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Could the presence of larger fractions of non-cyanobacterial species be used as a predictor of microcystin production under variable nutrient regimes?

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Abstract

The occurrence of cyanobacteria and microcystin is highly dynamic in natural environments and poses one of the biggest challenges to water resources management. While a number of drivers are known to be responsible for the occurrence of cyanobacterial blooms, the drivers of microcystins production are not adequately known. This study aims to quantify the effects of the changes in the structures of phytoplankton and cyanobacterial communities on the dynamics of microcystin production under highly variable nutrient concentration. In our study, nutrient variability could explain 64\% of the variability in microcystin production. When changes in the fractions of non-cyanobacteria versus cyanobacteria genera were additionally included, 80\% of the variability in microcystin production could be explained; under high nutrient concentrations, non-cyanobacterial phytoplankton groups were dominant over cyanobacteria and cyanobacteria produced more toxins. In contrast, changes in the cyanobacterial community structures could only explain a further 4\% of the dynamics of microcystin production. As such, the dominance of non-cyanobacterial groups appears to be a useful factor to explain microcystin occurrence in
addition to traditionally used factors such as absolute cyanobacterial cell numbers, especially when the nutrient regime is taken into account. This information could help to further refine the risk assessment frameworks which are currently used to manage the risk posed by cyanobacterial blooms.

**Keywords** Phytoplankton; cyanobacteria; microcystin production; allelopathic interaction; nutrients; public health

**Introduction**

An adequate understanding on the dynamics of microcystin production will lead to a better management of cyanobacterial blooms and reduce their impact on water resources. While the occurrence of algal blooms is considered to be a significant threat to water safety and security around the world, the presence of high level of cyanotoxins, such as microcystins, significantly increase the risk to the environment and the public. As such, it is critical to understand the key drivers behind the toxin production during a bloom situation.

The dynamics of microcystin production in a bloom depends on the toxin production on a cellular level, caused by changes in environmental factors such as nutrients and light (Chorus and Bartram 1999, Van de Waal et al. 2009, Zurawell et al. 2005), and has been shown to be associated with the changes in the planktonic communities’ structure, in particular phytoplankton populations, including cyanobacterial as well as non-cyanobacterial species (Holland and Kinnear 2013, Joung et al. 2011, Pflugmacher 2002, Yang et al. 2014), and with changes in the ratio of toxic to non-toxic genotypes (Janse et al. 2005, Kardinaal et al. 2007). To date, many causation factors leading to changes in the community structure of phytoplankton populations and the development of cyanobacterial blooms have been proposed and debated (Dadheech et al. 2014, Macías et al. 2008, O'Neil et al. 2012, Paerl and Otten 2013).
Allelopathy has been suggested as a key mechanism in determining the community structure and the
dynamics of phytoplankton populations (Holland and Kinnear 2013, Macías et al. 2008). The theory of
allelopathy suggests that the ability of certain phytoplankton groups to dominate the community may
be enhanced by the production of toxic or non-toxic compounds (Camacho 2008, Graneli and Hansen
2006, Li and Li 2011, O’Neil et al. 2012). This theory has been used in an attempt to explain the
ecological role of microcystin production in cyanobacteria; however, this has not been extensively
supported by experimental data (Babica et al. 2006, Berry et al. 2008, Jang et al. 2006). As such,
cyanobacteria species could increase their competitive advantage through the production of
microcystin (Huisman and Hulot 2005), especially under certain conditions of nutrient limitation
(Graneli et al. 2008). Phytoplankton exposure to microcystin has been suggested to be associated with
growth inhibition and deterioration of photosynthesis and enzymatic activities in some non-
cyanobacteria species (Babica et al. 2006, O’Neil et al. 2012). Consequently, it can be hypothesized
that the dynamics of microcystin production is related to the inter-species interaction between
cyanobacteria and other phytoplankton species, interaction within cyanobacterial communities, as well
as the direct effects of environmental factors on phytoplankton. While the concept of allelopathy is
hard to prove in situ, as the effect of the chemical on the receiving organism is alleviated by the effects
of other environmental factors, an increase in the production of toxins by cyanobacteria in the presence
of a larger fraction of non-cyanobacterial genera might be an indirect indicator for allelopathy.

Many studies have been focusing on the effect of nutrients on microcystin production (Chorus and
Bartram 1999, Orihel et al. 2012, Zurawell et al. 2005); while a limited number of studies have also
investigated the combined effects of phytoplankton community structure and cyanobacterial species
composition on microcystin production (Engström-Öst et al. 2011, Kardinaal et al. 2007, Millie et al.
2009), none has studied this in relation to changes in nutrient levels. This information is essential and
would enable managers to assess the potential toxicity of cyanobacterial blooms from the structure of
the phytoplankton community and cyanobacterial composition under different environmental
conditions. The present study aims to quantify the effects of the changes in the structure of
phytoplankton communities, including cyanobacterial species composition, on the dynamics of microcystin production under highly variable nutrient regimes. It is anticipated that nutrient dynamics, specifically phosphorus, nitrogen and iron, alone are not good predictors of cyanotoxin production and that key changes in the phytoplankton communities, especially a dominance of non-toxin producing classes, such as chlorophytes or diatoms, may be responsible for triggering an allopathetic response leading to a higher toxin production.

**Materials and Methods**

**Study area**

This study was carried out in Jackadder Lake (31°54′30″S, 115°47′36″E), Bibra Lake (32°5′25″S, 115°49′16″E) and Yangebup Lake (32°6′56″S, 115°49′33″E) located on the Swan Coastal Plain, Western Australia. These lakes are shallow with mean depth of 2.1 m, 1.1 m, and 2.5 m in Jackadder Lake, Bibra Lake and Yangebup Lake, respectively. Jackadder Lake is surrounded by 6.6 ha of parkland (Arnold 1990, Woodward 2008), while Bibra Lake is surrounded by urban areas and a golf course. Yangebup Lake is surrounded by residential, agriculture and industrial areas. Jackadder Lake and Yangebup Lake are permanent lakes, while Bibra Lake is subject to seasonal drying. Prolonged stable thermal stratification is usually prevented in these lakes during summer due to continuous or intermittent wind mixing that creates a homogeneous environment throughout the water column (Arnold and Oldham 1997, Davis et al. 1993). These lakes were selected due to levels of cyanobacterial biomass and microcystin concentration reported in earlier extensive sampling (Reichwaldt et al. 2013, Sinang et al. 2013). These lakes represent systems with low, medium and high cyanobacterial biomass and microcystin concentration. Mean cyanobacterial biomass in an earlier study was reported as 28 µg chl-α L⁻¹ in Jackadder Lake, 108 µg chl-α L⁻¹ in Bibra Lake, and 80 µg chl-α L⁻¹ in Yangebup Lake. Mean cellular microcystin concentrations (mg g⁻¹ cyanobacterial dry mass) was 4.8 mg g⁻¹ in Jackadder Lake, 35 mg g⁻¹ in Bibra Lake and 1.7 mg g⁻¹ in Yangebup Lake (Sinang et al. 2013).
**Sampling**

The study lakes were sampled twice a month between January and March 2010. Samples were collected from three points on each lake on every sampling occasion. Samples were taken from waist-deep water at the same point on every sampling occasion. Bibra Lake dried up in late February; therefore no samples were taken from this lake in March.

Grab water samples were taken from 15 cm below the surface and stored immediately in a cool container. Parameters analysed from these samples were total phosphorus (TP), total dissolved phosphorus (TDP), total iron (TFe), total dissolved iron (TDFe), total nitrogen (TN), total dissolved nitrogen (TDN), ammonium (NH$_4^+$), cyanobacterial biomass, total phytoplankton biomass, intracellular and extracellular microcystin fractions, and cellular microcystin concentration. Samples for dissolved nutrients analyses were pre-filtered with a 0.45µm syringe filter (Acrodisc, HT Tuffryn) before storing at -20°C. Water samples for phytoplankton enumeration were preserved with Lugol’s iodine (1 % final concentration).

**Nutrient analyses and phytoplankton biomass**

TP and TDP concentrations were analyzed using the ascorbic acid method, while TFe and TDFe concentrations were analyzed with the Phenanthroline method, according to standard methods (APHA 1998). TN, TDN, and NH$_4^+$ were analyzed at the South Coast Nutrients Analysis Laboratory (Albany, Western Australia) with the standard colorimetric methods on a segmented flow auto-analyser (Alpkem, Wilsonville, OR, USA). Cyanobacterial and total phytoplankton chlorophyll-α were measured with a benchtop version of a FluoroProbe (bbe Moldaenke, Germany), which is capable of identifying up to four spectral algal groups based on their specific excitation spectrum of chlorophyll fluorescence (Beutler et al. 2002, Ghadouani and Smith 2005). The biomass of each group is given in equivalent µg chl-α L$^{-1}$. Fluoroprobe measurements of total chlorophyll-α gave a good correlation.
against samples extracted according to standard methods (APHA 1998) (linear regression analysis: $R^2 = 0.94$, $N = 32$, $P < 0.05$) (Sinang, S.C., unpublished data).

Water samples were filtered through pre-combusted and pre-weighed 47mm GF/C filter papers to collect the phytoplankton biomass and to separate intracellular from the dissolved microcystin fraction. Filter papers containing particulate organic matter and phytoplankton biomass were dried at 60°C and re-weighed after 24 hours to obtain the dry biomass (Chorus and Bartram 1999). These filter papers were then moistened with Milli-Q water and stored at -20°C until intracellular microcystin extraction.

As we were interested in the actual toxic potential of cyanobacteria, cyanobacterial dry mass was calculated from the total dry mass by adjusting it to the percentage of cyanobacterial biomass measured with the FluoroProbe.

Cyanobacteria were identified and enumerated to the taxonomic level of genera with 10-50 mL water samples fixed in Lugol’s solution using an inverse microscope according to Utermöhl (Utermöhl 1958). A minimum of 200 cells or colonies of the most abundant cyanobacteria were counted, and the size of 20 individual cells or colonies of each occurring genus was measured by using an inverse microscope. Cell densities were converted to biovolume by means of geometric figures that best approximated the shape of individual taxa (Hillebrand et al. 1999). The biovolume of each cyanobacterial genera was then used to calculate the biovolume fraction of the respective cyanobacterial genera in the total cyanobacterial community.

**Microcystin extraction and quantification**

Filters were freeze-thawed twice to break the cells prior to methanol extraction (Lawton et al. 1994). Each filter was placed into a separate centrifuge tube and 5mL 75 % methanol-water (v/v) was added. Filters were sonicated on ice for 25 minutes, followed by gentle shaking for another 25 minutes. The extracts were then centrifuged at 3750 rpm for 10 minutes at room temperature. Extracts were carefully transferred into conical flasks, and two more extractions were done per filter. All three extracts (75 % methanol) were pooled and diluted with Milli-Q to 20 % methanol. Intracellular
microcystin extracts and the pre-filtered water containing dissolved microcystin were subjected to solid phase extraction (SPE) (Waters Oasis HLB) as a clean-up step and for concentrating the microcystins. Sample loading speed to the SPE was adjusted to <10 mL per minute. After the samples had passed through, each cartridge was rinsed with 10 mL 10, 20 and 30 % methanol-water (v/v), before microcystin was eluted with 100 % methanol + 0.1 % trifluoroacetic acid (TFA) into 6mL glass vials and samples were evaporated to dryness under a mild nitrogen stream in a 40°C water bath. Finally, samples were re-dissolved in 30 % acetonitrile and analysed with HPLC-PDA systems (Waters, Alliance 2695).

HPLC analysis was carried out by using the Alliance 2695 (Waters, Australia) with a PDA detector (1.2nm resolution) and an Atlantis T3 3μm column (4.6 x 150mm i.d). Mobile phases used were acetonitrile + 0.05 % v/v trifluoroacetic acid (TFA) and Milli-Q water + 0.05 % v/v TFA. Microcystin peak separation was achieved by using a linear gradient a flow rate of 0.6 mL min⁻¹ (ISO 2005). Column temperature was maintained at 37.5 ± 2.5 °C. The limit of detection per microcystin peak was 1.12ng. Microcystin was identified by PDA spectra at 238nm. Commercially available microcystin-LR standard (Sapphire Bioscience, Australia; purity ≥ 95 %) was used to quantify microcystin concentrations in all samples.

Statistical analyses

Redundancy analysis (RDA) (Legendre and Legendre 1998) was carried out in R language to quantify the effects of nutrients, and the structure of phytoplankton and cyanobacterial community on microcystin production. The RDA analysis was carried out using the combined data from three lakes as we emphasize on finding the general trend rather than individual lake pattern. Explanatory variables used in the RDA analysis were nutrient concentrations in the water column, the fraction of different phytoplankton groups of total phytoplankton biomass, and the fraction of different cyanobacterial genera of total cyanobacterial community. Response variables used in the RDA analysis were cellular microcystin concentration and total microcystin concentration in the water column. The canonical
relationship was computed with standardized explanatory and response variables and tested for the level of significant after 999 permutations. In all analyses, results were considered significant at $P < 0.05$.

**Results**

*Nutrient profiles of studied lakes*

The nutrient profiles in Jackadder, Bibra and Yangebup Lakes during the study period are shown in Fig. 1A-H. Nutrient concentrations varied on a temporal basis within lakes and spatially between lakes. Phosphorus concentrations were higher in Bibra Lake than in Jackadder and Yangebup Lakes throughout the sampling period. Mean TP concentrations (Fig. 1A) ranged from 22 up to ~92 µgL$^{-1}$, 230 to $>1000$ µgL$^{-1}$, and 28 to $>150$ µgL$^{-1}$ in Jackadder, Bibra and Yangebup Lakes, respectively. Meanwhile, mean TDP concentrations (Fig. 1B) ranged from 12 to 24 µgL$^{-1}$, 17 to 142 µgL$^{-1}$, and 14 to 37 µgL$^{-1}$ in Jackadder, Bibra and Yangebup Lakes, respectively.

TN (Fig. 1C) and TDN (Fig. 1D) concentrations in Bibra Lake were higher by up to one order of magnitude than concentrations in Jackadder and Yangebup Lakes. In contrast, mean molar TN:TP in Bibra Lake was lower than the ratios in Jackadder and Yangebup Lakes (Fig. 1E). Mean TN:TP ranged from 40 to 132, 36 to 84, and 65 to 255 in Jackadder, Bibra and Yangebup Lakes, respectively. NH$_4^+$ decreased over time in Jackadder and Yangebup Lakes (Fig. 1F). Mean NH$_4^+$ concentrations ranged from 43 to 170 µgL$^{-1}$, 157 to 239, and 40 to 143 in Jackadder, Bibra and Yangebup Lakes, respectively. Mean TFe (Fig. 1G) and TDFe (Fig. 1H) concentrations were higher in Bibra Lake than in the other two lakes during the beginning of the study. Mean TFe ranged from 77 to 247 µgL$^{-1}$, 147 to 220 µgL$^{-1}$, and 51 to 110 µgL$^{-1}$ in Jackadder, Bibra and Yangebup Lakes, respectively. Mean TDFe ranged from 24 to 174 µgL$^{-1}$, 61 to 117 µgL$^{-1}$, and 21 to 89 µgL$^{-1}$ in Jackadder, Bibra and Yangebup Lakes, respectively.
The phytoplankton composition in Jackadder, Bibra and Yangebup Lakes changed during the study period (Fig. 2). Phytoplankton composition varied on a temporal basis within lakes and spatially between lakes. In Jackadder Lake, cyanobacteria were dominant (68%) from January to early February. In late February, the phytoplankton community shifted to mainly chlorophytes and diatoms, and cyanobacteria only accounted for 7% of the community. Then in early March, cyanobacteria started to increase and contributed to 67% of the total phytoplankton biomass by the end of March. In Bibra Lake, chlorophytes dominated with the biomass up to 66%, while cyanobacteria only contributed 27% to the total phytoplankton biomass in January. Cyanobacteria contributed up to 49% of the total phytoplankton biomass in early February, and the dominant phytoplankton changed to chlorophytes in late February. In Yangebup Lake, cyanobacteria were dominant in January and cyanobacteria contributed up to 67% to the total phytoplankton biomass. In February, cyanobacterial fraction in the total phytoplankton biomass decreased from 64 to 17%, and chlorophytes took over the major fraction of total phytoplankton biomass in late February. In early March, cyanobacteria contributed 48% to the total phytoplankton biomass in Yangebup Lake, and the fraction decreased to 32% in late March.

At the cyanobacterial genus level, cyanobacterial composition was highly variable between lakes, and, within a lake, in time. The cyanobacterial community in Jackadder Lake was mainly composed of three potential microcystin producing genera, namely *Microcystis* spp., *Planktothrix* spp. and *Anabaena* spp. (Fig. 3A). There was a shift of dominance from *Microcystis* spp. to *Planktothrix* spp. within a 7 day period in January, after which *Microcystis* spp. was again dominant (Fig. 3A). *Planktothrix* spp. biovolume reached $2.208 \times 10^3 \text{ mm}^3 \text{ mL}^{-1}$ in late January and decreased continuously towards the end of the study period (Fig. 4A). From February until the end of the study period, *Microcystis* spp. was the major fraction in the cyanobacterial community with biovolumes ranging from $0.318 \times 10^3$ to $1.896 \times 10^3 \text{ mm}^3 \text{ mL}^{-1}$ (Fig. 4A). *Anabaena* spp. was present as a small fraction in
January and February and increased to 50% of the total cyanobacterial community in March (Fig. 3A).

In Bibra Lake, *Anabaenopsis* spp., *Anabaena* spp., *Planktothrix* spp., *Nodularia* spp. and *Microcystis* spp. were the cyanobacteria detected, and their percentage biomass varied over time (Fig. 3B). In January, there was a change in dominance from *Anabaenopsis* spp. to *Microcystis* spp. within a 7 day period (Fig. 3B). During its dominance in early January, *Anabaenopsis* spp. reached a biovolume of 4.884 x 10³ mm³ mL⁻¹, but it was no longer detected after the end of February (Fig. 4B).

Simultaneously with the decrease of *Anabaenopsis* spp, the fraction of *Microcystis* spp. increased from early January and remained the dominant cyanobacterial genera throughout the study period (Fig. 3B). *Microcystis* spp. biovolumes ranged from 1.865 x 10³ to 111.34 x 10³ mm³ mL⁻¹ (Fig. 4B). *Anabaena* spp. also coexisted, but the total biovolume was always less than 50% of total cyanobacteria biomass (Fig. 3B). The highest *Anabaena* spp. biovolume was detected at 80.2 x 10³ mm³ mL⁻¹ in early February (Fig. 4B). *Planktothrix* spp. and *Nodularia* spp. contributed less than 5% of the total cyanobacterial biomass in Bibra Lake (Fig. 3B). In Yangebup Lake, *Microcystis* spp. was the dominant genera throughout the study period (Fig. 3C). *Microcystis* spp. biovolumes ranged from 2.766 x 10³ to 9.839 x 10³ mm³ mL⁻¹ (Fig. 4C). *Planktothrix* spp. and *Nodularia* spp. contributed less than 20 and 1% to the total cyanobacterial biomass, respectively (Fig. 3C).

Cellular microcystin concentration (mg MC-LR<sub>equiv</sub> g⁻¹ cyanobacterial dry mass) and total microcystin concentration in the water were highly variable over time in all lakes (Fig. 5). Cellular microcystin concentration ranged in three orders of magnitude in Jackadder Lake, and two orders of magnitude in Yangebup and Bibra Lakes. In all lakes, cellular microcystin concentration increased from January to February and decreased in March. The highest mean cellular microcystin concentration occurred in late February in all lakes. The highest mean cellular microcystin concentration was 1.4 mg g⁻¹ dry mass in Jackadder Lake, 0.6 mg g⁻¹ dry mass in Bibra Lake, and 0.5 mg g⁻¹ dry mass in Yangebup Lake. Total microcystin concentration in the water varied by an order of magnitude in all lakes. In Bibra Lake, total microcystin concentration increased significantly from January to February, and mean concentrations ranged between 0.7 µg L⁻¹ in January to 6.9 µg L⁻¹ in February. In Jackadder
Lake, mean total microcystin concentration ranged between 0.2 and 1.3 µg L⁻¹, and the highest concentration occurred in early March. In Yangebup Lake, mean total microcystin varied between 0.4 and 2.1 µg L⁻¹, and the highest mean concentration was also detected in early March.

Correlations between nutrients, phytoplankton and cyanobacterial community structure and microcystin production

RDA analysis showed that the dynamics of microcystin production in the studied lakes were significantly correlated to nutrient concentrations in the water column. Nutrients explained 64% of variation in cellular microcystin concentration (Fig. 6). Cellular microcystin concentration was closely correlated to iron concentration, while total microcystin concentration in the water was closely correlated to phosphorus and nitrogen availability in the systems. Inclusion of cyanobacterial composition as an explanatory variable in addition to nutrients increased the percentage explained from 64% to 68% (P < 0.05), suggesting that nutrients were more important as a factor influencing the dynamics of cellular microcystin production than the cyanobacterial composition (data not shown). In contrast, including the phytoplankton community as an explanatory variable in addition to nutrients, 80% of variability in microcystin production could be explained (P < 0.05) (Fig. 7). The combination of the phytoplankton community and nutrients as explanatory variables increased the ability to predict the dynamics of microcystin production by 16%, indicating the significance of both variables on microcystin production. As shown in the RDA biplot (Fig. 7), high microcystin production correlated to high nutrient concentrations in the presence of a high fraction of chlorophytes and diatoms and a low fraction of cyanobacteria in the phytoplankton community. In addition, higher cellular and total microcystin concentrations were associated with lower fractions of cyanobacteria present in the systems (Fig. 8). There was a significant, but very weak correlation between total cyanobacterial biomass and total microcystin concentration in the water (R² = 0.175; P < 0.05; y = 0.99 + 0.05*x).
Changes in water levels during dry months can have strong effects on nutrient dynamics, which subsequently could affect the structure of phytoplankton communities in lakes (Coops et al. 2003, Jeppesen et al. 2015). As such, this study has taken into account both nutrient and phytoplankton dynamics to understand the dynamics of microcystin production. The results of our study suggested that high microcystin production in the studied lakes was significantly correlated to high nutrient concentration and low TN:TP. Our results agree with previous studies, which have reported that increased phosphorus, nitrogen and iron concentrations could enhance microcystin production (Albay et al. 2005, Jiang et al. 2008, Scott et al. 2014), and that higher microcystin concentrations can be found at lower TN:TP ratios (Orihel et al. 2012). The observed close positive relationships between microcystin production with phosphorus and nitrogen are potentially due to the high cyanobacterial cellular requirement of phosphorus for microcystin biosynthesis and biomass production (Vezie et al. 2002). In addition, a positive correlation between iron and microcystin production may be related to an earlier suggestion that microcystin production involves enzyme synthetase, in which the enzyme's activity is controlled by the amount of iron present (Utkilen and Gjolme 1995).

In addition to the possible direct effect of nutrients on the dynamic of microcystin production, previous studies have also suggested that nutrients might indirectly regulate microcystin production through their effects on cyanobacterial abundance and its distribution (Dai et al. 2008, Millie et al. 2009, Wu et al. 2014). Unlike chlorophytes and diatoms, a high cyanobacterial fraction was pointing in the opposite direction to high nutrient availability (Fig. 7). This result indicates that high nutrient concentrations in our systems did not favor the dominance of cyanobacterial genera. Instead, high phosphorus, nitrogen and iron concentrations in the water column may have triggered the dominance of chlorophytes and diatoms, due to their ability to grow faster than cyanobacteria (Huisman et al. 2005, Jensen et al. 1994, Reynolds et al. 2006).

Microcystin production is complex and various environmental factors including nutrients have been shown to have direct effects on the dynamics of microcystin production (Rinta-Kanto et al. 2009). Our
study suggests that the dominance of non-cyanobacterial groups is an additional factor to be considered and that changes in the structure of the phytoplankton community may have a large effect on microcystin production. Changes in the structure of the phytoplankton community explained up to 16% of the variability in microcystin production. Moreover, cyanobacteria tend to increase microcystin production in the presence of a high fraction of other phytoplankton groups. This result supports the theory of allelopathic interactions in cyanobacteria through the higher production of toxin microcystin with an increasing number of competitors (Holland and Kinnear 2013, Huisman and Hulot 2005, Rzymski et al. 2014). Earlier studies on allelopathy suggest that cyanobacteria may be capable of react to the presence of competitors by increasing microcystin production stimulated by the presence of extracellular products released by competitors such as chlorophytes (Bittencourt-Oliveira et al. 2014, Kearns and Hunter 2000, Pinheiro et al. 2013). In addition to the effects of changes in the structure of phytoplankton community, the dynamics of microcystin production could also be related to the succession of toxic and non-toxic cyanobacterial genotypes (Janse et al. 2005, Kardinaal et al. 2007, O’Neil et al. 2012), which was not analyzed in this study.

Based on the available cyanobacterial genera composition data, our results suggest that the changes in the structure of the cyanobacterial community did not affect the dynamics of microcystin production to a large extent. This result indicates that the succession of *Planktothrix* spp., *Anabaena* spp., *Nodularia* sp., *Anabaenopsis* spp., and *Microcystis* spp. in our lakes may not be related to changes in microcystin production in response to cyanobacterial competitors, and, as such would not support the theory of allelopathic interaction between different cyanobacteria genera (Paerl and Otten 2013, Švercel 2013). Rather, the succession of different cyanobacteria genera may be related to different cyanobacterial genera benefitting from different concentrations of phosphorus, nitrogen and iron species (Chorus and Bartram 1999, Huisman and Hulot 2005). Drastic changes to the structure of cyanobacterial communities are possible even with only slight changes in the cyanobacterial total biomass (Soares et al. 2009). It was suggested that this could be due to the short doubling times of cyanobacteria in
addition to different cyanobacterial genera occupying different niches, which develop due to temporal
changes in the water’s physicochemical properties (Reynolds 1998).

As an example, *Microcystis* spp. are known to be capable to store high amounts of phosphorus and to
use phosphorus very efficiently (Baldia et al. 2007), while *Planktothrix* spp. has a lower affinity
towards phosphorus (Istvánovics et al. 2002). In addition, Nagai *et al.* (2007), has suggested that
*Planktothrix agardhii* requires an iron concentration 30 times higher compared to *Microcystis
aeruginosa* to achieve the same growth rate. This indicates that cyanobacteria may react differently to
environmental conditions created by other genera, which could lead to drastic changes in the structure
of the cyanobacterial community over short periods of time (Beversdorf 2013). As an example, the
higher iron requirement of *Planktothrix* spp. might reduce the iron availability in the water column,
and it would thus become favorable for *Microcystis* spp. in the system. Therefore, the succession of
different cyanobacteria genera is a driven by a complex interaction of environmental conditions,
including the availability of nutrients, and allelopathic interactions through cyanotoxins or other
metabolites (Śvercel 2013).

**Conclusions**

Our results indicate that the changes in the structure of the phytoplankton community and changes in
nutrient concentrations are important factors that determine the dynamics of microcystin production.
Under high nutrient concentration, non-cyanobacterial phytoplankton groups were dominant over
cyanobacteria, and cyanobacteria produced more microcystin. Our results might therefore support the
theory of allelopathic interactions between cyanobacteria and non-cyanobacterial competitors. We
conclude that eutrophication could lead to the occurrence of more toxic cyanobacterial blooms directly
through increased nutrient concentrations, and indirectly through changes in the phytoplankton
community leading to increased toxin production within the cyanobacterial cells.

Our results also suggest that changes in cyanobacterial community composition may not be of major
importance for the dynamics of microcystin production. In relation to the microcystin risk assessment
during toxic cyanobacterial blooms, our results therefore suggest to consider the fraction of total cyanobacteria in addition to the absolute cyanobacterial biomass, which is currently the basis of existing WHO alert levels for microcystin risk assessment (Churro et al. 2012). Our conclusion is drawn based on the quantification of total biomass for each cyanobacterial genera without differentiating between microcystin producing strains and non-producing strains. The quantification of toxic and non-toxic cyanobacteria strains would have allowed for a deeper understanding of the microcystin dynamics and the dominance within the cyanobacteria community and we therefore suggest to include molecular characterisation of cyanobacterial communities in future studies to get a clearer picture of the role of strain dynamics on microcystin production, which would help to develop a more assertive microcystin risk assessment.

Additionally, a direct relationship between the structure of the phytoplankton/cyanobacteria community and microcystin production is likely to be established. Such a relationship illustrates the possibility of using the cyanobacterial fraction in the phytoplankton community as an ecological indicator to infer the level of microcystin production or risk of microcystin contamination in shallow water bodies. The relationship observed in this study was in agreement with the theory of allelopathic interaction between cyanobacteria and non-cyanobacteria. As direct microcystin quantification is not regularly conducted in water quality monitoring, the observation of high nutrient concentrations in the presence of potentially toxic cyanobacterial species, together with the dominance of non-cyanobacterial phytoplankton groups over cyanobacteria could be used as an indication that toxin production per cell is high and should prompt direct microcystin quantification. Even so, we propose further testing of this relationship with a larger number of lakes, including verification for single lakes, to prove its application for lake management.

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Fig. 1 Mean nutrient concentrations (A: TP; B: TDP; C: TN; D: TDN; E: TN:TP; F: NH₄⁺; G: TFe; H: TDFe) in Jackadder, Bibra and Yangebup Lakes from January to March 2010.

Fig. 2 Phytoplankton community composition (% of total) in (A) Jackadder, (B) Bibra and (C) Yangebup Lakes from January to March 2010. Bibra Lake dried up in early March; therefore no samples were taken from this lake in March.

Fig. 3 Cyanobacterial community composition (% of total) in (A) Jackadder, (B) Bibra and (C) Yangebup Lakes from January to March 2010. Bibra Lake dried up in early March; therefore no samples were taken from this lake in March.

Fig. 4 Biovolume concentrations (mm³ mL⁻¹) of cyanobacterial genera over time in (A) Jackadder, (B) Bibra and (C) Yangebup Lakes from January to March 2010. Bibra Lake dried up in early March; therefore no samples were taken from this lake in March.

Fig. 5 Mean cellular microcystin concentration (mg g⁻¹ cyanobacterial dry mass) and mean total microcystin concentration in the water (µg L⁻¹) in Jackadder, Bibra and Yangebup Lakes from January to March 2010; error bars represent standard errors.

Fig. 6 RDA biplot of cellular microcystin (cMC) and total microcystin (tMC) concentration with nutrients; solid lines = environmental variables; short dashed lines = response variables. Canonical axis 1 and 2 represent a linear combination of the environmental variables.

Fig. 7 RDA biplot of cellular microcystin (cMC) and total microcystin (tMC) with nutrients and phytoplankton community (Chloro = chlorophytes; Crypto = cryptophytes; Diatom; and Cyano =
cyanobacteria); solid lines = environmental variables; short dashed lines = response variables. Coalition axis 1 and 2 represent a linear combination of the environmental variables.

Fig. 8 Correlation between cyanobacterial fraction and cellular microcystin concentration (mg g⁻¹ cyanobacterial dry mass) (solid line; $R^2 = 0.542; P < 0.05; y = 2.85 - 1.78x$) or total microcystin concentration (µg L⁻¹) (dashed line; $R^2 = 0.105, P < 0.05, y = 2.96 - 3.08x$).
Fig. 2
Fig. 3

(A) Jackadder Lake

(B) Bibra Lake

(C) Yangebup Lake

Mean phytoplankton biomass (% of total biomass)

Sampling date
Fig. 4

(A) Jackadder Lake

(B) Bibra Lake

(C) Yangebup Lake

Mean cyanobacterial genera biomass (% of total cyanobacterial biomass)

Sampling date: 19 Jan, 26 Jan, 9 Feb, 23 Feb, 9 Mar, 23 Mar
Fig. 5

(A) Jackadder Lake
- Cellular microcystin
- Total microcystin

(B) Bibra Lake

(C) Yangebup Lake

Mean cellular microcystin concentration (mg/g cyanobacterial dry mass) vs. Sampling date (19 Jan, 26 Jan, 9 Feb, 23 Feb, 9 Mar, 23 Mar)
Fig. 8

A scatter plot showing the relationship between cellular microcystin concentration (mg/g cyanobacterial dry mass) and cyanobacterial fraction. The plot includes data points for total microcystin and cellular microcystins, with a trend line for each.