appeared to be affected by the presence of the cyanobacteria in the water, or at least by the seasonal changes experienced in the water body which coincided with the increase in cyanobacterial growth. As the cyanobacteria became more dominant in the treated and control areas from October, there appeared to be a shift in the bacterioplankton population. Species of Actinobacteria and Bacteroidetes were present in both the treated and control areas only until October, with one species of Actinobacteria only being present in the treated area (represented by band 6). From November, the bacterioplankton population was dominated by β- and δ-proteobacteria. An α-proteobacteria, represented by band 2, was present in both areas throughout the months sampled. Van der Gught *et al*. (2005) investigated the bacterial community composition of four lakes with different nutrient loads (eutrophic and hypertrophic) and turbidity (turbid and clearwater). They found that in shollow eutrophic and hypertrophic lakes, the bacterioplankton was dominated by α- and β-proteobacteria, Bacteroidetes and Actinobacteria, with a low frequency of δ-proteobacteria. In the hypertrophic turbid Lake Blankaart, Actinobacteria were dominant, whereas in the eutrophic turbid Lake Visvijver, β-proteobacteria were dominant. In both clearwater lakes (one hypertrophic and one eutrophic) β-proteobacteria were dominant. The clearwater lakes had a higher percentage of Bacteroidetes, and the turbid lakes a higher percentage of cyanobacteria. This is in agreement with Zwart *et al*. (2002) who found a similar species composition in eutrophic water bodies. The results from this study agree in part with these findings. The treated and control areas revealed an almost identical species composition to those investigated by Van der Gught *et al*. (2005). The treated and control areas were both turbid from November, but were dominated by β- and δ-proteobacteria rather than Actinobacteria. In fact, the Actinobacteria species were not present after October in either the treated or control area, but the Bacteroidetes disappeared as expected as the water shifted from a clearwater to a turbid state. The β- and δ-proteobacteria present

appear to be tolerant to the turbid conditions resulting from a cyanobacterial bloom, and in fact may be species that associate with the bloom. The species composition of the bacterioplankton population therefore appeared to be affected more by the turbidity caused from the presence of cyanobacteria than from the nutrient composition of the water, as there was very little difference between the control and treated areas.

Although the Phoslock® treatment did appear to affect the cyanobacterial species composition in the treated area when compared to the control area, in both the treated and control areas the greatest effect on the cyanobacterial and bacterial populations seemed to be related to seasonal changes. The Phoslock® treatment did not prevent the development of an algal bloom, but this is likely due to the fact that a large amount of nutrient rich water flowed into the treated site at the start of the rainy season in October (Chapter 4).

5. Conclusion

It can be seen from the results that using cyanobacterial specific primers to analyse the cyanobacterial community composition by DGGE was necessary, as general bacterial primers did not give a detailed picture of the cyanobacterial species present in a sample. Using the 16S rRNA gene as a target was practical, as the database of these sequences is the largest. However, the resolution of certain species, the most notable of these being *Microcystis* spp., is low when this region is used. If resolution is required below species level for *Microcystis*, DGGE of the rRNA-ITS region should be considered.

The lower phosphorus concentration in the treated area encouraged the presence of diatoms, which are indicators of healthy species diversity. In terms of the cyanobacteria, the difference in trophic status between the treated and control areas had a greater effect on the filamentous cyanobacterial population, which were more prevalent in the treated area during the summer months than in the control area. The unicellular cyanobacteria were present in both areas, but there appeared to be a lag in the appearance of these species in the treated area. The Phoslock® treatment therefore appeared to affect the cyanobacterial species composition, resulting in an increase in diversity and a slower bloom time.

The bacterioplankton species in both the treated and control areas were similar to those found in other eutrophic and hypertrophic dams. The presence of cyanobacteria in the water appeared to cause a population shift in the bacterial population, which was most likely due to an increase in the turbidity of the water as the cyanobacterial bloom developed. The Phoslock® treatment did not appear to affect the bacterial population, as the treated and control areas displayed similar patterns.

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