The role of nutrients in cyanobacterial blooms in a shallow reservoir

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Certificate of original authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature Date

Acknowledgments

I have been looking forward to writing this section for several months now as I felt that it would mark the completion of my thesis and I should save it up for the very end when every graph would have been plotted, every chapter written and every section typeset. Having now reached this point and looking back, I feel grateful to have had the opportunity to do a PhD thesis in the first place.

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Abstract

This thesis examines potential causes for algal and cyanobacterial blooms in Grahamstown Dam, a shallow mesotrophic drinking water reservoir in coastal NSW, Australia. The objective was to understand the role of nitrogen and phosphorus in algal and cyanobacterial growth and to elucidate other chemical and physical processes that may enhance cyanobacterial growth in the lake.

Algal and cyanobacterial nutrient limitation was examined on different spatial and temporal scales in in situ assays. Other aspects that have been found to promote cyanobacteria, i.e. high irradiance levels as may occur during thermal stratification and trace metal nutrient additions, were investigated in situ. The effects of different nutrient supply ratios and different light climates on growth rate and yield of the prominent potentially toxic cyanobacterium *Anabaena circinalis* were tested in laboratory experiments. Different aspects of nutrient release from the sediments were examined under conditions that may occur during persistent thermal stratification, i.e. bottom water anoxia. Further experiments elucidated the influence of organic substrate on microbially mediated nutrient release process in the sediments.

Phytoplankton biomass and most individual genera were colimited by nitrogen and phosphorus. Further, the growth response of potentially toxic cyanobacteria lagged behind the response of most other phytoplankton. Many algae responded with increased growth to the combination of high irradiance and nutrient enrichment. The response of potentially toxic cyanobacteria was inconclusive. Trace metal nutrient additions enhanced the growth of one potentially toxic cyanobacterium and most non toxic genera. Nitrogen concentration and not nutrient ratio or phosphorus concentration determined yield of *A. circinalis*. This effect was increased by higher irradiance levels. Growth rates were enhanced

by high irradiance and high N concentration. The sediments were a source of N under oxic and anoxic conditions. Small amounts of phosphorus were released during anoxia only when the availability of dissolved organic C was improved, indicating microbiological activity as the cause of phosphorus release. Moreover, iron and phosphorus release was not caused by the same processes in the sediments.

These findings imply that a pulse of nutrients is not likely to lead to cyanobacterial blooms in Grahamstown Dam but it cannot be excluded that a gradual increase in nutrient load would not. Persistent thermal stratification may increase the risk of cyanobacterial growth by providing increased levels of nitrogen and an improved light climate. Unexpected results, such as insensitivity of cyanobacteria to nutrient enrichment, phytoplankton colimitation and decoupling of iron and phosphorus cycling in the sediments suggest that further research on shallow coastal lakes would be useful.

Chapter 1

Introduction

1.1 Scope and need for this study

Cyanobacteria are part of the natural planktonic community in freshwater systems and some species have been identified as the source of potent toxins. Mass occurrences of toxin producing cyanobacteria in lakes and reservoirs pose a major threat to drinking water supplies and can also render waterbodies unfit for irrigation and recreational use.

An increase in the frequency and magnitude of blooms has been observed worldwide (Carmichael, 2008). Affected freshwater systems include some of the world's largest inland waterbodies, for example Lake Victoria (Africa), Lake Erie and Lake Michigan (USA, Canada), Lake Okeechobee (Florida, USA), Lake Taihu (China), Kasumigaura (Japan) and the Caspian Sea in West Asia ((Paerl et al., 2011; Paerl and Otten, 2013). Further, global warming is likely to contribute to the severity of the problem in the future (Paerl and Huisman, 2008; Wagner and Adrian, 2009; Markensten et al., 2010). Already, economic losses due to poor quality of drinking and recreational water caused by cyanobacteria have been estimated to exceed two billion dollars (US) per year in the USA (Dodds et al., 2009).

In Australia, cyanobacterial blooms have been reported since the 19^{th} century (as cited in Stewart et al. (2008) and Codd et al. (2005)) and they occur frequently in rivers and reservoirs. For example, major river blooms include the ones occuring in the Barwon-Darling River in the summer of 1991 (Bowling and Baker,

1996) and in the Murray River in 2009 (Al-Tebrineh et al., 2012). Both affected about 1000 km of each river, threatening livestock and recreational use. Drinking water reservoirs can also be affected as the cyanobacterial outbreaks in Lake Cargelligo in New South Wales in the summer of 1990/91 (Bowling, 1994) and in Sydney's water supply (Lake Burragorang) in 2007 (SCA, 2010) illustrate. Being highly toxic, the bloom in Lake Cargelligo led to the first known closure of a town drinking water supply in New South Wales.

Although there are methods for removing cyanotoxins from drinking waters, they involve sophisticated technology and are costly. A more sustainable solution is the prevention of cyanobacterial mass occurrences through suitable management of waterbodies. Moreover, being capable of predicting the formation of blooms, would allow for provision of alternative, uncontaminated water supplies. Defining adequate management strategies and making predictions about cyanobacterial growth requires knowledge of the phytoplankton ecology and environmental factors influencing cyanobacterial dominance in the respective waterbody.

A substantial amount of research has been conducted on environmental factors favouring cyanobacteria and how cyanobacterial physiology can be advantageous in achieving dominance in phytoplankton communities (Shapiro, 1984; Reynolds et al., 1987; Robarts and Zohary, 1987; Paerl, 1988; Scheffer et al., 1997; Shapiro, 1997; Dokulil and Teubner, 2000). There is general agreement that eutrophication, nutrient enrichment with nitrogen (N) and phosphorus (P) of water bodies, is a main cause for cyanobacterial dominance and bloom formation. Cyanobacterial growth is often limited by the concentrations of single nutrients that can differ from those limiting the growth of other phytoplankton. This characteristic can then give cyanobacteria a competitive advantage.

A universal method for prediction or prevention of excessive cyanobacterial growth that could be applied to all waterbodies does not exist. Individual qualities of each waterbody, such as phytoplankton assemblage structure, degree of nutrient enrichment, morphology and hydrology are of importance in determining when and which phytoplankton will dominate. Thus, there is a need to investigate the conditions leading to cyanobacterial dominance and blooms at a

reservoir or river reach scale.

This study aims at satisfying this need for Grahamstown Dam, one of the main drinking water reservoirs of Newcastle and the lower Hunter Region in New South Wales. Grahamstown Dam currently provides drinking water for half a million people. However, the regional population is predicted to increase by 160 000 by 2031 (Hunter Water, 2011). As a consequence, increased demands on water supply as well as an increased risk for water quality through rising input of nutrients are expected.

Currently, the usually well mixed shallow reservoir can be classified as mesotrophic, i.e. containing a moderate amount of nutrients with moderate levels of phytoplankton productivity. Cyanobacterial blooms necessitating treatment of the water prior to delivery to customers have occurred in the reservoir but are not common. As the lake's catchment is too small to meet demands; it comprises 99 km^2 of which the lake surface takes up almost a third (28 km^2); dam levels are maintained by extracting water from the nearby relatively nutrient rich Williams River. With the increasing need of water supply by growing local communities, the water extraction practice may pose an increasing threat to water quality and subsequent cyanobacterial growth. Thus, this study pursues a proactive approach to control cyanobacteria by identifying nutrient dynamics conducive for cyanobacteria, nutrient sources and their appropriate management in Grahamstown Dam.

Grahamstown Dam water supply is managed by Hunter Water (a state owned corporation) who provided the major part of the funding for this study.

1.2 General features of cyanobacteria

1.2.1 Description of cyanobacteria

Cyanobacteria are prokaryotic organisms occurring in a wide range of terrestrial, marine and aquatic habitats, sometimes under extreme environmental conditions. Stromatolithic fossil records suggest that the evolution of cyanobacteria dates back 2.7 billion years (Lee, 2008). Cyanobacteria, similar to eukaryotic algae, perform oxygenic photosynthesis as a means of nutrition. However, chloro-

plasts are absent and photosynthetic pigments are bound in thylakoid membranes which are arranged concentrically at the cell periphery. Primary pigments are chlorophyll a or d and accessory pigments comprise phycobilins, carotenoids and sometimes chlorophyll b (Graham et al., 2009).

According to certain physiological qualities cyanobacteria can be divided into five groups: Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales (Waterbury, 2006). These do not necessarily correspond to evolutionary lineages and are thus not referred to as classical orders (Graham et al., 2009).

Members of the group Chroococcales are unicellular rods or cocci which reproduce by binary fission. They can form aggregates that are held together by extracellular mucilage. Pleurocapsales also occur as single cells but may undergo multiple fission, producing small, easily dispersed cells called baeocytes. Cyanobacteria belonging to the other three groups form filaments of undifferentiated (Oscillatoriales) or differentiated (Nostocales, Stigonematales) cells. Cells divide by binary fission at right angles to the long axis of the trichome. Filaments reproduce by detaching a short motile piece, the hormogonium, which will form a new trichome.

Differentiation in the Nostocales and Stigonematales may lead to the production of specialised cells: heterocysts, akinetes and tapered trichomes. Heterocysts serve the purpose of atmospheric N fixation and lack photosynthetic capabilities, presumably because oxygen is toxic to the nitrogenase enzyme (Sigee, 2005; Cohen and Gurevitz, 2006). Trichomes can become tapered when heterocysts are located terminally, resulting in a N gradient. Akinetes are resting cells, enabling cyanobacteria to survive adverse conditions by accumulating storage granules containing glycogen. Due to their higher density and larger size, akinetes sink to the sediment where they await improved conditions which lead to germination and formation of new filaments. Filaments of the order Stigonematales are further distinguished by forming complicated branching patterns.

1.2.2 Bloom formation

Cyanobacteria are ubiquitous in marine and freshwater systems where they can form mass occurrences known as cyanobacterial blooms. During a cyanobacterial bloom cell densities rapidly increase from low initial numbers of a few hundred cells per millilitre or less to 100 000 - 300 000 cells per millilitre (Bowling, 1994; Mitrovic et al., 2003; Hudnell et al., 2010).

Phytoplankton blooms are generally attributed to an increase of nutrients and/or increased light and temperature availability (Sigee, 2005). However, physiological traits such as tolerance of low (Scheffer et al., 1997) or high (De Tezanos Pinto and Litchman, 2010b) light levels, strongly alkaline conditions and low carbon dioxide levels (Shapiro, 1984, 1997) as well as the ability to regulate buoyancy (Reynolds et al., 1987), fix atmospheric N (De Tezanos Pinto and Litchman, 2010b) and resist zooplankton grazing (Lampert, 1987; Haney, 1987) occur in various cyanobacteria species and may aid in outcompeting other members of the phytoplankton assemblage.

Some species have been identified as the source of potent toxins, known as cyanotoxins, which can affect human and animal health. High concentrations of these toxins may be released during and after cyanobacterial blooms. Due to the release of the toxins from decaying cells and their resistance to degradation in the water column, water quality can be affected even several months after a cyanobacterial bloom has occurred (Sivonen and Jones, 1999).

Toxic freshwater cyanobacterial blooms have been observed in most parts of the world (Sivonen and Jones, 1999; Bianchi et al., 2000; Codd et al., 2005; Carmichael, 2008) with early reports dating back to 1878. Recent research indicates that their frequency has increased worldwide since the 1960s (Carmichael, 2008) and may increase in the future due to global warming (Paerl and Huisman, 2008; Wagner and Adrian, 2009; Markensten et al., 2010).

1.2.3 Impact of cyanobacterial blooms on human health

The associated impact of toxic cyanobacterial blooms ranges from deaths of livestock and domestic and wild animals, as described in detail by Falconer (2005)

and Stewart et al. (2008), to illness and deaths in humans (Texeira et al., 1993; Jochimsen et al., 1998; Carmichael et al., 2001; Soares et al., 2006). One of the most severe and often cited incidents associated with cyanotoxins was reported from Brazil (Texeira et al., 1993). In 1988, after the flooding of the Itaparica Dam a bloom of the cyanobacterial genera *Microcystis* and *Anabaena*[1] developed. Subsequently, serious cases of gastroenteritis, resulting in 88 deaths, occurred in areas supplied with drinking water from the dam. Further fatalities and illness have occurred in patients of a dialysis center in Caruaru, Brazil, in 1996. Water used in the hemodialysis treatment was contaminated with cyanotoxins and caused the intoxication of at least 116 patients, resulting in acute liver failure and subsequent deaths of 26 patients (Jochimsen et al., 1998; Carmichael et al., 2001; Soares et al., 2006). In Australia, an outbreak of severe hepato-enteritis occurred among the population of an island off the coast of Queensland in 1979 (Kuiper-Goodman et al., 1999). The local drinking water reservoir was affected by a cyanobacterial bloom containing the species *Cylindrospermopsis raciborskii*. The cytotoxin cylindrospermopsin produced by this species was made responsible for the illness of 140 children and 10 adults.

There are several kinds of cyanotoxins which can be grouped into categories according to the systems or organs they target (Funari and Testai, 2008):

Hepatotoxins damage the liver and can promote the growth of tumors (microcystins and nodularins).

Neurotoxins affect the neuromuscular or the peripheral nervous system (homoanatoxin a, anatoxin a, anatoxin a-(s), saxitoxin variants).

Cytotoxins affect the liver and kidneys (cylindrospermopsin).

Irritants and gastrointestinal toxins irritate skin and mucosa (aplysiatoxin, debromoaplysiatoxin, lyngbyatoxin (produced by marine cyanobacteria); lipopolysaccharidic (LPS) endotoxins).

Other cyanotoxins whose toxicological or ecotoxicological profile is only partially known.

[1]The genus *Anabaena* was recently reclassified and planctonic forms were renamed *Dolichospermum* (Wacklin et al., 2009). For reasons of simplicity, the old name is used in this thesis.

Human health problems are usually the result of drinking contaminated water but there are further routes of exposure to cyanotoxins and they are not always obvious as illustrated by the intoxication of hemodialysis patients described above. Skin contact, especially with the toxins lyngbyatoxin A and debromoaplysiatoxin of the marine species *Lyngbya majuscula*, during recreational activities has been found to cause, sometimes severe, contact dermatitis (Osborne et al., 2001, 2007). Common freshwater species can also cause skin irritation in about 20 % of people (Pilotto et al., 2007). However, effects of skin exposure were minor compared to exposure through ingestion of the cyanobacteria tested.

Intoxication has been caused by consumption of aquatic organisms that have accumulated cyanotoxins or toxic cyanobacteria (Funari and Testai, 2008). Other routes of potential exposure include the inhalation of aerosolised cyanotoxins during recreational activity (Backer et al., 2010) and the consumption of plants that have accumulated toxins as they were irrigated with contaminated water (Codd et al., 1999; Crush et al., 2008; Mohamed and Al Shehrib, 2009).

There is still a lack of understanding of the longterm effects of cyanotoxins on human health. The literature shows that cancer and neurological disorders seem to be the main illnesses arising from sublethal chronic exposure to cyanotoxins. Hernández et al. (2009) propose that longterm exposure to tap water containing microcystins at lower doses than suggested by the WHO guideline may be a risk factor for liver and colorectal cancer. Support for this hypothesis comes, amongst other studies, from the correlation of microcystin contaminated drinking water and cases of colorectal cancer in Haining City of Zhejiang Province, China (Zhou et al., 2002). Falconer and Humpage (2005) suspect cylindrospermopsin to be carcinogenic due to its structure which may enable it to interact with DNA or RNA. Studies of the carcinogenicity of cylindrospermopsin are of importance as the distribution and detection of *C. raciborskii* is growing (Padisák, 1997; Kling, 2009) and the toxin has the potential to bioaccumulate (Kinnear, 2010).

Human neurodegenerative diseases may be promoted by the waterborne neurotoxin beta-N-methylamino-L-alanine (BMAA) which can be produced by freshwater cyanobacteria as recently discovered (Cox et al., 2005) and which is also found in Alzheimer's disease and amyotrophic lateral sclerosis patients (Metcalf

and Codd, 2009).

Azevedo et al. (2008) summarised the current state of longterm toxicology studies and identified the need for carcinogenicity studies with cylindrospermopsin, neurodevelopmental and immunotoxicology studies with all groups of cyanotoxins and examination of the effects of acute, episodic and chronic exposures at sublethal concentrations as well as the examination of oral, inhalation and cutaneous pathways.

1.3 Eutrophication, cyanobacteria and water management

Eutrophication is generally understood as the process of enrichment of natural waters with inorganic nutrients, in particular N and P. Consequences of this process in freshwater systems include enhanced primary production and increased biomass of phytoplankton and macrophytes, increased turbidity and thus decreased light penetration, increased sedimentation and decomposition of organic matter followed by oxygen depletion, increased potential for fish kills and a decrease in perceived aesthetic value (Smith, 2003; Dokulil and Teubner, 2010).

The origin of nutrient enrichment is usually found in anthropogenic activities hence the process is often referred to as cultural eutrophication. It is frequently accompanied by a shift in composition of primary producers and cyanobacterial dominance of the phytoplankton assemblage (Keating, 1978; Robarts, 1985; Willén, 2001; Smith, 2003; Qin et al., 2010). The following description of two particular cases illustrates the issue and its potentially severe consequences.

Robarts (1985) describes how Hartbeestpoort Dam, a drinking water reservoir in South Africa, turned from first being oligotrophic (low nutrient concentrations, low phytoplankton density) to a hypertrophic state (overabundant nutrients, high primary production) within a few decades after its construction in 1923. The main source of inflow and also of nutrients of Hartbeestpoort Dam was the Crocodile River which carried domestic and industrial sewage from Johannesburg and other densely populated urban areas. The cyanobacterium *Microcystis aeroguinosa* became the dominant phytoplankton species in the hyper-

trophic lake for 10 months each year.

A further, more recent, example comes from China's Lake Taihu which is used as drinking water supply and receptacle for waste water. Substantial cyanobacterial blooms occurred in 2007 and were attributed to the steadily increasing nutrient load of the lake since 1980 (Qin et al., 2010). Being highly toxic, the bloom rendered the drinking water of about two million people unpotable.

The nutrient load of freshwater systems has increased dramatically during the 20^{th} century. For example, Triplett et al. (2009) examined the sediment of Lake St. Croix, a natural impoundment of the Upper Mississippi River, USA, using paleolimnological methods. They reported that since European settlement in the 1850s, P levels had increased eight times compared to those prior to settlement. The greatest increase in P occurred in the 1940s. During early settlement, until about 1890, activities only had a minor impact.

The effects of cultural eutrophication have been examined since the early 20^{th} century, but only in the 1960s researchers started to identify human activities as the major cause of nutrient enrichment of freshwater systems (Schindler, 2006; Schindler et al., 2008). Schindler et al. (2008) attribute the increasing introduction of nutrients to natural waters during the course of the 20^{th} century to five main issues:

- Introduction of water mains, flush toilets and sewage drains,
- Change of land-use which diminishes natural flows and deprives wetlands and riparian vegetation of water,
- Increased fertiliser production and use,
- Increased livestock farming and
- Increased cost for treating sewage.

Anthropogenic nutrients enter aquatic systems from point sources such as localised inflows of sewage or industrial effluent or from diffuse sources such as urbanised areas and land areas used for agriculture. In the late 1960s and during the 1970s, water management authorities started to control the input of nutrients from point sources which proved to successfully reduce eutrophication in many lakes and rivers in the USA and Europe (Edmondson and Lehman, 1981; Willén,

2001; Schindler, 2006; Schindler et al., 2008; Edlund et al., 2009). The control of point sources included more efficient sewage treatment leading to the removal of P from sewage and also banning P containing detergents. Also, nutrient rich effluent, provided it does not contain toxic substances, is sometimes used as fertiliser, e.g. to promote forest growth in parts of Pennsylvania and Michigan, USA (Schindler et al., 2008).

After the reduction of nutrient input from point sources, diffuse sources created by growing agricultural and urban development, have become the major contributor to eutrophication. An illustrative example of the importance of non-point sources is given by Soranno et al. (1996) who modelled the P loading of an eutrophic lake surrounded by agriculture and urban lands in North America. They estimated that the present nutrient loading was six times higher than the loading prior to settlement. Further, if the lake's entire watershed was converted into urban area, the P loading would double and water quality would decline immensely. Carpenter et al. (1998) identified various agricultural practices as the source of surplus nutrients in waterways and emphasised that if these practices were not changed, eutrophication would certainly increase. Input from diffuse sources prevails over input from point sources in most countries but due to its low settlement densities this applies even more so to Australia (Davis and Koop, 2006).

Aside from the extent of urbanisation and agriculture, other factors, such as transport capacity and soil drainage, impact on the amount of nutrients introduced to surface waters (Meynendonckx et al., 2006; Fraterrigo and Downing, 2008). Water renewal time (as residence time) and the internal nutrient loading can influence eutrophication of freshwater systems (Schindler, 2006). For example, low flows in the Hawkesbury-Nepean River near Sydney led to a concentration of discharge from sewage treatment plants and caused large cyanobacterial blooms in 1994 (Davis and Koop, 2006).

The regeneration of nutrients, especially P, from sediments may be a consequence of eutrophication and may impede the effectiveness of restoration measures (Søndergaard et al., 2001, 2003; Carpenter, 2005). The resuspension of sediments in reservoirs by refilling after draw down can also release nutrients, lead-

ing to algal and cyanobacterial blooms (Davis and Koop, 2006). The mechanisms and further details of nutrient release from sediments are discussed in section 1.4.5.

The consequences of eutrophication are reversible provided nutrient loads are sufficiently reduced and enough recovery time is given. Willén (2001) describes how biomass of cyanobacteria and other phytoplankton and the duration of blooms decreased in four large Swedish lakes after their P loading was reduced by 90-95% in the mid 1970s. Jeppesen et al. (2005) synthesise recovery studies of 35 shallow and deep water lakes in Europe and the USA after reoligotrophication measures, i.e. the reduction of total P (TP) input and in some cases total N (TN) input. The consequences of the nutrient reduction were a decline in lake TP and also phosphate, an increase in the ratio of TN to TP, a decline in total phytoplankton biomass and a shift in the phytoplankton composition with decreasing cyanobacteria dominance in favour of other taxa.

1.4 Nutrient influence on cyanobacterial growth

1.4.1 Nutrient limitation

Phytoplankton growth depends on the availability of resources such as light, macronutrients (e.g. P, N, carbon (C), silicon (Si)) and micronutrients (trace elements, e.g. iron and manganese) (Tilman et al., 1982). If any of the nutrient resources are not available in excess, they can limit primary production. Nutrient limitation, in relation to nutrient enrichment, can be defined as the limitation of the potential rate of net primary production (Howarth, 1988). This means, if a phytoplankton population is limited by a certain nutrient and this nutrient is added, primary production will increase.

Eutrophication research in the Experimental Lakes Area (ELA) in Canada has promoted the limitation of phytoplankton growth by P in freshwater systems (Schindler, 1977, 1978; Hecky and Kilham, 1988). This research has been cited frequently and P limitation has become a prevalent paradigm (Tilman et al., 1982; Smith, 1984; Hecky and Kilham, 1988; Sterner, 2008; Lewis and Wurtsbaugh, 2008) (and references therein). This was supported by whole-lake fertilisation

of an oligotrohpic lake with P, N and C which led to an increase in chlorophyll a concentration, while the addition of N and C alone did not.

Recent studies are showing that N may also be important in limiting phytoplankton biomass. Elser et al. (1990) reviewed short term nutrient enrichment assays and also whole-lake fertilisation experiments in North America and reported that a combined enrichment with N and P enhanced algal growth more frequently and to a greater extent than enrichment with N or P alone. Some lakes in the West Midlands in the U.K. display strong N limitation, while some are P limited and others had no clear limitation pattern (James et al., 2003). In contrast to the ELA lakes examined by Schindler (1977, 1978), the lakes in the U.K. were generally characterised by high P concentrations wich may have caused these different limitation patterns.

These observations suggest that nutrient limitation of a lake may depend on its trophic state. Downing and McCauley (1992) examined the variability of TN and TP with trophic state and found that the ratio of TN:TP was high in oligotrophic lakes but very low in eutrophic lakes. Moreover, ratios depended on the nutrient source in the watershed, i.e. oligotrophic lakes received their nutrients from natural sources that export less P than N, while in eutrophic lakes the nutrient ratio resembled that of sewage.

Whether the enrichment with N or P or with both is responsible for eutrophication and its inherent problems has recently been subject of a debate concerning the regulation of nutrients in freshwater systems. While in particular Schindler and colleagues remain convinced that managing P alone would prevent cyanobacteria outbreaks and eutrophication (examples can be found in Schindler et al. (2008); Schindler and Hecky (2008, 2009)), Paerl and colleagues argue that N was as important as P, especially when estuarine and coastal waters were concerend and the threat of climate change was taken into consideration (examples can be found in Howarth and Paerl (2008); Paerl (2009); Paerl and Scott (2010); Paerl et al. (2011); Paerl and Otten (2013)).

The relationship between environmental nutrient ratios and trophic state can be interpreted in relation to the Redfield ratio. The Redfield ratio is the fixed atomic ratio of C, N and P in algal cells, amounting to 106:16:1 (C:N:P) (Sigee,

2005). It does not vary with environmental nutrient ratios. Thus, a high environmental N:P ratio (higher than 16:1) would equate to an oversupply of N which cannot be taken up by phytoplankton. However, if additional P is supplied, algal growth may be stimulated.

By adding one or several macronutrients, other macro- or micronutrients may become a limiting factor for phytoplankton growth (Beardall et al., 2001). This occurred in a nutrient enrichment study where a lake was fertilised with P from 1990 until 2005, and consequently, TN, N:P ratio, chlorophyll a concentration and phytoplankton biomass decreased (Scott and McCarthy, 2010).

1.4.2 Investigating nutrient limitation

Nutrient enrichment bioassays are widely applied and there are several methods of investigating nutrient limitation in freshwater systems. They generally involve the artificial enrichment of a waterbody, or subsample thereof, with one or several nutrients and examining the growth response of primary producers. Nutrients are added to natural phytoplankton populations or single species contained in natural waters or culture media (Beardall et al., 2001; Howarth, 1988). The response to the enrichment can be measured as change in growth rate, biomass production, chlorophyll concentration or C^{14} uptake. If those response variables experience an increase, it is deduced that the added nutrient limits phytoplankton growth (Howarth, 1988; Beardall et al., 2001).

Nutrient enrichment experiments have been conducted over a broad range of scales, starting with single species growth potential experiments under laboratory conditions lasting for hours (Horvatić et al., 2006) and ending with the enrichment of whole ecosystems over several decades (Schindler, 1977; Schindler et al., 2008).

While being comparably cheap, easy to maintain and analyse, laboratory assays also offer the ability to exactly control and monitor environmental conditions. However, laboratory assays may accidentally eliminate natural variables which would interact with phytoplankton growth. Laboratory assays may not represent natural responses to nutrient enrichment adequately and thus, deductions for higher levels of ecosystem organisation may be difficult (Hecky and

Kilham, 1988). Using a single species in a nutrient enrichment assay may have similar effects. The natural competition for resources in phytoplankton communities, for example, would not be accounted for.

Contrarily, whole ecosystem enrichment experiments offer natural conditions at natural scales but little control for the researcher other than the nutrient addition itself. Thus, results from these experiments must be unambiguous in order to be considered valid (Hecky and Kilham, 1988). Elser et al. (1990) reviewed whole lake fertilisation experiments in North America in regards to N and P limitation. In this study it was suggested to use a multi-lake approach in order to successfully distinguish between the roles of the tested nutrients. Although sensible from a statistical point of view, this would certainly add to the challenging logistics involved in this type of experiment.

Mesocosm enrichment experiments present a compromise between laboratory and whole ecosystem assays regarding control, analysis and logisistics. The use of factorial designs, as suggested by Elser et al. (1990) and widely applied (Vrede et al., 2009; Mitrovic et al., 2001a; Perkins and Underwood, 2000; Levine and Schindler, 1999), to investigate the interactions of several nutrients is more feasible at mesocosm scale and replication is more easily incorporated compared to whole lake enrichment. In situ mesocosms can range from bottles containing little more than a litre (Mitrovic et al., 2001a) to plastic bags with volumes ranging from 20 to 2500 litres (Perkins and Underwood, 2000; Vrede et al., 2009) to large enclosures incorporating the sediment and many cubic metres of water (Levine and Schindler, 1999).

Although exposed to natural conditions, the confinement of small scale mesocosms may alter conditions, such as gas concentrations or pH values, compared to those in surrounding waters. This can be tested for easily and the duration of the experiment adjusted accordingly. Small mesocosms containing 1.25 L may be deployed for six days (Mitrovic et al., 2001a) while large ones may be used for several months (Vrede et al., 2009; Levine and Schindler, 1999). Large scale experiments may include grazing by zooplankton or consumption by planktivorous fish to make conditions more realistic (Vrede et al., 2009). In small scale enrichment assays, however, zooplankton grazing may be unwanted as it might

interact with phytoplankton responses over shorter timescales (Mitrovic et al., 2001a). Zooplankton can be eliminated through filtering or effects of grazing can be controlled using special treatments (Perkins and Underwood, 2000).

1.4.3 Competition for nutrients and cyanobacterial dominance

Not all taxa in a phytoplankton population have the same affinity to different resources and thus may not be limited by the same nutrient at the same concentration. Algal and cyanobacterial species have fastest growth rates when nutrients are available at their individual optimum nutrient ratio (Tilman et al., 1982; Sigee, 2005). The optimum nutrient ratio is the ratio of two or more nutrients at which neither of their concentrations is limiting. If one nutrient is supplied in relatively higher quantities than dictated by the ratio, the remaining nutrients will become limiting. As species in a phytoplankton population have different optimum nutrient ratios, the composition of the population can change, if the nutrient supply ratio changes (Tilman et al., 1982; Hecky and Kilham, 1988; Suttle and Harrison, 1988; Bulgakov and Levich, 1999; Beardall et al., 2001; Sigee, 2005).

Changing nutrient supply ratios are often used to explain the dominance of cyanobacteria in phytoplankton communities in nutrient enriched waters. Resource ratio hypothesis argues that in particular, a low N to P (N:P) ratio is favourable for cyanobacteria (Smith, 1983). The ability of some cyanobacterial taxa to fix N is viewed as especially advantageous under such conditions.

The important work by Schindler (1977) on limitation of phytoplankton by N showed that a low ratio of N to P was conducive for cyanobacteria in the Canadian Experimental Lake Area (ELA). Two whole lake fertilisation experiments showed that when N:P ratios were reduced from 14 to 5 (by weight), N fixing cyanobacteria (*Anabaena* spp. and *Aphanizomenon gracile*) became dominant in formerly oligotrohpic ELA lakes. Similar results were observed in oligotrophic Kennedy Lake on Vancouver Island, Canada, when selected areas of the lake were fertilized with inorganic N and P, creating high P levels and a low N:P ratio (Stockner and Shortreed, 1988). In response to this treatment, *Anabaena circinalis* developed late summer blooms in 1981 and 1982.

Bulgakov and Levich (1999) reviewed laboratory and field studies examining

the influence of nutrient supply ratios and found further support for cyanobacteria being successful under a low N:P ratio, while high N:P ratios inhibited their growth. Dominance of N fixing species and also of *Microcystis aeruginosa* was observed at N:P ratios of 2 and 5 (Haarhoff et al., 1992; Bulgakov and Levich, 1999). This is surprising as *Microcystis aeruginosa* does not fix N and was found to show optimal growth at N:P supply ratios of 8:1 and 18:1 in laboratory cultures (as cited in Pick and Lean (1987)). Bulgakov and Levich (1999) concluded that both cyanobacteria and algae responded more intensely to relative supplies of nutrients than to absolute quantities which eventually shaped the phytoplankton composition.

There are a number of more recent examples of field studies where a low N:P ratio was found to be beneficial for cyanobacterial dominance. For example, in subtropical Lake Okeechobee in Florida, USA, the concentration of TP increased from the mid 1970s to the late 1990s from 50 μg/L to more than 100 μg/L which led to a decrease of the N:P ratio from 30:1 to less than 15:1 by mass and 15:1 to 6:1 of soluble N:P, respectively (Havens et al., 2003). The phytoplankton biovolume consisted of 50 to 80 % of cyanobacteria at the time the study was conducted and the main taxa present were *Oscillatoria* and *Lyngbya*.

There are further findings justifying the resource ratio hypothesis. Wang et al. (2005) examined cyanobacterial temporal and spatial abundance in regards to physicochemical parameters and found a strong negative correlation between cyanobacterial biovolume and TN and nitrate concentrations in a eutrohpic lake. Cyanobacterial blooms were attributed to low levels of N coupled with internal P release from the sediment in the lake investigated by Wang et al. (2005). Vrede et al. (2009) hypothesized that if N loading relative to P loading was reduced, N fixing cyanobacteria would become dominant and confirmed it in mesocosm experiments in a naturally eutrohpic lake. An increase in heterocyst formation in *Anabaena planktonica* was associated with lowest observed ratios of dissolved inorganic N to soluble reactive P before a bloom of this taxa occurred in a New Zealand reservoir (Wood et al., 2010). A low N:P ratio may not only be beneficial for planktonic cyanobacteria but also for those in benthic communities: Vis et al. (2008) observed a shift from benthic chlorophytes to N fixing benthic cyanobacteria along a gradient of N depletion in a fluvial lake.

The logic and simplicity of a low N:P ratio being responsible for cyanobacterial dominance and the number of field and laboratory studies where this was shown, make it tempting to accept this explanation. However, there are also studies presenting contrary results. Pick and Lean (1987) reviewed the role of macronutrients in controlling cyanobacterial dominance in temperate lakes and concluded that while TN:TP ratios above 30 seemed to inhibit cyanobacterial dominance, rates below 30 did not necessarily promote their growth. A late summer bloom of *Aphanizomenon klebhanii* in Lake Verevi, Estonia, also does not fit into this model as it occurred despite N:P ratios fluctuating between 60 and 81 in the summer months (Kõiv and Kangro, 2005).

Levine and Schindler (1999) specifically tested the hypothesis that cyanobacteria were favoured in low N:P conditions in ELA lakes. A bloom of the heterocystous N fixing genus *Anabaena* was observed in some treatments but evidence was not enough to confirm the hypothesis. Contrary to Havens et al. (2003)'s study, a later study found the proportion of cyanobacteria increased with increasing TN:TP ratio while the proportion of Bacillariophyceae was decreasing (McCarthy et al., 2009).

Some cyanobacteria have even been observed to thrive in high N:P conditions. *Synechococcus*, for example, has been observed to outcompete Bacillariophyceae (Suttle and Harrison, 1988) and other cyanobacteria (Stockner and Shortreed, 1988) at N:P ratios of 45:1 and 35:1.

N fixing cyanobacteria may need other conditions to be ideal, e.g. high light intensities, in order to achieve dominance. De Tezanos Pinto and Litchman (2010b) examined the influence of N:P ratios and light on *Anabaena flos-aquae* in a multispecies assemblage. N fixation only occurred when N:P ratios were low and increased with increasing light intensity. Only when light intensities were high did *A. flos-aquae* dominate the species assemblage. The increase of N fixation with increasing light intensities may be explained by the fact that N fixation is very energy intensive (Tilzer, 1987; Graham et al., 2009). It competes for energy and reductant with CO_2 in photosynthesis (Tilzer, 1987). If light availability and thus energy increase, competition between N fixation and photosynthesis should decrease and N fixation can become advantageous.

The ability to fix atmospheric N may not be the preferred or only strategy of acquiring N and obtaining an advantage over other phytoplankton taxa. According to Blomqvist et al. (1994), non-N fixing cyanobacteria have a low competitiveness for nitrate N but a high competitiveness for ammonium N and cyanobacteria gain dominance in N deficient systems by either fixing atmospheric N or utilising their superior abilities of ammonium acquisition.

Testing of the hypothesis that cyanobacteria dominate because they fix atmospheric N revealed that dominance depended on other factors (Ferber et al., 2004; Burford et al., 2006). Tapping benthic sources of ammonium and the formation of shading surface scums were strategies used by *Aphanizomenon* and *Ananbaena* species to outcompete other phytoplankton (Ferber et al., 2004).

Support for cyanobacterial dominance due to utilisation of ammonium rather than N fixation comes from several studies. In Lake Okeechobee, a positive relationship between the ammonium to nitrate/nitrite (NH_4^+:NO_x) ratio and cyanobacteria proportions was observed while diatoms responded in an inverse fashion (McCarthy et al., 2009). In a study of phytoplankton assemblage composition of the coastal Myall Lakes, Australia, growth of *Anabaena* was positively related to ammonium concentrations (Ryan et al., 2008).

Apart from nitrate, molecular N and ammonium, other sources of N may be accessible to cyanobacteria. During a bloom of heterocystous *Aphanizomenon ovalisporum* in Lake Kinneret, N was derived from dissolved organic N (DON) and N fixation did not occur (Berman, 2001). However, little is known of the availability of this source of N in natural waters.

While low N:P ratios can be used to explain the dominance and blooms of cyanobacteria in some cases, it has been suggested that TN and TP concentrations may play a more important role in promoting cyanobacterial dominance. An analysis of 100 temperate unmanipulated lakes showed that TP, and especially TN concentrations, were strongly correlated with increasing proportions of cyanobacteria in the phytoplankton assemblage while the ratio of N:P was only weakly correlated with cyanobacterial dominance (Downing et al., 2001). Kõiv and Kangro (2005) proposed that total concentrations of limiting resources in combination with stratification and increased temperatures were responsible for

the late summer bloom of *Aphanizomenon klebhanii* in the stratified, hypertrophic Lake Verevi.

1.4.4 Nutrient inflow events

Inflows to a waterbody can change conditions to favour cyanobacterial development by introducing nutrients and altering hydrological conditions such as stratification and turbidity. This includes intermittent or continuous inflow from anthropogenic point sources and non-point sources as well as natural events.

Inflows from floodwaters can create a nutrient enriched water column which can initiate blooms of cyanobacteria. Examples include blooms of *A. circinalis*, *Anabaenopsis elenkinii* and *Aphanizomenon issatschenkoi* in an impoundment of the Fitzroy River, Australia (Bormans et al., 2005) and *A. circinalis* and some other taxa in Lake Cargelligo (Bowling, 1994). In both cases concentrations of P increased in the receiving waterbody due to the inflows.

The cyanobacterial bloom in Lake Burragorang in 2007 was caused by several inflow events that led to the disturbance of thermal stratification and thus mixing of nutrient rich hypolimnion water into the epilimnion. In combination with the high nutrient content of the inflow water, this resulted in prolific growth of *Microcystis aeruginosa* (SCA, 2010).

Not only intermittent flows but more constant inflows containing high nutrient loads can be responsible for cyanobacterial blooms. Izydorczyk et al. (2008) identified a stream inlet into a shallow bay as an initiator of *Microcystis* blooms. The observed TP loading of the stream was higher than the lake and in combination with physical protection in the inlet, was considered responsible for triggering the blooms.

Complex dynamics may also result from storm mediated inflows. Storm discharge can increase light limitation but decrease nutrient limitation, while also flushing phytoplankton from a lake (Vanni et al., 2006). However, no influence of inflows on cyanobacteria was observed in this study.

1.4.5 Nutrient release from sediments

The sediments of a waterbody can act as a source or a sink of nutrients and thus play an important role in nutrient dynamics in aquatic systems (Boström et al., 1988; Petterson, 1998; Saunders and Kalff, 2001; Søndergaard et al., 2001; Baldwin et al., 2002; Søndergaard et al., 2003; Nowlin et al., 2005; Spears et al., 2008). Transport to the waterbody, incorporation into and release from the sediments depend on the type of nutrient, following different mechanisms for N and P.

Phosphorus

P is present in dissolved mineral or organic particulate form. It occurs in four pools in the aquatic environment, namely, dissolved in the water column, associated with suspended particles, associated with bed sediment and incorporated in biota (Baldwin et al., 2002).

Most lakes experience a net deposit of P in the sediment (Boström et al., 1988) and the amount of P in sediments is much larger (often more than 100 times (Søndergaard et al., 2003)) than the amount contained in the water column (Petterson, 1998). This can greatly increase lake water concentrations, if released from the sediments. Retention and release of P in lake sediment can follow a seasonal pattern, with P being accumulated during the winter and released during the summer (Søndergaard et al., 1999).

The transfer mechanisms of P to the sediment, described by Boström et al. (1988), comprise:

- sedimentation of detrital P minerals from the watershed,
- precipitation of P with organic compounds,
- sedimentation of P with allochthonous or autochthonous organic matter,
- assimilation from the water column by benthic biota and
- adsorption of dissolved phosphorous onto particles in the sediment.

While most of these mechanisms appear to be straightforward, precipitation and adsorption reactions are more complicated. The former removes two or more components from the water column by combining them into a new solid compound and the latter removes a compound from solution by its concentration in

a solid phase (Holtan et al., 1988). In contrast to physical adsorption, which is easily reversible, chemisorption is irreversible or only partly reversible.

Important binding partners for anionic phosphate are iron (Fe) and aluminium (Al) minerals, more precisely Fe(III) and Al oxides and oxyhydroxides (Lijklema, 1980; Baldwin et al., 2002; Perrone et al., 2008). Adsorption depends on pH (Lijklema, 1980; Holtan et al., 1988; Boers, 1991; Nemeth et al., 1998), number of hydroxyl groups on the surface of minerals in the sediment and the specific surface area of the sediment (Baldwin et al., 2002). Fe(III) and Al oxides and oxyhydroxides have positive surface charges at slightly acidic to neutral pH and therefore high affinity for anions. Phosphate anions are bound through ligand exchange with hydroxyl groups on the mineral surface (Holtan et al., 1988). Manganese dioxides may also play a role as binding partners for phosphate anions. Although manganese dioxide only displays a neutral or positive surface charge at pH values of 2 (as cited in Baldwin et al. (2002)), adsorption of P to manganese dioxide has been observed in seawater at typical pH (Yao and Millero, 1996). The more surface area a mineral possesses, the more binding sites are present. Hence, amorphous oxyhydroxides offer more possibilities for binding than older more crystalline minerals (Baldwin et al., 2002).

Precipitation of phosphate minerals occurs in the sediments if the porewater is supersaturated with the respective phosphate mineral, e.g. calcium- or Fe(III) phosphate (Baldwin et al., 2002). The formation of apatite, a calcium phosphate mineral, through direct precipitation or coprecipitation of calcium carbonate has been observed especially in eutrophic hardwater lakes (Golterman, 2001).

Remobilisation of P from the sediment requires the release of P from sediment particles and subsequent transfer from the interstitial water to the water column. Baldwin et al. (2002) identified four main pathways of P release:

- changes in solution chemistry,
- excretion or autolysis of cells from biota,
- hydrolysis of sedimentary P compounds and
- reduction of iron oxides and oxyhydroxides.

Changes in solution chemistry affect the equilibrium between dissolved P and

sediment bound P. If dissolved P is removed, e.g. by biological uptake, more P may be released from the sediment in order to compensate for the loss. Thus, the equilibrium between dissolved and sediment bound phosphate may be shifted towards a higher release of dissolved phosphate (Baldwin et al., 2002). The pH also influences the desorption of phosphate from sediment particles. Phosphate release from hydroxide minerals is positively correlated with pH (Boers, 1991; Nemeth et al., 1998). It has been shown that a high pH, as found in eutrophic lakes in summer can increase the internal P loading in combination with intensive resuspension of sediment (Koski-Vähälä and Hartikainen, 2001).

P contained in bacterial biomass in the sediment may contribute a large proportion of P available from the sediment. Gächter and Meyer (1993) reported that in eutrophic lakes the amount of bacteria bound P equalled the amount that settled with organic detritus per year. In oligotrophic lakes, the bacteria bound P pool may even exceed the annual sedimentation of organic P. Bacteria bound P may be released through cell lysis or excretion. Jin et al. (2007) found that the presence of organisms, such as benthic bacteria, algae and protists, in the sediment had a decreasing effect on the release of P from sediment compared to the release from sediment that had been sterilised. However, the sterilisation process may have destroyed cells and thus increased the mobility of P previously contained in the organisms.

Organic P compounds can be hydrolysed biotically or abiotically. Biotically mediated processes include the extracellular enzymatic hydrolysis of organic P compounds with free orthophosphate as a product. It has been suggested that hydrolysis through phosphatase may substantially accelerate eutrophication by providing more bioavailable P (Song et al., 2006). The abiotic pathway for hydrolysis of organic P compounds can be facilitated by Fe, Al, manganese (Mn) and titanium (Ti) minerals (Baldwin et al., 2002).

Apart from pH, other environmental factors such as temperature and redox potential influence the release of P. Increased temperatures lead to increased biological activity which in turn may alter the chemical environment (Boström et al., 1988). Microbial activity can deplete the hypolimnion and sediment of oxygen. Mortimer (1971) reported that oxygen concentrations below 2 mg/L resulted in

a decrease in electrode potential in the top millimetres of sediment and mobilisation of Mn and Fe(II). The release of large amounts of P into the water were observed simultaneously. A widely accepted explanation for this phenomenon is that the released P was bound in Fe(III) oxyhydroxides and its release was the consequence of the reduction of Fe(III) to Fe(II) under anoxic conditions. The inhibition of phosphate release in oxygen rich conditions, supports this explanation (Malecki et al., 2004; Beutel et al., 2008). Some studies suggest that the reduction of ferric (Fe(III)) oxyhydroxides to ferrous (Fe(II)) oxyhydroxides and the release of P is due to microbial activities (Roden and Edmonds, 1997; Mitchell and Baldwin, 1998). Anaerobic microbial sulfate reduction has been linked to phosphate release from sediment (Caraco et al., 1989). Hydrogen sulfide, the end product of the reduction, may act as a reducing agent, turning ferric minerals into ferrous ones while releasing phosphate.

The release of phosphate through reduction mechanisms under anoxic conditions has received some criticism. Golterman (2001) argued that although P may be released by hydrogen sulfide reduction, this required excess amounts of hydrogen sulfide which would usually not be present in lakes. Instead, the solubilisation of apatite and microbial release of polyphosphates were proposed to be more likely pathways of P mobilisation. Moreover, P is not always mobilised under anoxic conditions, even if Fe(II) is released (Baldwin and Williams, 2007). Kopáček et al. (2007) and Hupfer and Lewandowski (2008) contended that P release could be completely inhibited under anoxic conditions by the presence of redox-insensitive P binding systems such as Al(III) hydroxide and unreducible Fe(III).

Once released into the porewater, P is transported into the water column by disturbance of the sediment-water interface. Disturbances include high flow events resulting in sediment scouring, anthropogenic causes such as dredging, formation and release of gas bubbles from the sediment, foraging of bottom feeding animals and bio-irrigation (benthic organisms flushing their burrows) (Baldwin et al., 2002).

Mixing events caused by high winds may be especially important in shallow lakes. Suspended solids and TP increased by a factor of five to ten in a shal-

low Danish lake within a few days of increasing wind speed (Søndergaard et al., 2003). Similarly, Quin et al. (2006) found that TP release in shallow Lake Taihu, China, was greater during periods of wind induced turbulence and concomitant sediment resuspension than under static conditions. Microstratification in a shallow lake may also lead to temporary anoxia and increased release of P through microbial reduction activity (Boström et al., 1988). The released P may then be transported into the water column during the next mixing event.

Nitrogen

N is present as dissolved inorganic N (nitrate, nitrite, ammonium and dinitrogen gas), insoluble particulate material derived from rock and sediment, dissolved organic authochthonous and allochthonous forms and in biota in aquatic systems (Sigee, 2005). Nitrate entering aquatic systems undergoes a sequence of transformations. It is taken up by primary producers and incorporated as organic N into animals and plants which release insoluble organic N through cell death or excretion. Insoluble organic N is transported to the sediment where remineralisation, nitrification and/or denitrification occur (Sigee, 2005).

Remineralisation or ammonification is the conversion of insoluble organic N into soluble inorganic N in the form of ammonium. This process is facilitated by bacteria, fungi and protozoans in the lower part of the water column and in the sediments. Ammonium is microbially oxidised to nitrite and nitrate in a process called nitrification, occurring in the upper oxygenated layers of the sediment. Nitrification exerts a major demand on the oxygen pool and can lead to anoxia of the bottom water of a lake (Sigee, 2005). In turn, ammonium accumulates in bottom waters if oxygen is depleted and nitrification impaired (Beutel, 2006). Denitrification, the microbially mediated reduction of nitrate and nitrite to dinitrogen gas, uses organic matter as an electron donor and occurs under anaerobic conditions in deeper parts of the sediment (Knowles, 1982). Dissimilatory nitrate reduction to ammonium (DNRA) is another anaerobic microbially meditated process, using nitrate as an electron acceptor with ammonium as the product (Smolders et al., 2006). The products of nitrification and denitrification, i.e. nitrate and dinitrogen, diffuse from the sediments into the water column. N is permanently

removed from the aquatic system by the denitrification process as the product is not available to most primary producers and is released into the atmosphere (Scheffer, 1998; Saunders and Kalff, 2001). Denitrification has been identified as a primary mechanism for N retention (the difference between N input and output to a freshwater system) followed by sedimentation and uptake by aquatic plants in wetlands, lakes and rivers (Saunders and Kalff, 2001).

Unlike phosphate, ammonium and nitrate do not undergo sorption or precipitation reactions (Scheffer, 1998). Hence, an accumulation of N in the sediment does not occur (as cited in Scheffer (1998)). However, release of ammonium from anoxic sediments is common and has been observed in microcosms and in situ (Malecki et al., 2004; Nowlin et al., 2005; Baldwin and Williams, 2007; Beutel et al., 2008). It contributes to internal loading and affects water quality (Malecki et al., 2004; Beutel, 2006; Beutel et al., 2008).

1.5 Management of cyanobacteria

1.5.1 Nutrient management

As previously discussed, eutrophication often leads to cyanobacterial blooms and subsequent drinking water problems. In order to reduce the nutrient enrichment of aquatic systems, it is important to target both external and internal nutrient loading (Søndergaard et al., 2001, 2003; Cooke, 2005; Smolders et al., 2006).

External loading

The reduction of external loading comprises the control of nutrient introduction from point sources (as described in section 1.3) and identification and reduction of diffuse sources.

Diffuse sources of P can be identified using the P index (PI) (Gburek et al., 2000), which can be useful to lake managers as it provides a watershed-scale evaluation of non-point P sources (Cooke, 2005). This is achieved by first separating source characteristics, such as high soil concentrations and fertiliser application rates, and transport characteristics, such as soil erosion and distance to water. The

potential of the site to add P to streams is determined by an index that weights the individual importance of the source and transport characteristics (Gburek et al., 2000; Cooke, 2005).

There are several options to target the load of N and P from diffuse sources. However, all are indirect measures and do not directly address the land use which is the actual source of the diffuse nutrient pollution. In order to achieve a reduction of input from diffuse sources, Carpenter et al. (1998) and Cooke (2005) propose to apply best management practices in agriculture, e.g. matching fertiliser use with actual crop needs, treating sewage from high density livestock farming or using waste from these operations as crop fertiliser. Urban runoff, apart from well designed sewer systems, can be controlled by retention ponds and wetlands, the reduction of impervious areas, litter control and street sweeping (Carpenter et al., 1998; Cooke, 2005). Moreover, the maintenance of healthy riparian vegetation may aid in retaining nutrients (Davis and Koop, 2006; Cooke, 2005).

Several studies have shown that a riparian buffer zone in agricultural watersheds reduces nutrients from non-point sources to aquatic systems (Peterjohn and Correll, 1984; Osborne and Kovacic, 1993; Hill, 1996). Correll (2005) and Cooke (2005) described the characteristics of these zones and identified that a buffer strip of at least five metres width along both sides of a stream with a variety of native grassy and woody plants was essential for the effectivity of buffer zones.

Constructed wetlands can be used to retain particulate and dissolved nutrients in urban and agricultural run off and thus protect natural water bodies and reservoirs (Cooke, 2005; Klapper, 2003). The restoration of wetlands may increase denitrification and thus remove N from aquatic systems (Carpenter et al., 1998). However, N in the form of nitrate does not necessarily increase eutrophication but may actually aid in decreasing it (Smolders et al., 2006). The proposed mechanism is nitrate reduction under anaerobic conditions as this leads to the oxidation of Fe(II) and metal sulfides which in turn results in the increase of binding sites for phosphates.

Internal loading

Measures addressing the internal loading of nutrients, specifically in shallow or non-stratifying lakes, include nutrient inactivation by physical and/or chemical capping of the sediment, nutrient inactivation by chemical injection of the sediment, flushing and removal of sediment.

The capping of sediments involves passive or active capping agents which trap nutrients, in particular P, in the sediment. Passive agents include sand, gravel and clay (Kim et al., 2007; Hickey and Gibbs, 2009) which inhibit the diffusion of nutrients from the sediment into the overlying water column and reduce the oxygen demand of sediments by burying organic matter. Experiments have shown that capping with sand can successfully turn P into a more stable form compared with P in uncapped systems (Kim et al., 2007). Typically, layers of these agents are thicker than five centimetres and thus, application is restricted to smaller water bodies (Hickey and Gibbs, 2009).

Active capping agents remove P by chemically binding it. These agents include alum (aluminum sulfate) (Welch and Cooke, 1999; Cooke, 2005; Hickey and Gibbs, 2009), modified zeolite (microporous, aluminosilicate minerals) (Hickey and Gibbs, 2009), polyaluminium chloride (Łopata and Gawrońska, 2008), calcite materials (Berg et al., 2004), and Phoslock^{TM} (Hickey and Gibbs, 2009). Alum also acts as a flocculation agent, removing dissolved phosphate, algae and suspended solids from the water column before settling as floc on the sediment surface. Modified zeolite also has the capability to remove N in the form of ammonia and does not, unlike alum, affect the pH (Hickey and Gibbs, 2009). Polyaluminium chloride can completely remove phosphates from the water column, reduce total P by 50-60% and decrease the amount of mobile P by 40% (Łopata and Gawrońska, 2008). Phoslock^{TM} consists of lanthanium modified bentonite clay which precipitates orthophosphate and also polyphosphates, leading to almost complete removal of phosphates. Capping treatment has been successfully applied in deep, stratifying lakes as well as shallow ones and can be effective for up to 20 years (Welch and Cooke, 1999; Cooke, 2005). All capping agents potentially smother benthic organisms, while some, such as sand, may create improved habitat (Hickey and Gibbs, 2009). Chemical capping agents may also be poten-

tially toxic to benthic organisms. Other disadvantages include the slow sedimentation rate of flocculents and fine particles, necessity for a restriction period for drinking water and difficulties in maintaining accuracy and evenness of application (Hickey and Gibbs, 2009). Application of capping agents depends strongly on timing, as the P needs to be in the sediment (flocculation agent alum being the exception) and should be administered when the lake is fully mixed (Hickey and Gibbs, 2009).

Aside from capping the sediment to inhibit nutrient release, other chemical treatments exist, such as oxygenation of the sediment to increase nutrient binding capacity. In the RIPLOX method, ferric chloride, calcium nitrate and calcium carbonate (lime) are injected into the sediment (Straškraba and Tundisi, 1999; Hickey and Gibbs, 2009). Ferric chloride increases the P binding capacity, calcium nitrate enhances the redox potential and leads to oxidation of Fe in sulfide compounds, increasing their P binding capability. Calcium carbonate can be added to optimise conditions for denitrification by increasing the pH.

The RIPLOX method can be applied directly to the sediment, with minimal disturbance, but injection techniques require a shallow waterbody with a flat bottom. Further disadvantages include high cost of chemicals and sophisticated injection equipment. Beutel (2006) also raises concern about adding calcium nitrate, especially to lakes affected by urban or agricultural development as external P control was often difficult in these lakes and additional nitrate would lead to enhanced eutrophication. Moreover, he argues that nitrate enriched lake water may eventually reach the ocean where N is often the limiting nutrient. Smolders et al. (2006) describe the control of P by addition of lime as this supersaturated the water column with Ca^{2+} which in turn resulted in the precipitation of the P mineral hydroxyapatite. However, hydroxyapatite has its lowest solubility at a pH above 9.5, and P sorbs strongly to it at high pH (Cooke, 2005). So if the pH falls, P may again be released. Addition of lime may also not be effective in soft water lakes, as the water may require large amounts of lime before becoming supersaturated.

The removal of nutrient enriched sediment by dredging is another option to reduce the internal nutrient load (Straškraba and Tundisi, 1999; Cooke, 2005; Smolders et al., 2006; Hickey and Gibbs, 2009). Dredging requires adequate knowl-

edge of the sediment structure, specialised equipment and suitable disposal methods and areas for the removed sediment. These issues render the removal of sediment a costly operation (Hickey and Gibbs, 2009). Dredging is also not free of environmental risks, as it may significantly disturb the ecosystem physically and liberate nutrient and other contaminants from the sediment into the water column (Cooke, 2005; Hickey and Gibbs, 2009). According to Cooke (2005), there is little documentation concerning the success or failure of most dredging projects. Some successful cases where sediment removal led to lasting decline in nutrient concentration have been documented (Straškraba and Tundisi, 1999; Brouwer and Roelofs, 2002).

Apart from these established techniques, new methods of nutrient management are being developed, especially for P. For example, De Vicente et al. (2010) investigated the efficiency of magnetic nanoparticles in P removal and Chen et al. (2007) examined fly ash as a potential P binding agent.

1.5.2 Inflow management

Increased nutrient concentration and hydrological conditions near an inlet of a lake or reservoir can lead to algal and cyanobacterial blooms (see section 1.4.4). Aside from constructed wetlands and vegetation buffer zones as described in section 1.5.1, pre-dams or pre-basins can be used to control the load of nutrients arriving through a defined inlet.

Pre-basins and pre-dams are constructed along a stream inlet upstream from the main reservoir or lake (Klapper, 2003; Cooke, 2005). Their purpose consists of trapping and removing particulate nutrients by sedimentation before the water leaves the pre-dam via a surface overflow and enters the main lake. Dissolved nutrients are removed by phytoplankton – ideally diatoms because of fast sedimentation rates – and macrophyte growth. Pre-dams can remove up to 90% of soluble reactive P (Salvia-Castellvi et al., 2001), depending on water retention time (to allow settlement of particulates) and depth. The depth should not be greater than the euphotic zone depth to prevent internal loading (Cooke, 2005). Siltation decreases the volume and can decrease retention time of a pre-dam. Thus, sediment removal may be necessary to keep it in working order (Paul, 2003).

1.5.3 Stratification management

Stratification can affect cyanobacterial growth in two ways. Firstly, it may lead to anoxia in bottom waters and subsequent increase of nutrient release from the sediment (Boström et al., 1988). Released nutrients will be made available to phytoplankton growth in the euphotic zone when stratification breaks down. Secondly, some cyanobacteria, e.g. *A. circinalis* and *Microcystis* spp., benefit from a stratified water column as they rely on internal buoyancy control to stay at a level in the euphotic zone that is ideal for their growth (Ibelings et al., 1991; Walsby et al., 1997; McCausland et al., 2005). Blooms of *A. circinalis* have been linked with stratified water columns in several cases (Sherman et al., 1998; Mitrovic et al., 2003).

Destratification or artificial circulation methods include various techniques to mix the water column. These include different techniques of aeration (e.g. bubble plume mixing), pumping to induce water circulation and mixing fans (Straškraba and Tundisi, 1999; Klapper, 2003; Cooke, 2005; Hudnell et al., 2010). Artificial circulation is applied to reduce anoxia in bottom waters to prevent release of P from the sediment, increase dissolved oxygen concentration and induce light limitation in phytoplankton (Straškraba and Tundisi, 1999; Cooke, 2005; Hickey and Gibbs, 2009).

Although usually applied in deeper lakes which permanently stratify during summer, destratification may be useful for polymicitic lakes where stratification only occurs during periods of calm weather for days or weeks. Oxygen in bottom water may be depleted quickly, especially if the organic matter content is high in the sediment and development of *A. circinalis* has been observed to start after several days of stable stratification (Mitrovic et al., 2003).

There are adverse effects of artificial circulation, including an increase in temperature, especially in the hypolimnion, which may lead to a loss of cold water species (Cooke, 2005). The mobilisation of TP from the sediment and potential mineralisation to phosphate which is then available to phytoplankton, pose another risk (Straškraba and Tundisi, 1999; Cooke, 2005).

Destratification by aeration in order to prevent cyanobacterial blooms was successfully applied to a South Korean reservoir, receiving agricultural run off (Heo and Kim, 2004). The authors report that artificial circulation created ho-

mogeneous physical and chemical parameters throughout the lake, while not increasing TP concentrations. A switch from cyanobacterial to Bacillariophyceae dominance was attributed to the increased ratio of mixing depth to euphotic depth and resulting net reduction in underwater irradiance. Similarly, *Microcystis* dominance could be shifted to flagellate, Chlorophyceae and Bacillariophyceae dominance through artificial mixing without increase of TN or TP in Lake Nieuwe Meer in the Netherlands (Jungo et al., 2001).

Other studies describing the application of destratification in order to control cyanobacteria in lakes or reservoirs report mixed results. Tsukada et al. (2006) and Sherman et al. (2000) both found that the aeration they were applying to lakes was not strong enough to break stratification and had no or little influence on preventing cyanobacterial blooms. However, Sherman et al. (2000) found artificial mixing did reduce the internal P load from the sediments by over 80 % compared to the load during stratified anoxic conditions.

A study by Lindenschmidt and Chorus (1997) illustrates that the prevention of cyanobacterial dominance by artificial mixing may depend on the cyanobacteria species present. The authors found that increased mixing during summer favoured population growth of *Microcystis* spp. and *Aphanizomenon flos-aquae*, while other species, *Planktothrix agardhii* and *Anabaena* spp., grew better under stratified conditions.

1.6 Aims and overview of this study

The objective of this study was to understand the role of N and P in algal and cyanobacterial bloom development and to elucidate physical and chemical aspects that may lead to enhanced cyanobacterial growth and dominance in the initially low nutrient environment of Grahamstown Dam. Reservoir specific characteristics and dynamics that influence the availability of these nutrients for phytoplankton growth in the lake were also to be identified. Cyanobacterial outbreaks are not common in Grahamstown Dam and cyanobacterial growth has rarely been examined in waterbodies where that is the case. Thus, this study pursued a novel approach to understanding development of problematic cyanobacterial

growth. This approach was also applied to provide proactive recommendations for the management of cyanobacteria in the lake. In light of this, the logical link between chapters and their individual aims is described in the following.

Chapter 2 This chapter addresses the basic need to find the limiting nutrient for algal and cyanobacterial growth in Grahamstown Dam. Management strategies had previously targeted P but there was no lake specific information on the role of P in phytoplankton growth limitation as nutrient limitation had not been examined in the lake before.

Specific aims were:

- to find the limiting nutrient for phytoplankton growth at an assemblage and genus level,
- to determine if nutrient limitation is seasonally different and
- to examine if the experimental period influences the phytoplankton response to nutrient enrichment at an assemblage and genus level.

This chapter has been submitted as a manuscript to the journal *Hydrobiologia*.

Chapter 3 This chapter built on the results from chapter 2 which revealed that phytoplankton biomass and many individual algal genera were colimited by N and P. However, most cyanobacteria, especially potentially toxic genera like *Anabaena* and *Microcystis*, did not respond to nutrient enrichment with N or P or both in combination. It seemed likely that resources other than or in addition to N and P were needed to induce cyanobacterial growth in Grahamstown Dam. The effects of the two resources light and trace metals were examined.

Specific aims were:

- to determine if light availability affects cyanobacterial growth responses to nutrient additions and
- to determine if cyanobacteria are stimulated by trace metal additions in Grahamstown Dam.

Chapter 4 Growth characteristics of a prominent cyanobacterium, *A. circinalis*, which is often a main constituent of toxic blooms in Australian waterways and a concern species in Grahamstown Dam, were examined in this chapter. The genus

Anabaena is present in Grahamstown Dam and an outbreak of *A. circinalis* may potentially threaten drinking water supply as there are no facilities in place to treat water for saxitoxins produced by this species. There was little information available on certain growth characteristics of *A. circinalis*, in particular in regards to growth at different nutrient concentrations and supply ratios. This chapter aimed to narrow this knowledge gap and to provide valuable information for improving water management in Grahamstown Dam.

Specific aims were:

- to examine how different nutrient ratios and concentrations affect growth rates and abundances of *A. circinalis* and
- to test if different light levels affect the response.

It was hypothesised that external P supply was more important than external N supply for the N fixing cyanobacterium *A. circinalis* and that absolute concentrations were not as important as nutrient ratios. Thus, *A. circinalis* growth rates would be higher under low N:P ratios irrespective of nutrient concentrations. Also, more energy for N fixation should promote this effect. Thus, high light availability would further increase *A. circinalis* growth rates under low N:P ratios.

Chapter 5 This chapter, and the following chapter, examined sediment nutrient dynamics in Grahamstown Dam. As large amounts of nutrients can be present in lake sediments, it was important to identify conditions under which nutrients were released to the water column. Nutrients, especially P, are often released under anoxic conditions. In shallow lakes, like Grahamstown Dam, bottom water anoxia may occur during thermal stratification events and nutrients released during these events can be mixed into the euphotic zone when the stratification breaks down. Thus, it was essential to find out if events of persistent thermal stratification occurred and how long they would last for.

Specific aims were:

- to determine which types of major algal nutrients are released from the sediment,
- to determine how anoxic and oxic conditions affect the release of these nu-

trients and

- to determine the nature of thermal stratification in the lake.

It was hypothesised that sediments release higher amounts of nutrients, in particular of P, in anoxic conditions than in oxic conditions.

Chapter 6 Results from chapter 5 revealed unusual patterns of nutrient release: P was not released from the sediments in oxic or anoxic conditions while substantial concentrations of oxidised N species (nitrate and nitrite) were released in anoxic conditions. Chapter 4 had shown that *A. circinalis* abundance depended more on the amount of available N than on the availability of P. Thus, there was a need to further investigate sediment nutrient dynamics under anoxic conditions to gain insight into conditions that may promote *A. circinalis* growth. The unexpected nutrient release patterns in chapter 5 may have been due to a lack of bioavailable C as many transformation processes in the sediments are microbially mediated and rely on suitable C sources. In order to gain insight into these unexpected findings the role of different C sources on nutrient release was examined.

Specific aims were:

- to investigate the effect of organic substrate on nutrient release and
- to determine the content of total P, potential P binding partners and organic matter in the sediments.

These aims were based on two hypotheses: (1) anaerobic nutrient cycling processes, i.e. denitrification and the release of P, are limited by availability of organic substrate and (2) P concentrations in the sediments are low and thus, at least partly responsible for the lack of P release in anoxic conditions.

Chapter 7 In this chapter, results of chapters 2, 3, 4, 5 and 6 are discussed and overall conclusions are drawn. Recommendations for management of algal and cyanobacterial growth for Grahamstown Dam are also provided.

Chapter 2

Nutrient limitation in Grahamstown Dam

2.1 Introduction

Phytoplankton blooms, especially if dominated by toxic cyanobacteria, can have serious consequences for the supply of drinking water due to the production of potent toxins (Bowling, 1994; Qin et al., 2010). Hence, one goal of drinking water management is to reduce the main cause of phytoplankton blooms – the enrichment of water supplies with the macro nutrients N and P. Of these two main algal nutrients, P has traditionally been the focus of nutrient management in freshwater systems (Lewis and Wurtsbaugh, 2008; Sterner, 2008) and the reduction of P loading to lakes and reservoirs has successfully led to a decrease in algal and cyanobacterial biomass in many cases (Willén, 2001; Jeppesen et al., 2005). However, N alone can also limit phytoplankton productivity (James et al., 2003; Bergström et al., 2008) and there is increasing evidence that phytoplankton colimitation by both nutrients sometimes occurs (Elser et al., 2007; Harpole et al., 2011). Thus, in some lakes controlling N may be as important as controlling P.

Moreover, seasonal changes in nutrient limitation can occur and different scenarios have been observed in different lakes. Colimitation by P and silicate in spring and P and N in autumn may occur although limitation by P prevails during the rest of the year (Moon and Carrick, 2007). No limitation by N or P in some months and limitation by N only in others has been observed in a mainly P

limited lake (Xu et al., 2013). In contrast, lakes that are governed by N limitation may become P limited for short periods in early summer followed by colimitation by N and P before switching back to N limitation (Bergström et al., 2008). As these changes in nutrient limitation fall into growth periods that may be critical for bloom formation, the management of certain nutrients may be necessary before or during these periods of change while the management of other nutrients may be more important throughout the rest of the year.

Whether phytoplankton growth is controlled by P or N is commonly assessed using in situ nutrient enrichment assays. Based on the law of the minimum first conceived by von Liebig, the nutrient that evokes the greatest amount of biomass increase is deemed the limiting nutrient for phytoplankton growth (de Baar, 1994). Biomass increase is often determined as chlorophyll a and, in fewer cases, as cell counts. In situ enrichment assays vary in spatial and temporal scale, ranging from whole lake fertilisation lasting for years (Schindler et al., 2008) to microcosm assays using small volumes (3 L) over a few days (Burger et al., 2007). The extrapolation from microcosm assays to whole ecosystem processes has received criticism in the past. Schindler (1998) and Carpenter (1996) argue that downscaling on the temporal and spatial level, as occurs in microcosm experiments, led to ecologically unrealistic responses. Reasons for this include the partial exclusion of communities and the failure to capture slow processes (Schindler, 1998). However, research has shown that in nutrient enrichment studies algal response does not vary greatly with container volume or surface to volume ratio even when comparing mesocosms ranging from several litres (4 L, 0.15 m diametre, 0.25 m depth and 4 L, 0.32 m * 0.18 m * 0.11 m depth) to small ponds (500 000 L, 46.05 m * 15.56 m * 3.00 m depth) (Spivak et al., 2011). Thus, it is likely that small scale assays examining the direct response of phytoplankton to nutrient addition realistically represent processes occurring at larger scales. In terms of environmental impact, cost and ability to replicate, microcosm assays are the preferred option for investigating nutrient limitation in drinking water storages.

Temporal scale plays an important role in the outcome of nutrient enrichment assays. Algal biomass has been observed to be first limited by N then to not be nutrient limited and finally to be limited by P in bottle assays over a 11 day period

(Carignan and Planas, 1994). Spivak et al. (2011) also reported strong variation of algal responses to nutrient enrichment with time during their seven day experiments. The growth response at a species or genus level may be even more affected by time than the overall growth or biomass increase. Each phytoplankton species has its own optimum growth rate which depends on growth conditions, i.e. temperature, light and nutrient availability. Optimum growth rates can vary greatly between species (Lürling et al., 2013) leading to different species growing at different rates under the same conditions (Litchman, 2000; Schabhüttl et al., 2013). This is important to consider, if the growth response at a species or genus level is to be used in predicting the growth of nuisance groups. In particular, many cyanobacteria have slower growth rates than chlorophyta under similar temperature regimes (Lürling et al., 2013) which implies that cyanobacterial responses to nutrient enrichment may not be detected as fast.

The N and P nutrient limitation of the phytoplankton in Grahamstown Dam, a shallow mesotrophic drinking water reservoir in south east Australia, was examined. The reservoir has a small natural forested catchment and water levels are maintained by pumping water from a nearby river when required. This process introduces nutrients into the lake as the river carries a comparatively higher nutrient concentration. Potentially toxic cyanobacteria are present in the lake but they do not dominate the phytoplankton. However, there is concern that cyanobacterial blooms may develop as a result of increased nutrient levels. The aims were to find the limiting nutrient for phytoplankton growth at an assemblage and genus level, to determine if nutrient limitation is seasonally different and to examine if the experimental period influences the phytoplankton response to nutrient enrichment at an assemblage and genus level.

2.2 Methods

2.2.1 Study site

Grahamstown Dam is a shallow reservoir located 20 kilometres north of Newcastle on the east coast of New South Wales, Australia (Figure 2.1). The lake is usually well mixed due to its surface area of 28 km^2, which provides a large

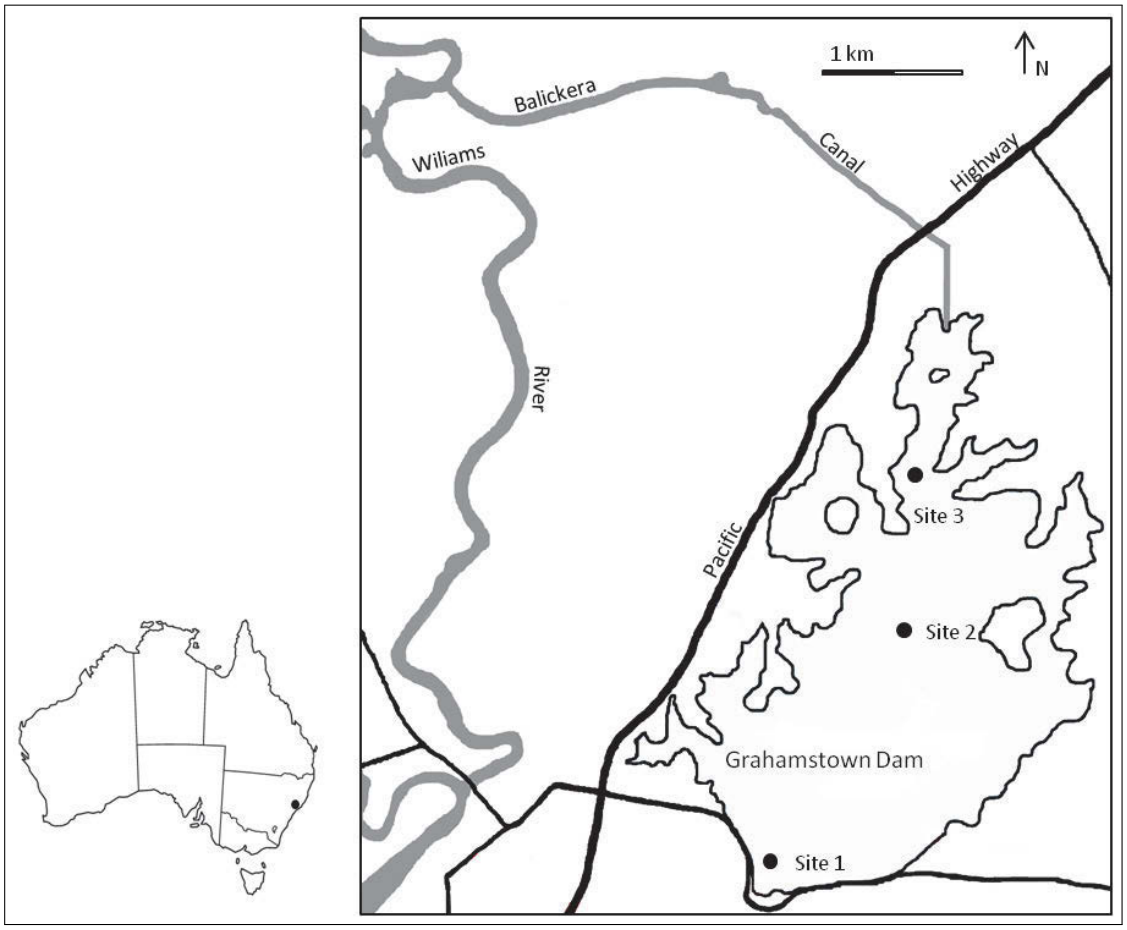

Figure 2.1: Location of Grahamstown Dam on the Australian continent and location of the three experimental sites within the lake. Solid circles mark approximate positions.

fetch, and its relatively shallow mean depth of 7 m (the full supply level is 12.8 m). Short periods of thermal stratification may occur for several days during the summer months.

The catchment comprises 73 km^2 which may not be sufficient to maintain levels necessary for drinking water supply. To compensate, water levels are increased by pumping water from the nearby Williams River through a canal (Balickera Canal) into the northern end of the lake. The land use of the river's catchment is dominated by agriculture (80 %), in particular cattle farming (Hunter Water, 2011), resulting in higher nutrient concentrations in the river compared with Grahamstown Dam (Figure 2.2). As about 50 % of the lake's inflow are extracted from Williams River (Hunter Water, 2011), there is a threat that nutrient concentrations will increase in the reservoir. Currently, the lake has been classified as mesotrophic according to OECD guidelines (Cole and Williams, 2011).

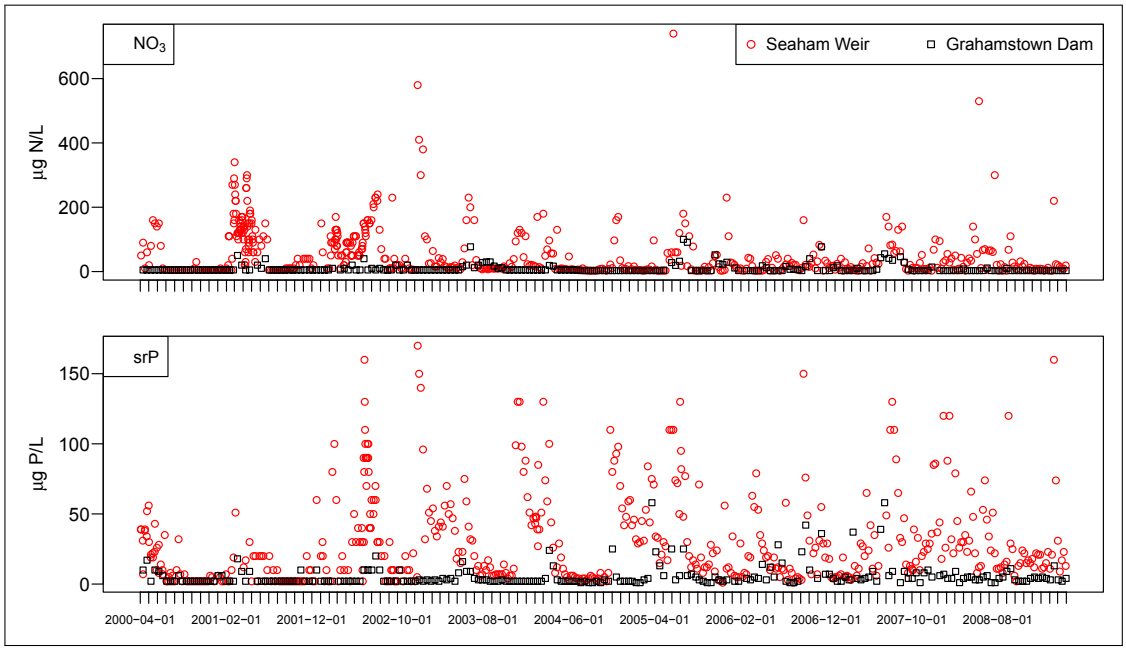

Figure 2.2: Biweekly monitoring of nitrate (NO_3) and srP at Seaham Weir on the Williams River and at the monitoring site close to Balickera Canal in Grahamstown Dam. Data presented were collected between 1/04/2000 and 1/04/2009 by Hunter Water.

For this study, three different sites in the lake were chosen for their individual environmental characteristics. Site 3, close to Balickera Canal, is the most protected and shallowest (4-6 m, depending on capacity of the lake) while Site 2, closest to the centre of the lake, is the most wind exposed and deepest (9-10 m). Site 1, close to the drinking water out take, is between 7 and 9 m deep. Each site is permanently marked with a buoy (Figure 2.1).

Concentrations of nitrate and soluble reactive phosphorus (srP) vary between 3 and 100 μg N/L and 1 and 58 μg P/L in Grahamstown Dam (Hunter Water monitoring data, Figure 2.2). Compared with the lake, concentrations of N and P are higher in the Williams River at Seaham Weir from where water is pumped into the dam via Balickera Canal (Hunter Water monitoring data). Concentrations of nitrate and srP vary between 3 and 740 μg N/L and 1 and 170 μg P/L at Seaham Weir.

The lake's phytoplankton is dominated by Chlorophyceae and Bacillariophyceae by biovolume and by non toxic colonial cyanobacteria by cell counts. Potentially toxic cyanobacteria of the genera *Microcystis*, *Anabaena* and *Aphanizomenon* are present in the lake. Cell densities of *Microcystis* are often around 1000 cells/ml

while *Anabaena* rarely reaches 1000 cells/ml. Cell counts have increased up to 30 000 cells/ml for *Microcystis* and 20 000 cells/ml for *Anabaena* on two separate occasions since 1992 while *Aphanizomenon* has only been recorded intermittently since 2000 (Hunter Water monitoring data).

2.2.2 Seasonal microcosm enrichment assays

In order to identify the limiting nutrient for phytoplankton growth and potential changes due to seasonalities, in situ microcosm nutrient enrichment assays were conducted in April 2009, August 2009, February 2010, May 2010, December 2010, May 2011 and August 2011 at the three sites. Each microcosm enrichment experiment lasted for four days (day 0 to day 4).

Three different nutrient treatments and one untreated control (C) were tested in triplicate for each of the three sites: 500 μg N/L in the form KNO_3 (treatment N), 200 μg P/L in the form of KH_2PO_4 (treatment P), a combination of both in the same concentration as in the single treatments (treatment PN). Nutrient concentrations were chosen so that nutrients were available in excess but within the natural range expected in Australian freshwater systems. Any limitation by carbon was assumed to be minimal compared to limitation by N and P as initial tests showed a large response to N and P. Also, the extent of limitation by N or P was less of interest than knowing the nutrient that elicited the greatest response.

The experimental set up at each site consisted of two racks each holding six 1.25 L clear PET bottles parallel to the water surface in order to ensure an even light exposure. Racks were placed in the euphotic zone at half of the Secchi depth which approximated 25 % surface irradiance. At each site, about 25 L of surface water were filtered through a 63 μm plankton net and pooled in a plastic bin. Bottles were filled from the bin, leaving some space at the top. Nutrient solutions of P and N were added to make up the intended concentrations, then the bottles were filled to the top and mixed by rotation. Lake water was filtered in order to exclude zooplankton and thus, prevent any changes in phytoplankton dynamics due to zooplankton grazing.

Samples for phytoplankton, chlorophyll a and nutrient concentrations were collected on day 0 and day 4. Day 0 samples were taken in triplicate from the

bin of filtered but otherwise untreated lake water. Phytoplankton samples were preserved in Lugol's iodine. Concentrations of the added dissolved nutrients were measured in surrogate bottles on day 0. Surrogate bottles were prepared in triplicate and in the same way as the ones for the PN treatment. Nutrient samples were filtered through a 0.45 μm filter.

2.2.3 Mesocosm enrichment assays

In situ mesocosm assays were conducted to examine the effect of greater time periods on nutrient enrichment. Assays were conducted from the 17/1/2013 until the 4/2/2013 and from the 21/3/2013 until the 8/4/2013. Mesocosm assays were deployed at Site 3 as the previous microcosm assays had shown that phytoplankton growth at this site was generally higher compared with the other sites and cyanobacterial numbers were potentially higher. Moreover, Site 3 was the most sheltered of the three. Simultaneously, microcosm assays were conducted at Site 3 as described in section 2.2.2.

Nutrient treatments were the same as in the microcosm assays (C, N, P, PN, n=3) . Mesocosms were made of polyethylene sheeting heat sealed into bags of 2 m length and 1.5 m width. A cylindrical shape (1 m diameter, 1.5 m length), open only at the water surface, was maintained by stiff plastic tubing attached at the top and bottom of the bags. The mesocosm bags were attached to three floating frames (2$*$2$*$2 m). Each level of nutrient treatment was represented within each frame to ensure randomisation. Mesocosms were filled with approximately 1000 L of unfiltered lake water.

Integrated depth samples for phytoplankton, chlorophyll a and nutrient concentrations were collected on day 0, 4, 8, 12 and 18. Nutrient samples were taken before and after nutrient additions on day 0. Dissolved oxygen, pH and temperature were measured in the mesocosms on each sampling day.

Chlorophyll a analysis

Chlorophyll a samples were filtered via vacuum filtration onto glass fibre filters on site or in the laboratory on the same day of sampling. Chlorophyll a was analysed according to Gregor and Maršálek (2004) but omitting the acidification

step. The glass fibre filters were extracted in 90 % boiling ethanol for five minutes. Remains of the filters were eliminated by centrifuging. The supernatant was analysed immediately using a Varian Cary 50 Bio UV spectrophotometer.

Phytoplankton enumeration

Phytoplankton numbers were determined microscopically at 200 times magnification using a Sedgwick-Rafter counting chamber. Samples were concentrated by factors 3.3, 5 or 10 as required prior to counting. To achieve a realistic representation of the phytoplankton assemblage, individuals of each genus were counted until numbers of the most abundant genus reached at least 120 individuals. At least three short traverses of the chamber were counted for each sample. Cyanobacteria and algae were identified to genus level using identification literature by Prescott (1978), Streble and Krauter (2008) and Entwistle et al. (1997). Biovolumes of the most abundant genera were determined by using the most appropriate conversion factors from NRMMC (2006) (in review) and Olenina et al. (2005).

Nutrient analysis

Nutrient concentrations were determined photometrically with a QuikChem 8500 Lachat nutrient analyser. Soluble reactive phosphorus (srP) was measured with the molybdate blue method using ascorbic acid as reductant. Nitrate and nitrite (NO_x) were analysed as surrogate of nitrate (after establishing that nitrite concentrations were negligible) with the sulphanilamide method after reduction by a cadmium column (APHA, 1995).

2.2.4 Data analysis

In the microcosm assays examining potential seasonal changes in nutrient limitation, chlorophyll a concentrations were analysed with two factorial repeated measures analysis of variance (ANOVA) with a significance level of α=0.05 using SPSS Statistics 19.0. The Wilk's Lambda statistic was interpreted and interactions between treatments and sites were analysed using Tukey's pairwise comparison. Data for phytoplankton genera and chlorophyll a were analysed with two factorial ANOVA and Tukey's pairwise comparison for each assay. Homogeneity

of variance was tested with the Levene statistic. Consequentially, chlorophyll a data from the seasonal microcosm assays were logarithmically transformed to satisfy the assumptions of ANOVA. Chlorophyll a concentrations on day 4 in the mesocosm assays and in the simultaneously conducted microcosm assays were compared using two factorial ANOVA. Graphs were prepared with the statistics software R (version 2.13.0, R Development Core Team (2011)).

2.3 Results

2.3.1 Seasonal microcosm enrichment assays

Chlorophyll a response to nutrient addition

Phytoplankton growth was recorded in the experimental bottles in each of the seven assays as indicated by increased chlorophyll a concentrations in at least one of the treatments compared with initial concentrations. Figure 2.3 and Table 2.2 show that the PN treatment evoked significantly higher phytoplankton growth compared with all other treatments at all three sites in all but the August 2009 assay. This suggests colimitation of the phytoplankton assemblage by both N and P. In August 2009, there was a significant response to the addition of P, indicating P limitation of phytoplankton growth.

There was a significant interaction ($p \leq 0.001$) between time, nutrient enrichment treatment and site (Table 2.1). This was due to a varying magnitude of the response to the PN treatment at different sites and different assays and a response mainly to P additions in August 2009. Also, a lack of a distinct response pattern at Site 3 in May and August 2011 likely contributed to the interaction. Site 3 generally had the highest chlorophyll a concentration in the PN treatment, followed by Site 1 and then Site 2. The magnitude of the response to the PN treatment was greater in the assays carried out during the warmer seasons, in particular in the February and December 2010 assays. The responses to nutrient treatments as well as overall growth were lowest in the August 2009 and 2011 assays, i.e. assays conducted in winter.

Time and site as well as time and treatment interaction terms were also significant and according to Tukey's Pairwise Comparison the nutrient treatments can

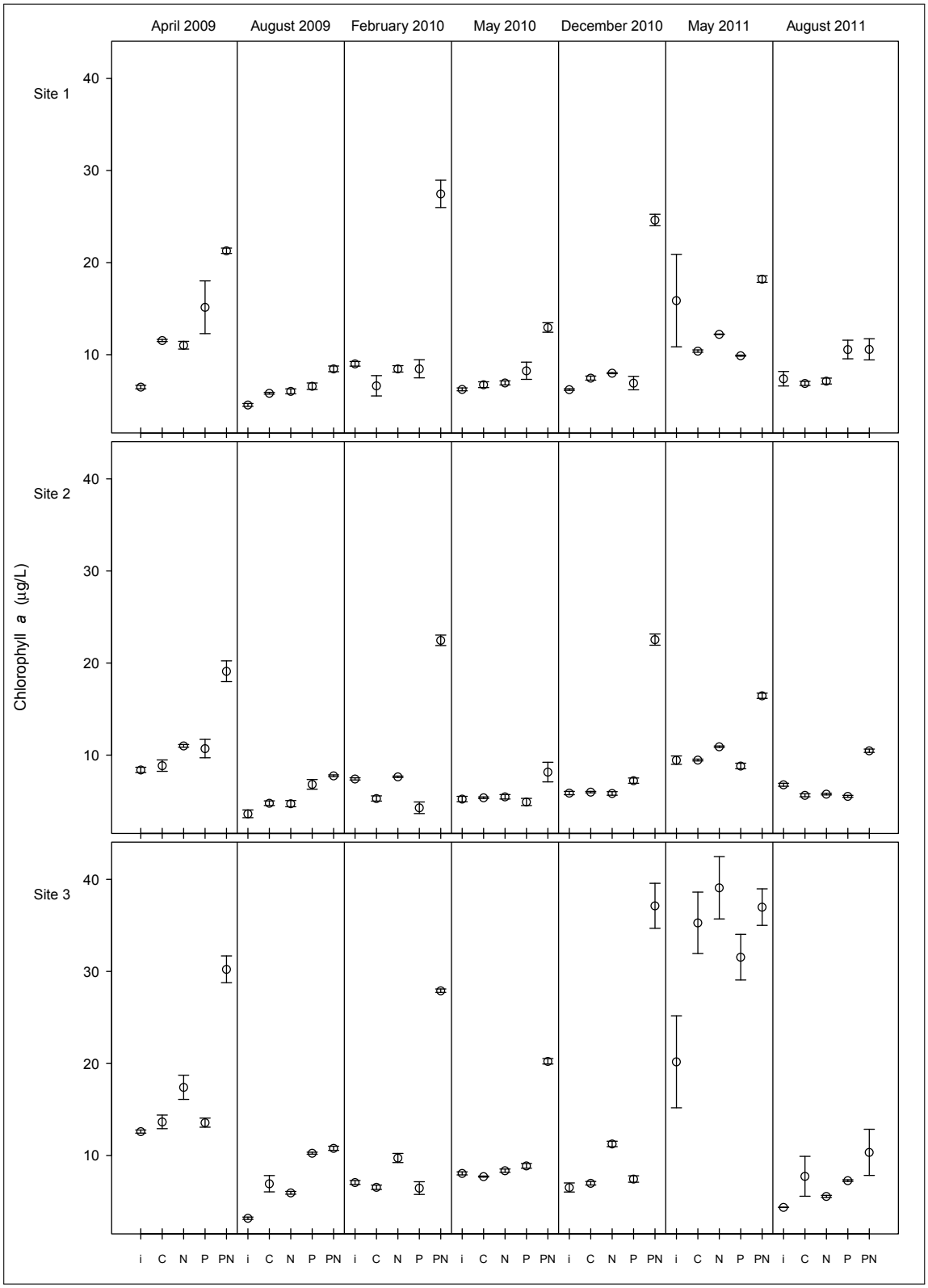

Figure 2.3: Chlorophyll a concentration for all three sites during the seasonal microcosm nutrient enrichment assays conducted from April 2009 until August 2011. Treatments with N only addition (N), P only addition (P) and N and P added in combination (PN) and the control (C) display values on day 4. Initial concentrations (i) were measured on day 0 prior to nutrient additions. Error bars are one standard error from the mean, n=3.

Table 2.1: Result of repeated measures ANOVA of the chlorophyll a response to the four nutrient treatments (C, N, P and PN) at the three sites in seven microcosm assays (time).

Effect	Wilk's λ	F	Hypothesis df	Error df	P
time	0.011	294.993	6	19.000	0.001
time*site	0.013	24.957	12	38.000	0.001
time*treatment	0.004	19.012	18	54.225	0.001
time*site*treatment	0.008	4.895	36	86.196	0.001

be grouped into the following three homogeneous subsets:

1. PN treatment,
2. P treatment and N treatment and
3. controls (C).

When analysed individually with two factorial ANOVA, most assays had the highest response to nutrient enrichment in the PN treatments. According to Tukey's pairwise comparison, this was significant in the assays conducted in February 2010 ($p \leq 0.001$), May 2010 ($p \leq 0.010$) and December 2010 ($p \leq 0.001$) at all three sites and at Site 2 and 3 in April 2009 ($p \leq 0.001$), at Site 1 in August 2009 ($p \leq 0.008$) and Site 2 in August 2011 ($p \leq 0.033$). There were also signs of P limitation. In August 2009, chlorophyll a values in the P and PN treatments were significantly greater than values in the remaining treatments ($p \leq 0.006$) at Sites 2 and 3.

Genera response to nutrient addition

About 35 of the most abundant genera were counted in each assay of which 23 responded to the nutrient treatment as shown in Table 2.2. Responses to the PN treatment were the most frequently recorded ones and were significant on 63 occasions when compared to the controls. There were also responses by individual genera to N only and P only additions. Growth responses to P were significant on 25 occasions and there was one significant growth response to N. Most of the growth responses to the PN treatment were found in the assays conducted in February and December 2010. In contrast, most growth responses were due to additions of P in the August 2009 assay.

Chlorophyceae and Bacillariophyceae mainly responded to additions of combined P and N or P alone with significantly increased growth but there were also

Table 2.2: Growth responses of the phytoplankton genera that responded to the nutrient enrichment treatments for each site and each assay. Treatment names given in the table indicate which treatments evoked enhanced growth and asterisks show whether the growth response was significantly higher compared with the control. Superscript a indicate that there was no significant difference between marked treatments. No entry for any genus means there was no distinct growth in any of the treatments compared with controls. P values were derived from fully factorial two way ANOVA of site and nutrient enrichment treatment for each assay followed by Tukey's Pairwise Comparison.

		April 2009			August 2009			February 2010			May 2010		
Taxonomic group	Genus	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Cyanobacteria	*Aphanothece*	PN^{**at}, P^{a}	PN^{**at}, P^{a}	PN^{**at}, P^{a}	P**		P**, PN				PN		
	Aphanocapsa			PN			PN						PN
Chlorophyceae	*Ankistrodesmus*	PN^{**t}	PN^{**t}	PN^{**t}	PN	P, PN	P, PN	PN^{**2}	PN^{**3}	PN^{**1}	PN^{**a2}, P^{a}	PN^{3}, P	PN^{**1}
	Chodatella				P, PN			PN^{**t}	PN^{**t}	PN^{**t}	P^{**t}	P^{**t}	P^{**t}
	Cosmarium							PN^{**t}	PN^{**t}	PN^{**t}			
	Crucigenia	PN	PN	P, PN	P^{**at}, PN^{a}	P^{**at}, PN^{a}	P^{**at}, PN^{a}	P		PN	PN^{*t}	PN^{*t}	PN^{*t}
	Dictyosphaerium	P ^{a}, PN^{a}	PN	P^{a}, PN^{a}	PN, P	PN, P	PN, P	PN, P	PN, P	PN, P	PN^{*a}, P^{a}	PN, P	PN^{**a}, P^{**a}
	Elakatothrix		PN			P*	P^{*a}, PN^{a}	PN^{**at}, P^{*a}	PN^{**at}, P^{*a}	PN^{**at}, P^{*a}	PN	P*	
	Nephrocytium	PN^{**t}	PN^{**t}	PN^{**t}	P		PN	PN^{**t}	PN^{**t}	PN^{**t}	P^{*t}, PN	P^{*t}, PN	P^{*t}
	Oocystis						PN, P	PN, P	PN	PN**			P
	Scenedesmus			PN**				PN^{**t}	PN^{**t}	PN^{**t}	PN, P		PN
	Sphaerocystis				P^{*at}, PN^{a}	P^{*at}, PN^{a}	P^{*at}, PN^{a}				PN, P		PN*
Bacillariophyceae	*Acanthoceras*	PN**	PN			PN	PN, P						
	Aulacoseira		PN**	PN**			PN						
	Cyclotella		PN**	PN**			PN	PN^{**t}	PN^{**t}	PN^{**t}	PN^{**a}, P^{**a}		
	Synedra							PN^{a}, P^{a}	PN**	PN**			
	Urosolenia	PN, N	PN	N			PN	PN, N, P	PN		PN**, P	PN	PN
Cryptophyceae	*Cryptomonas*	PN^{*t}	PN^{*t}	PN^{*t}			PN**>P**	PN^{**t}	PN^{**t}	PN^{**t}	PN, P		PN
Synurophyceae	*Mallomonas*	PN^{**t}	PN^{**t}	PN^{**t}			PN, P						
Euglenophyceae	*Trachelomonas*			N				PN**					
	Chlorophyll a	PN^{**a1,2}, P^{a}	PN^{**2}	PN^{**1}	PN^{**a1,2} P^{a2}	PN^{**a2}, P^{**a2}	PN^{**a1}, P^{**a1}	PN^{**t}	PN^{**t}	PN^{**t}	PN^{**2}	PN^{*3}	PN^{**1}

Table 2.2 continued:

		December 2010			May 2011			August 2011		
Taxonomic group	Genus	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Cyanobacteria	*Aphanothece*	PN	PN**		PN	PN, P	P			
	Aphanocapsa	N	N	PN*				PN	PN	
	Anabaena						P, N		P	
	Microcystis		N		PN, P		N			
Chlorophyceae	*Ankistrodesmus*	PN**[2]	PN**[a3], P*[a]	PN**[1]	PN			P	PN*	
	Chodatella	PN*[t]	PN*[t]	PN*[t]		PN				
	Crucigenia		P**	PN, P		PN				
	Dictyosphaerium	PN[2]>P	PN**[1]>P	PN*[2]	P*[ta], PN[a]	P*[ta], PN[a]	P*[ta], PN[a]			
	Elakatothrix		P**		P	PN				
	Nephrocytium	PN**	PN**		PN*[t], P	PN*[t], P	PN*[t], P	PN	P	
	Oocystis	PN**[t]	PN**[t]	PN**[t]				P	PN	
	Scenedesmus	PN**[1]		PN[2]				P*[t]	P*[t]	P*[t]
	Sphaerocystis	PN**[t]	PN**[t]	PN**[t]	PN**	PN**				
	Staurastrum	PN**[t]	PN**[t]	PN**[t]						
Bacillariophyceae	*Acanthoceras*	PN**	PN**> P**	PN**>N**	PN**	PN**				
	Aulacoseira	PN, P, N	P, PN	PN**						
	Cyclotella	PN**[1]>P**		PN**[2]				PN**[a], P**[a]	PN**	
	Synedra	PN[2]		PN**[1]						
	Urosolenia	PN**	PN[a], P[a]							
Cryptophyceae	*Cryptomonas*	PN**[t]	PN**[t]	PN**[t]		PN				
Synurophyceae	*Mallomonas*	PN**[1]	PN**[2]	PN**[2]						PN
Euglenophyceae	*Trachelomonas*						PN**			
	Chlorophyll a	PN**[2]	PN**[2]	PN**[1]> N**	PN**	PN**		PN**[t]	PN**[t]	PN**[t]

*p≤0.05 compared with control

**p≤0.005 compared with control

[a]nutrient treatments were not statistically different from each other within each site

[t] no interaction between treatment and site but treatment was significant (p≤0.05)

[1,2,3] indication of magnitude of response (1 = highest, 3 = lowest) to a nurient treatment, if treatments were different between sites

responses by *Cryptomonas*, *Mallomonas* and *Trachelomonas*. *Aphanothece* and *Aphanocapsa* were the only cyanobacteria that showed significant growth responses in any treatment. The former responded to P and PN treatments in the April 2009, August 2009 and December 2010 assays and the latter to the PN treatment in the December 2009 assay.

2.3.2 Mesocosm enrichment assays

Chlorophyll a response to nutrient addition

Similar to the seasonal microcosm assays, the highest response to nutrient enrichment, measured as chlorophyll a concentration, was found in the PN treatment in the two mesocosm and simultaneous microcosm assays conducted in 2013 (Figure 2.4). In both mesocosm assays, biomass continued to increase in the PN treatment until day 8. After day 8, biomass declined in both assays.

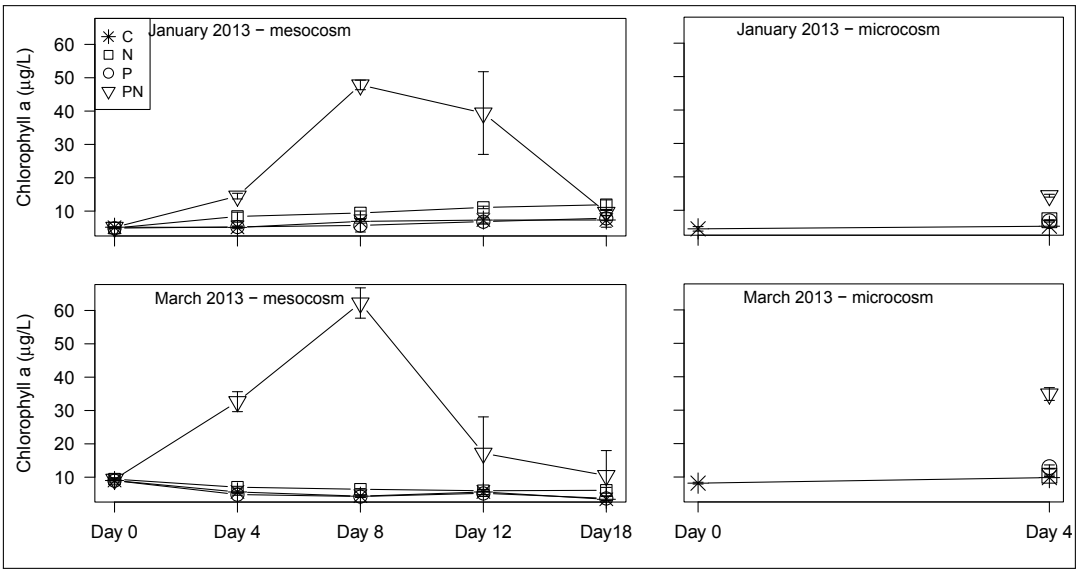

Figure 2.4: Chlorophyll a concentration in μg/L in the mesocosm and microcosm assays conducted in January and March 2013. Error bars are one standard error from the mean, n=3.

In the January assay, chlorophyll a concentrations increased almost three fold between days 0 (5.2 μg/L) and 4 (14 μg/L) and more than three fold between days 4 and 8 (48 μg/L) in the PN treatment. In the March assay, biomass increase was more than three fold in the PN treatment between days 0 (9.4 μg/L) and 4 (33 μg/L) and almost two fold between days 4 and 8 (62 μg/L).

Biomass in the simultaneously conducted microcosm assays were statistically not different from the biomass in the mesocosm assays on day 4 in the January assays (Table 2.3, Figure 2.4). In the March assays, concentrations were higher in the microcosm assays but the response pattern was the same, i.e. highest response to the PN treatment (Table 2.3, Figure 2.4).

There was no noticeable response to P only additions compared with controls in either assay while there was a small response to N only additions in the January assay, reaching highest values on day 18 (12 μg/L). Controls did not experience a biomass increase more than two fold in the January assays. In March, biomass declined in the controls from day 0 onwards. There was no statistical difference between chlorophyll a concentrations in treatments and controls on day 0 in either assay.

Table 2.3: Results of the comparison of the chlorophyll a concentration of the mesocosm and microcosm assays on day 4. Factor size had the levels microcosm and mesocosm, factor treatment had the levels C, N, P and PN in the two factorial ANOVA.

	January 2013			March 2013		
Source	adjusted MS	F	p	adjusted MS	F	p
size	0.08	0.01	0.939	122.19	24.48	0.000
treatment	105.74	93.49	0.000	962.79	192.89	0.000
size*treatment	2.11	1.87	0.176	10.21	2.05	0.148

Genera responses to nutrient enrichment

In both mesocosm assays, the most obvious response to nutrient additions by individual genera were observed in the treatment enriched with P and N in combination. The PN treatment evoked distinctly higher growth in 13 of the 25 most abundant phytoplankton genera when compared to the control and the other treatments in the January assay (Figures 2.5) and in 20 genera in the March assay (Figures 2.6). In the January assay, these genera included the cyanobacterium *Aphanocapsa*; the Chlorophyceae *Ankistrodesmus*, *Scenedesmus*, *Sphaerocystis*, *Oocystis*, *Chodatella*, *Pediastrum*, *Mougeotia* and *Tetraedron*; the Bacillariophyceae *Cyclotella*, *Urosolenia* and *Acanthoceras* and the Cryptophyceae *Cryptomonas*.

In the March assay, they comprised the cyanobacteria *Anabaena*, *Aphanocapsa*, *Aphanizomenon* and *Chroococcus*; the Chlorophyceae *Ankistrodesmus*, *Closterium*,

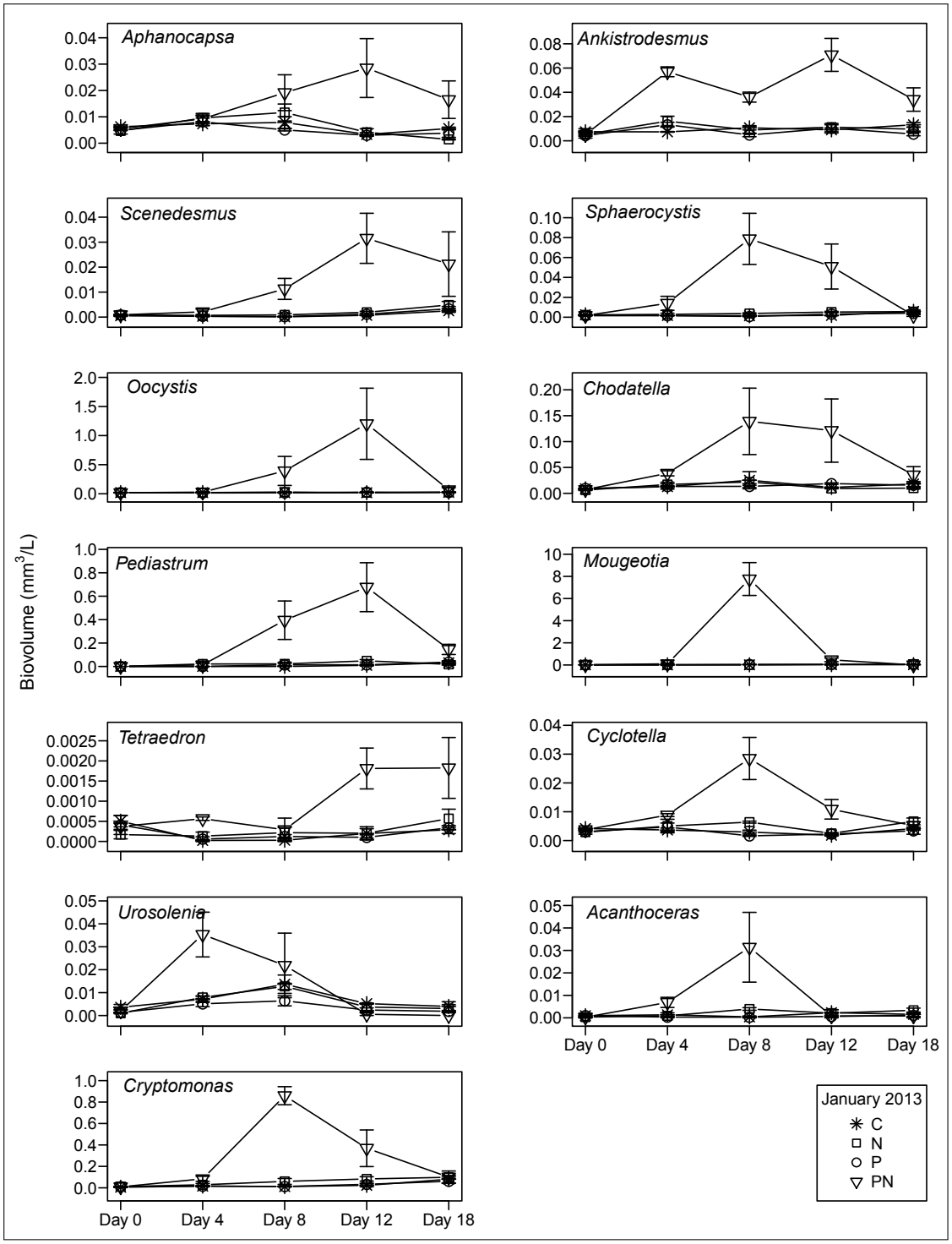

Figure 2.5: Biovolumes in mm^3/L of all phytoplankton genera responding to additions of P and N in combination in the mesocosm assays in January 2013. Error bars are one standard error from the mean, n=3.

Chodatella, *Crucigenia*, *Nephrocytium*, *Oocystis*, *Pediastrum*, *Scenedesmus* and *Sphaerocystis*; the Bacillariophyceae *Acanthoceras*, *Aulacoseira*, *Cyclotella* and *Synedra*; the Cryptophyceae *Chroomonas* and *Cryptomonas* and the Synurophyceae *Mallomonas*.

Single additions of N and P also evoked growth responses in some genera when compared with growth in the controls and the other treatments (Figure 2.7). *Chroococcus*, *Crucigenia*, *Staurastrum* (Chlorophyceae), *Aulacoseira*, *Synedra*, *Trachelomonas* (Euglenophyceae) and *Chroomonas* responded to the N treatment in the January assay. In the March assay, only *Staurastrum* responded to additions of N.

Some genera responded to different nutrient treatments during the course of the experiment (Figure 2.7). *Aphanizomenon* and *Anabaena* responded to additions of P and N in combination within the first 8 days of both experiments (Figures 2.6 and 2.7). Additions of P evoked a 24 fold increase in biovolume by *Aphanizomenon* and a 28 fold increase in biovolume by *Anabaena* between days 12 and 18 in the January assay. *Anabaena* also responded to the P treatment between days 8 and 12 in the March assay but its growth response to the PN treatment exceeded the growth response to the P treatment. In the January assay, *Crucigenia* and *Chroomonas* responded to the PN treatment within the first four days of the experiment but their biovolumes also increased in the N treatment between days 8 and 12. This increase continued until the end of the experiment while there was a decrease in biovolumes of both genera in the PN treatment after day 8 (*Crucigenia*) and day 12 (*Chroomonas*). Conversely, *Chroococcus* first showed a growth response to N additions on day 8 and also responded to the PN treatment between days 8 and 12. In the March assay, *Aulacoseira* biovolume increased in response to the PN and the N treatment on day 4 (Figure 2.6). After day 4, *Aulacoseira* biovolume decreased in the N treatment, while it continued to increase in the PN treatment.

The day a growth response to a nutrient treatment was first recorded varied between genera. In the January assay, a response to nutrient enrichment was recorded for *Anabaena* (Figure 2.7), *Ankistrodesmus*, *Chodatella*, *Sphaerocystis*, *Crucigenia*, *Acanthoceras*, *Cyclotella*, *Synedra* (Figure 2.5) and *Chroomonas* (Figure 2.7) on day 4 of the experiment. Genera for whom a response was first recorded

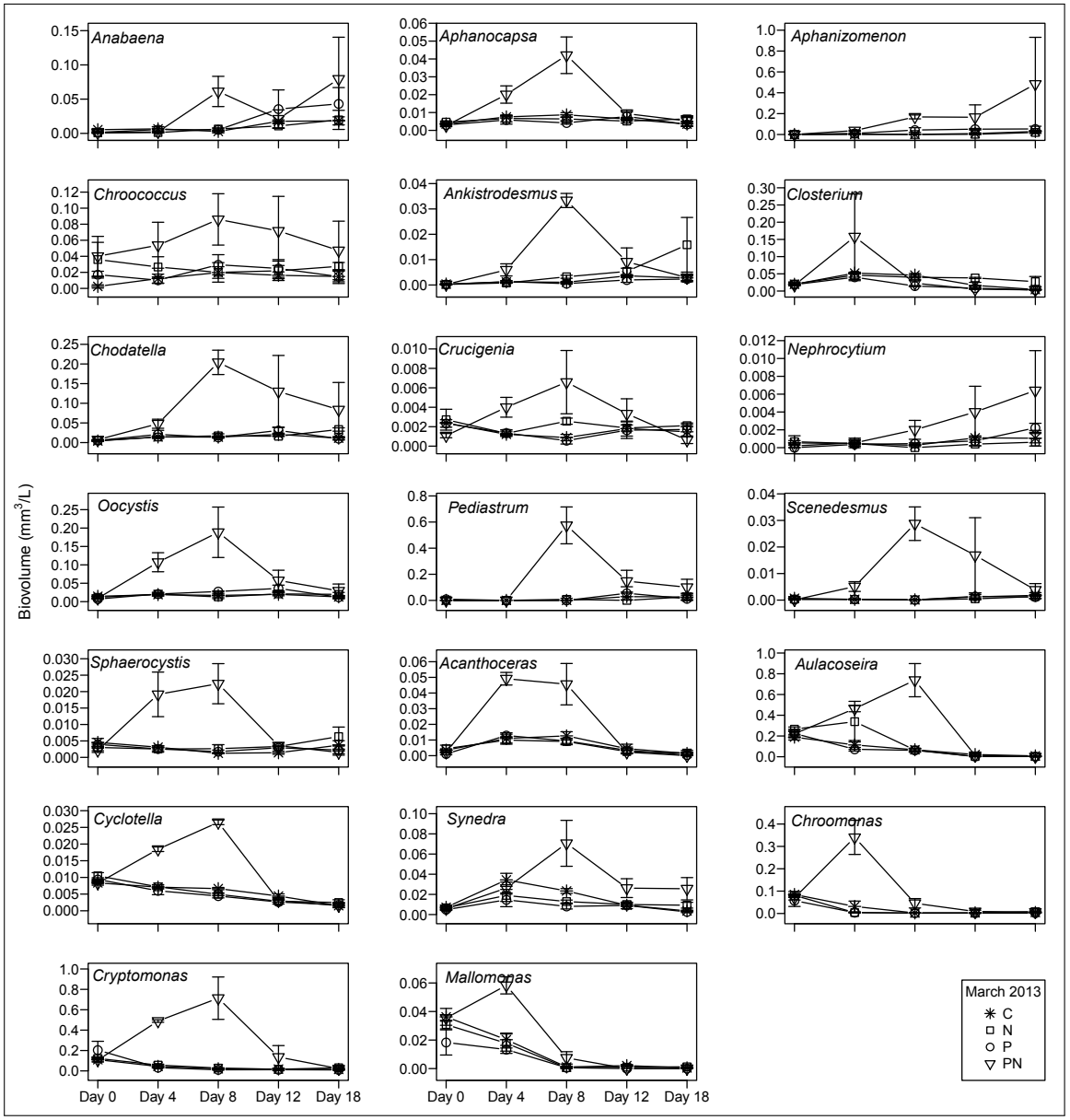

Figure 2.6: Biovolumes in mm^3/L of all phytoplankton genera responding to additions of P and N in combination in the mesocosm assays in March 2013. Error bars are one standard error from the mean, n=3.

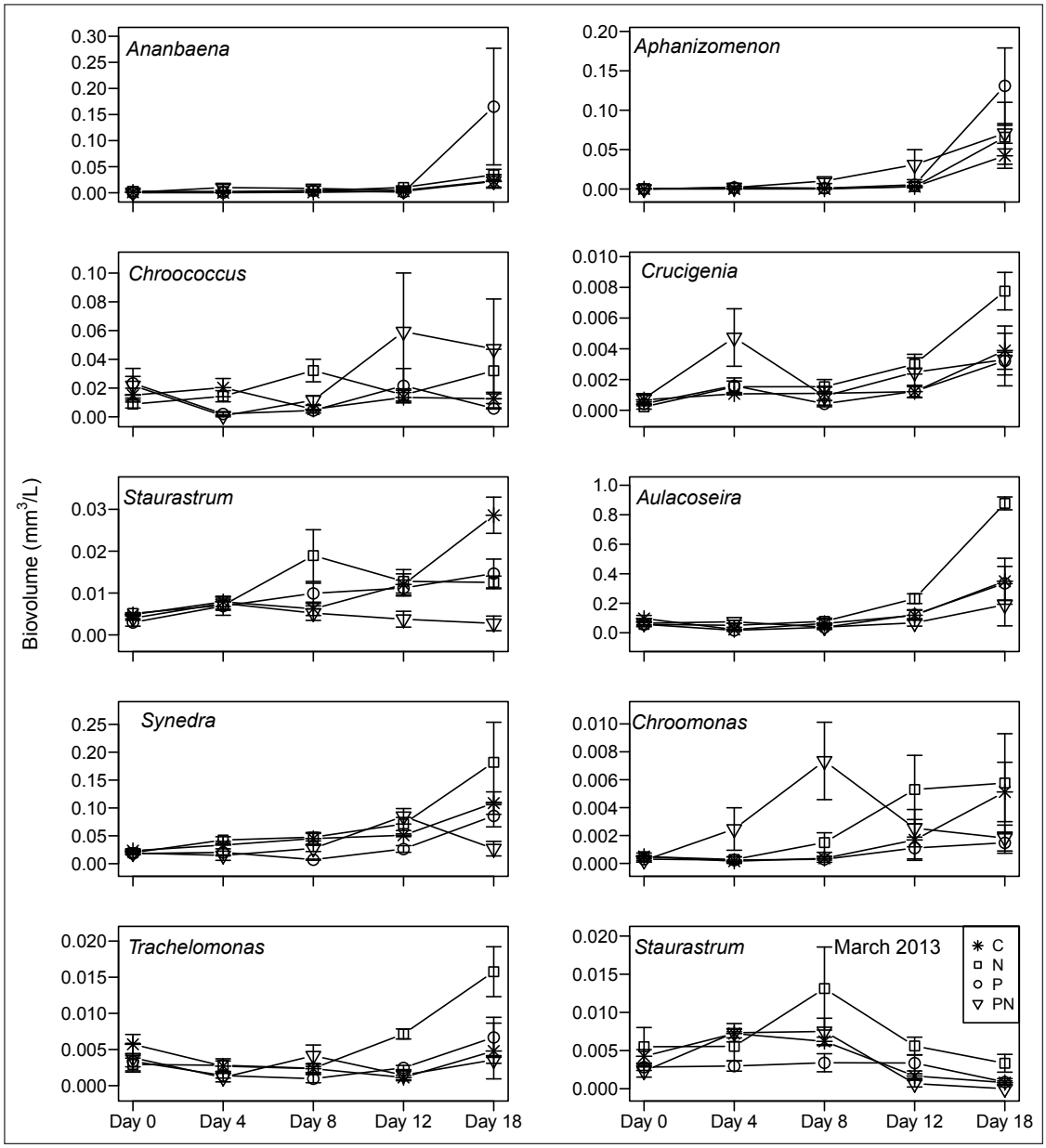

Figure 2.7: Biovolumes in mm^3/L of phytoplankton genera responding to additions of P or N and genera switching responses to different treatments. Biovolumes of *Anabaena* and *Aphanizomenon* have been square root transformed because of low values at the beginning of the experiment. Graphs not further labeled show responses of genera in the mesocosm assay in January 2013. Error bars are one standard error from the mean, n=3.

on day 8 included: *Aphanocapsa*, *Chroococcus*, *Scenedesmus*, *Oocystis*, *Pediastrum*, *Mougeotia*, *Staurastrum* and *Cryptomonas*. *Tetraedron*, *Aulacoseira* and *Trachelomonas* responded first to nutrient additions between days 8 and 12 and *Synedra* after day 12. In the March assay, most genera showed a growth response to nutrient enrichment on day 4 of the experiment. They included *Anabaena*, *Aphanocapsa*, *Ankistrodesmus*, *Closterium*, *Chodatella*, *Crucigenia*, *Oocystis*, *Scenedesmus*, *Sphaerocystis*, *Acanthoceras*, *Aulacoseira*, *Cyclotella*, *Cryptomonas*, *Chroomonas* and *Mallomonas*. A growth response of *Aphanizomenon*, *Chroococcus*, *Nephrocytium*, *Pediastrum*, *Staurastrum* and *Synedra* was first seen on day 8.

The day the highest biovolume was recorded varied between genera. In the January assay, *Urosolenia* was the only genus that reached the highest observed biovolume on day 4. Highest biovolumes of *Sphaerocystis*, *Chodatella*, *Mougeotia*, *Cyclotella*, *Acanthoceras*, *Cryptomonas* and *Chroomonas* were recorded on day 8. On day 12 highest biovolumes were observed for *Chroococcus*, *Aphanocapsa*, *Ankistrodemus*, *Scenedesmus*, *Oocystis*, *Pediastrum* and *Tetraedron*. Biovolumes of *Anabaena*, *Aphanizomenon*, *Crucigenia*, *Staurastrum*, *Aulacoseira*, *Synedra* and *Trachelomonas* were highest on day 18. In the March assay, biovolumes of *Closterium*, *Acanthoceras*, *Chroomonas* and *Mallomonas* were higher on day 4 than on any other sampling day while for most genera highest biovolumes were recorded on day 8. These genera included *Aphanocapsa*, *Chroococcus*, *Ankistrodesmus*, *Chodatella*, *Crucigenia*, *Oocystis*, *Pediastrum*, *Scenedesmus*, *Sphaerocystis*, *Staurastrum*, *Aulacoseira*, *Cyclotella*, *Synedra* and *Cryptomonas*. Biovolumes of *Anabaena*, *Aphanizomenon* and *Nephrocytium* were highest on day 18.

2.4 Discussion

The most frequent outcome of the nutrient enrichment assays was a response to the PN treatment while addition of single nutrients did not lead to a significant growth response. This was observed at the biomass level and at the level of individual genera. The response pattern to factorial nutrient addition where two simultaneously added nutrients evoke a greater growth response than their individual additions is often referred to as colimitation by both nutrients (Burger

et al., 2007; Ogbebo et al., 2009; Harpole et al., 2011). Harpole et al. (2011) further distinguish between two types of colimitation: simultaneous and independent. Responses to two resources in combination but not to their individual additions are classified as simultaneous colimitation. In the case of independent colimitation, two resources evoke an equal growth response when added individually. When added in combination, the growth response exceeds the ones caused by their individual additions.

Most results of the microcosm assays fall into the category of simultaneous colimitation: assemblage growth responses at all three sites in February and May 2010, at Sites 2 and 3 in April 2009, Sites 1 and 2 in December 2010, Sites 1 and 2 in May 2011 and at Site 1 in August 2009. Most of the genera responses to nutrient additions in these assays can also be classified as simultaneous colimitation (e.g. *Ankistrodesmus*, Table 2.2).

A significant response to the PN treatment together with a significant response to addition of either N or P can be classified as "serial limitation" (Harpole et al., 2011). This type of response was observed at the assemblage level in the microcosm assays at Sites 2 and 3 in August 2009, Site 1 in April 2009 and at Site 3 in December 2009. In August 2009, most genera responses reflected the assemblage response, i.e. most genera were serially limited by P and then by N (Table 2.2). In December 2010 and April 2009, some genera responses could be classified as serially limited (e.g. *Acanthoceras*, limited first by N and then by P, Table 2.2) but most genera would be considered simultaneously colimited. This indicates that in particular in August 2009, one nutrient was more important for the phytoplankton assemblage and its individual genera than the other.

Similarly, the biomass response in the January mesocosm assay may fall into the category of serial limitation as concentrations in the N treatment were increased throughout the assay compared with the controls. In contrast, the biomass response in the March mesocosm assay could be considered as simultaneous colimitation. Also, the biomass responses were reflected by most genera in both assays.

Colimitation of phytoplankton growth has been reported frequently in short term experiments using chlorophyll a or other composite measures to detect a

growth response (Dzialowski et al., 2005; Burger et al., 2007; Quiblier et al., 2008; Ogbebo et al., 2009). Phytoplankton growth was not only found to be colimited by P and N at an assemblage level but also at a genus level in two different types of nutrient enrichment assay, i.e. seasonal nutrient enrichment microcosm assays and two mesocosm assays.

According to the law of the minimum, only one nutrient can be limiting at a given time (de Baar, 1994) which implies that colimitation by two nutrients at the same time would not be possible. Danger et al. (2008) argue that the law was developed for individual crop plants and could not be applied to naturally diverse assemblages which would adapt to the stoichiometry of their available resources. Ultimately, this would result in colimitation at the assemblage level. When individual taxa responses are not considered and a composite measure alone detects colimitation, it seems obvious to explain colimitation as the combined effect of individual taxonomic groups being limited by different single nutrients as suggested in some studies (Harpole et al., 2011). It has been demonstrated that the entire assemblage can be colimited by N and P while individual species or genera are limited by either nutrient (Quiblier et al., 2008; Burger et al., 2007).

Although colimitation of the phytoplankton assemblage seems to be common, it has rarely been shown that growth of individual genera can also be colimited. Hyenstrand et al. (2001) demonstrated that growth of the cyanobacterium *Gloeotrichia echinulata* was colimited by trace metal nutrients and macronutrients (N and P) in combination. Different mechanisms for colimitation of algal growth rates by trace metals have been suggested and shown, e.g. biochemical substitution where two trace metals can fulfill the same biological function or biochemically dependent colimitation where the lack of one element prevents the acquisition of another (Saito et al., 2008). However, these mechanisms cannot explain colimitation by biochemically mutually exclusive nutrients such as N and P at the genus or species level.

In the assays conducted in this chapter, many genera from different families were simultaneously colimited in both types of nutrient enrichment assay. Harpole et al. (2011) found that simultaneous and independent colimitation were often observed in nutrient enrichment assays where environmental nutrient con-

centrations were low. This may also apply to our case as concentrations of srP and NO_x Grahamstown Dam are usually low (8-50 μg N/L and 5-30 μg P/L). The response evoked by additions of single nutrients might have been too small to be statistically significant and thus simultaneous colimitation may have masked serial limitation.

Simultaneous colimitation does not contradict the law of the minimum, if indeed small growth responses and low statistical power are responsible for this response and serial limitation was the actual type of nutrient limitation. Serial limitation may be interpreted as the limitation by one nutrient, i.e. the nutrient that evokes a response when added on its own, N or P in our case. This would alleviate limitation but it might also induce limitation by the second nutrient. When the second nutrient is also present, as in the PN treatment, further growth can occur.

It is possible that carbon was the above mentioned third nutrient that became limiting before N or P were depleted. Limitation by inorganic carbon would have been unlikely in the mesocosm experiments because the mesocosm bags were open to the atmosphere at the water surface and well mixed due to prevailing coastal wind. Carbon limitation could have affected the microcosm assays because the bottles used in the experiments allowed for little gas exchange. As the main concern was the limiting nutrients and not the level of response, this does not alter the conclusions in regards to nutrient limitation. Moreover, simultaneously conducted micro- and mesocoms experiments had very similar results, i.e. colimitation of the phytoplankton assemblage and many individual genera. Hence, it is unlikely that the phytoplankton response in the microcosms was affected by lack of inorganic carbon.

Different container sizes and the inclusion or exclusion of zooplankton may also have influenced phytoplankton responses to nutrient additions in the enrichment assays in this chapter. However, container size had only an effect in the experiments conducted in March 2013. Chlorophyll a concentrations were higher in all treatments of the microcosm assay compared with treatments of the mesocosm assay on day 4. Different mean light levels may have affected algal biomass in mesocosm and microcosm assays. Naturally, phytoplankton would

be dispersed throughout the mixed layer depending on wind conditions. Light intensities would change continuously which may lead to periods where light becomes growth limiting. The microcosm bottles were suspended at the same depth (at 25 % surface irradiance) during the course of the assay, while the mesocosms covered the first 1.5 m of the water column (which included the depth the microcosm bottles were placed at) and would better represent natural turbulence and light conditions. The light regime in the microcosms may have affected the magnitude of the growth response by ameliorating potential light limitation and thus, accelerating nutrient uptake and growth.

It is also likely that the exclusion of zooplankton from the microcosms affected algal growth positively. In turn, zooplankton grazing in the mesocosms may have caused the delay of growth responses in some genera such as *Mougeotia* in the January mesocosm assay and *Pediastrum* in both mesocosm assays. It is difficult to tease the specific effects of zooplankton grazing and container size apart without quantifying zooplankton densities.

Generally, mesocosms would provide a more natural environment than microcosms. When extrapolating from the microcosm results to the lake, one needs to take into account that temporary light limitation of phytoplankton growth may occur in mixed conditions. Thus, should nutrient concentrations in the lake increase to concentrations used in the nutrient enrichment assays, the magnitude of the phytoplankton growth response may be somewhat smaller than observed in the microcosm assays. However, the fact that the pattern of the nutrient limitation response of the phytoplankton assemblage was the same in both types of enrichment assay within the first four days, confirms and validates the results of the seasonal microcosm assays. Further, it suggests that the results from the seasonal microcosm assays are a good indicator of the nutrients limiting algal biomass in Grahamstown Dam.

Colimitation may not prevail throughout the year in Grahamstown Dam as there were signs of P limitation of phytoplankton biomass and of individual genera during winter. Although this may indicate a greater importance of P compared with N, growth responses were also much lower in winter, hinting at a combination of nutrient and light or temperature limitation. Seasonal changes in

nutrient limitation have been shown in other studies (Moon and Carrick, 2007; Bergström et al., 2008; Xu et al., 2013) and in contrast to the present results, they often occur during the phytoplankton growing season. It is possible that the sampling frequency was too low to pick up switches in nutrient limitation in Grahamstown Dam. However, several experiments were conducted during the main growing season in the lake (December to May) and all of those resulted in colimitation of phytoplankton biomass.

Not only seasonality but also experimental duration influenced the phytoplankton response to nutrient enrichment in Grahamstown Dam. Specifically, there were three different effects of experimental duration on the response to nutrient enrichment as shown in the two mesocosm experiments. Firstly, some genera switched from responding to one nutrient treatment to responding to another during the course of the experiment. This was most evident in the potentially toxic cyanobacteria *Anabaena* and *Aphanizomenon* in the January experiment. Both genera are capable of fixing atmospheric N and may have used this trait to their advantage in the P treatments. It has been observed, that low N:P ratios can result in increased N fixation rates (Piehler et al., 2009) and dominance of the phytoplankton by N fixing cyanobacteria (Vrede et al., 2009; De Tezanos Pinto and Litchman, 2010b). The utilisation of internal P storages might have enabled other algae such as *Crucigenia* and *Chroomonas* to increase their growth when additional N was available (in the N treatment). The formation of polyphosphate storage granules may take place after periods of P starvation (Eixler et al., 2006). As P concentrations are often low in Grahamstown Dam, this is not unlikely to occur.

Secondly, some genera showed a delay in the response to nutrient additions. Again, an explanation may be found in zooplankton grazing. Initial cell densities of these genera may have been at the detection limit but in balance, i.e. growth and loss through dying and grazing would have been in balance so that a stable population would have been present. Added nutrients would have increased the growth of these sparse genera. Eventually, the exponential nature of algal growth would have led to growth exceeding loss and thus, would have made the growth response detectable.

Thirdly, the day the highest growth response was measured varied between individual genera, i.e. the day the highest growth response was measured varied between day 4, 8, 12 or 18. This may have been due to different growth rates of different taxa within the phytoplankton assemblage, preferences of zooplankton grazing or a combination of both factors. Most cyanobacteria possess low growth rates compared with Chlorophyceae (Lürling et al., 2013). In particular, the highest responses of *Anabaena* and *Aphanizomenon* which were recorded at the end of the assays, may have been due comparatively low growth rates. In contrast, at the assemblage level, the highest magnitude of the growth response occurred on day 8 (or shortly thereafter as biomass on day 12 was lower than on day 8). In contrast, at the assemblage level, the highest magnitude of the growth response occurred on day 8 (or shortly thereafter as biomass on day 12 was lower than on day 8). Low cyanobacterial growth rates may also partly explain the lack of a response of most cyanobacteria in the microcosm experiments.

Assessing responses of potentially toxic cyanobacteria to nutrient enrichment in a lake where those cyanobacteria occur in low densities proved to be difficult. Although potentially toxic cyanobacteria were present during the seasonal microcosm experiments, growth responses to any nutrient treatment were not detectable. Despite even lower densities of the potentially toxic cyanobacteria in the mesocosm assays, the mesocosm assays enabled us to record the growth response of the two genera that were present in the lake at the time the assays were conducted. This can be attributed to the longer duration of the mesocosm assays.

The results of the present study confirm that one has to choose the experimental period of nutrient enrichment assays carefully. Although micro- and mesocosm assays conducted in 2013 revealed the same response pattern in terms of biomass within the first 4 days, the mesocosm assays have also shown that some effects of time would have been missed, had only microcosm experiments been conducted.

For the management of external nutrient loading to Grahamstown Dam, the results of this chapter imply that the input of N and P needs to be addressed to prevent extensive phytoplankton growth. Currently, water quality management is based on concentration of total phosphorus (TP) and cyanobacterial numbers in

Grahamstown Dam (Cole and Williams, 2011). Under this management plan, water can be extracted from the Williams River, if the concentration of TP is below 200 μg/L and cell counts of toxic cyanobacteria do not exceed 10 000 cells/ml. Thus, N would need to be included in the management plan and limits of external N loading would need to be defined. However, increased input of either nutrient on its own is less likely to enhance growth and the management of one nutrient alone may be effective in the short term. As P is likely to be more important for potentially toxic N fixing cyanobacteria, the control of P would be most effective. If, however, only P is controlled in the long term, phytoplankton in the reservoir may become severely P limited and any uncontrolled P input, e.g. from the sediments, may cause extensive growth.

Chapter 3

Effects of light and trace metals on cyanobacterial growth in Grahamstown Dam

3.1 Introduction

Cyanobacterial dominance and blooms are often attributed to anthropogenic eutrophication of freshwater systems. High phosphorus concentrations and a low nitrogen (N) to phosphorus (P) ratio can be conducive to cyanobacterial growth (Schindler et al., 2008; Vrede et al., 2009; Carvalho et al., 2013). However, these conditions do not necessarily result in dominance of cyanobacteria (Jensen et al., 1994) and other environmental conditions need to be considered to define effective management strategies (Carvalho et al., 2013). Two prominent factors that can affect cyanobacterial growth in combination with nutrient enrichment are light and trace metal availability.

It has been suggested that in eutrophic environments nutrient load determines phytoplankton assemblage composition while in oligotrophic environments nutrient ratios are decisive (Brauer et al., 2012). Competition for light is considered the driving mechanism in nutrient enriched environments: superior light competitors would outgrow and overshadow other species and would subsequently dominate the assemblage. Cyanobacteria, particularly those that can regulate their buoyancy, can become fierce competitors for light if environmental conditions, such as a stable water column, allow them to make use of this trait. Havens

et al. (1998) observed that underwater light availability could determine cyanobacterial species composition in a shallow turbid lake: windstill periods led to dominance of the high light adapted, buoyancy controlling species *Anabaena circinalis*, *A. flos-aquae* and *Microcystis* spp., while high winds and mixing periods reduced light availability and resulted in dominance of low light adapted *Lyngbya* species.

Stable stratification that enables access to high light intensities in surface waters has also been shown to greatly increase *Anabaena* abundance in nutrient rich, turbid weir pools (Sherman et al., 1998; Mitrovic et al., 2003), sometimes leading to the replacement of the Bacillariophyceae *Aulacoseira* (Sherman et al., 1998). Conversely, it has been observed that low light availability suppressed *Microcystis aeruginosa* blooms in a hypereutrophic lake (Tomioka et al., 2011). Access to high light intensities may lead to further advantages for cyanobacteria capable of fixing atmospheric N. While the fixation of N can enable these species to thrive in conditions of low N:P ratios (Vrede et al., 2009), it is also an energy intensive process that needs a high light environment (Tilzer, 1987). The N fixer *A. flos-aquae* has been shown to dominate an assemblage of cyanobacteria, Chlorophyceae, Bacillariophyceae and Cryptophyceae when N:P ratios are low but only in high light conditions (De Tezanos Pinto and Litchman, 2010b).

Micronutrients, for example the trace metals Fe and Mn, are vital for phytoplankton growth because they play major roles in the photosynthesis electron transport system (Raven et al., 1999). Thus, it is not surprising that micronutrients can limit algal growth – either as a primary limiting nutrient (Downs et al., 2008) or in combination with macronutrients (Twiss et al., 2000; Sterner et al., 2004; Vrede and Tranvik, 2006; North et al., 2007). Primary limitation by micronutrients does not appear to be related to trophic state or lake size (Downs et al., 2008). Cyanobacteria have a high requirement for Fe (Raven et al., 1999) and Fe additions often stimulate cyanobacterial growth (Xu et al., 2013; De Wever et al., 2008) and can even favour cyanobacteria relative to other taxa (Morton and Lee, 1974; Hyenstrand et al., 2000). In addition to Fe, boron (B) has been shown to stimulate the growth of the cyanobacterium *Gloeotrichia echinulata* when administered in combination with Fe and N and P (Hyenstrand et al., 2001). Further, nitrogenase,

the metalloprotein complex facilitating nitrogen fixation, requires either Fe, vanadium (V) or molybdenum (Mo) (Zhao et al., 2006) and colimitation of N fixation by Fe and P has been shown in the marine environment (Mills et al., 2004). Similarly, marine and freshwater heterocystous cyanobacteria can be colimited by Mo and N as the lack of Mo suppresses N fixation (Glass et al., 2010).

Experiments at Grahamstown Dam that have examined phytoplankton nutrient limitation at the genus and the assemblage level have shown that phytoplankton biomass and many individual algal genera were colimited by N and P (see chapter 2). However, most cyanobacteria, especially potentially toxic genera like *Anabaena* and *Microcystis*, did not respond to nutrient enrichment with N or P or both in combination. It seems likely that resources other than or in addition to macronutrients are needed to induce cyanobacterial growth in the lake. Identifying these resources would improve our understanding of cyanobacterial growth and management strategies of the drinking water storage. As Grahamstown Dam is a well mixed shallow lake, light limitation would be likely to restrict cyanobacterial growth, in particular, growth of those species that can control their buoyancy.

The aims of this chapter were to determine if light availability affects cyanobacterial growth and to determine if cyanobacteria are stimulated by micronutrient additions in Grahamstown Dam.

3.2 Methods

In order to determine if light or micronutrients affect cyanobacterial growth, in situ microcosm assays were carried out in January 2013. The assays lasted for four days (day 0 to day 4) and, in addition to manipulation of light intensity and trace metal enrichment, comprised three different nutrient treatments (N only, P only, P and N in combination) and one untreated control (C). The design and experimental set up followed the seasonal enrichment assays described in section 2.2.2. However, assays described here were only carried out at Site 3. This site was chosen because the seasonal enrichment assays showed that phytoplankton growth and cyanobacterial numbers were generally higher at Site 3 compared

with the other sites.

Samples for phytoplankton, chlorophyll a and nutrient concentrations were collected on day 0 and day 4 for both assays. Samples were processed as described in section 2.2.3.

Light level assay Three light levels were meant to be tested in combination with above mentioned nutrient enrichment treatments: 90 %, 25 % and 10 % surface irradiance. Racks holding microcosm bottles for each light level were suspended at the depth that corresponded to the proportion of surface irradiance of each treatment. The 90 % surface irradiance treatment was placed at about 0.1 m depth, the 25 % surface irradiance treatment was placed at 1.2 m and the 10 % surface irradiance treatment was placed at 2.4 m (see also Figure B.1). Irradiance was measured with a Licor light meter using a quantum sensor for determining light quanta in μmolm^{-2}s^{-1}. Unfortunately, the 10 % surface irradiance treatment was lost as racks holding the microcosm bottles of this treatment were accidentally not placed at the correct depth.

Micronutrient assay Macronutrient (C, P, N and PN) and micronutient treatments were tested in a fully factorial design. There were two treatment levels for micronutrients: micronutrients present in combination (trace metal treatment) and micronutrient absent (macronutrients only treatment). Microcosm bottles of the trace metal treatment were placed at a depth corresponding to 25 % surface irradiance and the 25 % treatment of the light level assay also served as macronutrients only treatment. Micronutrient additions for this assay consisted of the trace metal components, stock solutions V and VI, from the Swedish Standard (SIS) *Lemna* growth medium (complete composition is provided in appendix C) (OECD, 2006). This medium had proven to be a good experimental and culture medium for *A. circinalis* (see chapter 4) and its mixture of various trace metals was chosen to test if there is generally any trace metal limitation in Grahamstown Dam. Stock solutions V and VI were added to achieve the same concentrations in the microcosm bottles as would be required for the medium. Final concentrations of all trace metals are shown in Table 3.1.

Table 3.1: Elemental concentrations of trace metal solutions in the micronutrient assays. Stock solutions are from the Swedish Standard (SIS) *Lemna* growth medium (OECD, 2006).

Stock solution		Concentration in trace metal treatment
No.	Element	μg/L
V	B	170.0
	Mn	56.0
	Mo	4.0
	Zn	11.0
	Cu	1.3
	Co	2.0
VI	Fe	170.0

3.2.1 Data analysis

Chlorophyll a concentrations from the light level and micronutrient assays were individually analysed with two factorial analysis of variance (ANOVA) using the type III model. Interactions between light and macronutrient treatments and micro- and macronutrient treatments were individually analysed using Tukey's pairwise comparison. Biovolumes of individual cyanobacterial genera were analysed with two factorial ANOVA (type I model or type III model if the interaction term was significant) and Tukey's pairwise comparison for each assay. Homogeneity of variance was tested with the Levene statistic. Two factorial permutational multivariate analysis of variance (PERMANOVA) was used to analyse phytoplankton assemblage differences in the light level and the micronutrient assay. Data was fourth root transformed to amend homogeneity of group dispersions.

Non metric multidimensional scaling (NMDS) was performed to illustrate phytoplankton assemblage changes due to the different treatments for both assays. All analyses were carried out with the statistics software R (version 2.13.0, R Development Core Team (2011)) using the functions LeveneTest (with median as the centre), glm, aov, Anova (with type III model and F-test), TukeyHSD, betadisper (with Bray-Curtis distances), metaMDS (with Bray-Curtis distances) and adonis from the packages MASS, car and vegan.

3.3 Results

3.3.1 Light level assay

Cyanobacterial response The four most abundant cyanobacterial genera in the light level assay were *Anabaena*, *Aphanocapsa*, *Aphanizomenon* and *Chroococcus* (Figure 3.1). Of these four, only *Anabaena* showed a significant growth response in this experiment (Figure 3.1 and Table 3.2). *Anabaena* biovolumes were significantly higher in the 25 % surface irradiance treatments compared with the 90 % surface irradiance treatments ($p \leq 0.029$). The highest biovolume was found in the P treatment at 25 % surface irradiance but this was not statistically different from the other nutrient treatments. Similarly, the highest biovolume of *Aphanizomenon* was detected in the P treatment at 25 % surface irradiance, followed by the P treatment at 90 % surface irradiance. *Aphanizomenon* was not detected in any other treatment or the control. *Chroococcus* responded to P additions at 90 % surface irradiance only. *Aphanocapsa* biovolume was highest in the PN treatment at 25 % surface irradiance when compared to the control and the other treatments.

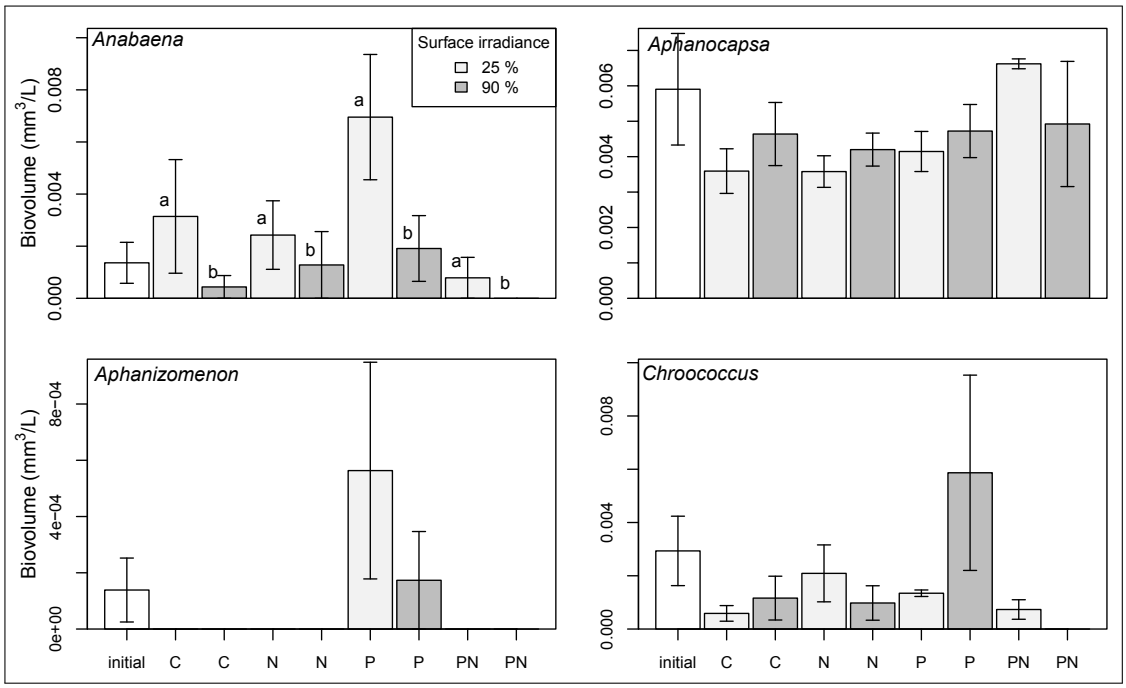

Figure 3.1: Mean biovolumes in the light level assay. Letters a and b indicate homogeneous subsets according to the result of Tukey's pairwise comparison (p=0.029). Initial values (samples from day 0) were not included in the Tukey's test. Error bars are one standard error from the mean, n=3.

Table 3.2: Results of two factorial ANOVA of cyanobacterial biovolumes from the light level assay. Factor light had the levels 90 and 25 % surface irradiance and factor macronutrients had the levels C, N, P and PN. Initial values (samples from day 0) were not included in the analysis.

	Source	MS	F	p
Anabaena				
	light	$3.51*10^{-5}$	5.747	0.029
	macronutrients	$1.70*10^{-5}$	2.784	0.075
	light*macronutrients	$5.63*10^{-6}$	0.920	0.453
Aphanocapsa				
	light	$1.12*10^{-7}$	0.053	0.821
	macronutrients	$4.27*10^{-6}$	2.018	0.152
	light*macronutrients	$2.31*10^{-6}$	1.095	0.380
Aphanizomenon				
	light	$5.70*10^{-8}$	0.853	0.369
	macronutrients	$2.04*10^{-7}$	3.042	0.059
	light*macronutrients	$5.70*10^{-8}$	0.853	0.485
Chroococcus				
	light	$3.69*10^{-6}$	0.664	0.427
	macronutrients	$3.64*10^{-5}$	2.036	0.149
	light*macronutrients	$9.55*10^{-5}$	1.668	0.214

Assemblage response The NMDS ordination plot, giving a very good representation of site and species scores (stress=0.068), showed that the phytoplankton assemblage structure changed due to different nutrient additions and different light levels (Figure 3.2). Site scores of the PN treatment at 90 % surface irradiance were close together, i.e. had a similar genera composition to each other and further away from site scores of all other treatments and controls.

This indicates that the genera composition in the PN treatment at 90 % surface irradiance was different compared with the genera composition in all other treatments and controls. Site scores of the PN treatment at 25 % surface irradiance were also close to each other and placed some distance away from site scores of the P treatment followed by the N treatments and controls at both light levels. They were also further away from site scores of the PN treatment at 90 % surface irradiance, indicating that the genera composition in the two different PN treatments was different from each other and that the genera composition of the

PN treatment at the lower light level was more similar to the genera composition in the remaining treatments, in particular, the P treatment. Site scores of the P treatments of both light levels were close to each other, i.e. had similar genera compositions. In contrast, genera composition of the controls and N treatments were more similar to each other within each light level than they were similar within nutrient treatments.

Species scores of the cyanobacteria were closer to site scores of the intial samples and the P treatments than to any other site scores in the NMDS ordination which means that the cyanobacteria were relatively more associated with these treatments than with any other treatments. The Chlorophyceae *Ankistrodesmus*, *Chodatella*, *Mougeotia*, *Nephrocytium*, *Oocystis*, *Scenedesmus* and *Sphaerocystis*, the Cryptopchyceae *Chroomonas* and the Chrysophyceae *Dinobryon* were associated with the PN treatment at 90 % surface irradiance as indicated by their species scores being close to site scores of the PN treatment at 90 % surface irradiance. Genera associated with the PN treatment at 25 % surface irradiance were the Chlorophyceae *Crucigenia* and *Dictyosphaerium* and the Bacillariophyceae *Acanthoceras*.

The NMDS ordination suggests that there was an interaction between nutrient treatment and light level. According to PERMANOVA, the interaction was significant ($p<0.001$) (Table 3.3). However, it is possible that the PERMANOVA result may misrepresent actual assemblage changes due to macronutrient additions because multivariate dispersion of factor macronutrients was not homogeneous despite fourth root transformation.

Table 3.3: Results of PERMANOVA of the light level assay. Factor micronutrients had the levels trace metals present and trace metals absent and factor macronutrients had the levels C, N, P and PN. Number of permutations was 999. Initial values (samples from day 0) were not included in the analysis.

Source	MS	F	R^2	p
light	0.0277	5.436	0.087	0.001
macronutrients	0.0503	9.865	0.474	0.001
light*macronutrients	0.0195	3.818	0.183	0.002

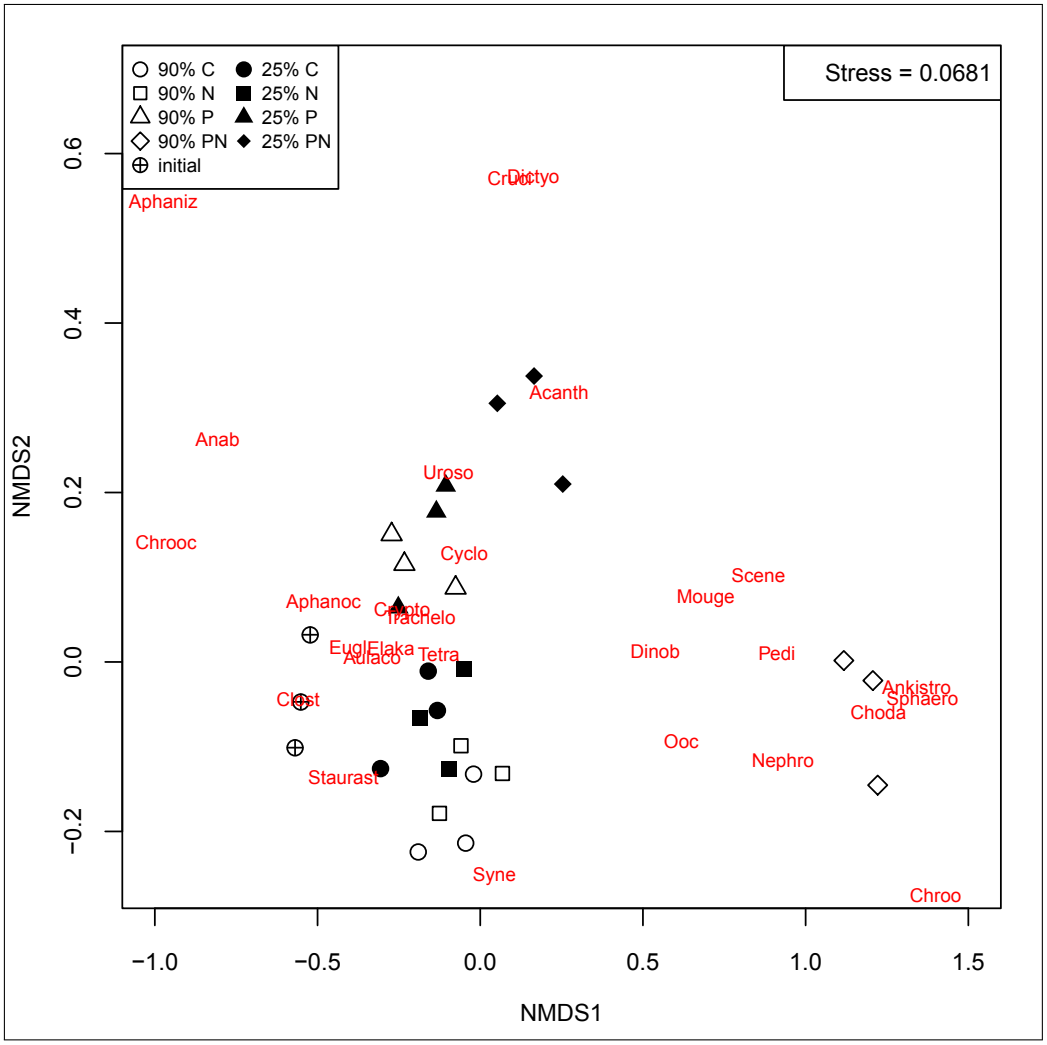

Figure 3.2: NMDS plot of the most abundant phytoplankton genera in the light level assay. Species scores (phytoplankton genera) are weighted and expanded averages of site scores (treatments) which leads to species scores being close to the site scores they are relatively more associated with. Names of genera were abbreviated and stand for Anab = *Anabaena*, Aphaniz = *Aphanizomenon*, Aphanoc = *Aphanocapsa*, Chrooc = *Chroococcus*, Ankistro = *Ankistrodesmus*, Choda = *Chodatella*, Clos = *Closterium*, Cruci = *Crucigenia*, Dictyo = *Dictyosphaerium*, Elaka = *Elakatothrix*, Mouge = *Mougeotia*, Nephro = *Nephrocytium*, Ooc = *Oocystis*, Pedi = *Pediastrum*, Scene = *Scenedesmus*, Sphaero = *Sphaerocystis*, Staurast = *Staurastrum*, Tetra = *Tetraedron*, Acanth = *Acanthoceras*, Aulaco =*Aulacoseira*, Cyclo = *Cyclotella*, Syne = *Synedra*, Uroso = *Urosolenia*, Eugl = *Euglena*, Trachelo = *Trachelomonas*, Chroo = *Chroomonas*, Crypto = *Cryptomonas* and Dinob = *Dinobryon*.

Biomass response Chlorophyll a concentrations showed that there was a growth response of the phytoplankton assemblage to additions of macronutrients and different light levels (Figure 3.3). There was a significant interaction ($p \leq 0.001$) between macronutrients and light level treatments (Table 3.4 and Figure 3.3). The highest chlorophyll a concentration was found in the PN treatment at 90 % surface irradiance. This growth response was significantly higher than the second highest one which was found in the PN treatment at 25 % surface irradiance ($p \leq 0.001$). Chlorophyll a concentrations in both PN treatments were significantly higher ($p \leq 0.001$) than concentrations in all other treatments and in the control.

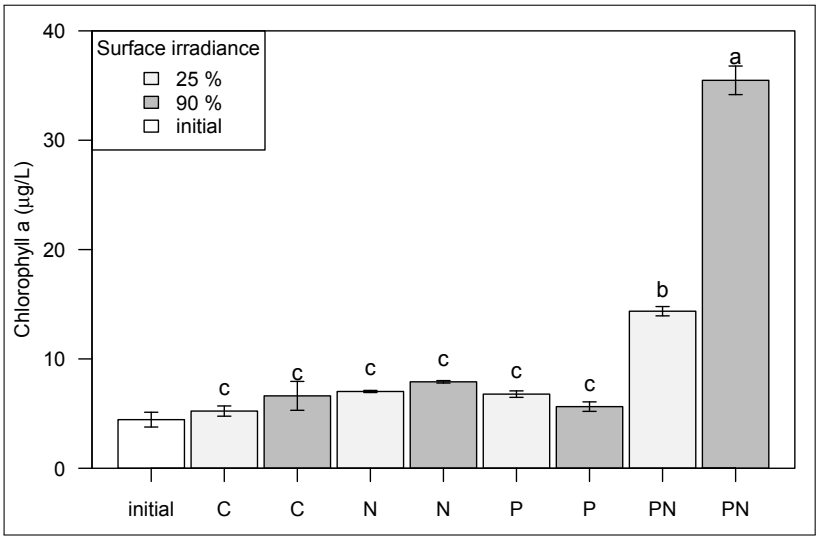

Figure 3.3: Mean chlorophyll a concentration in the light level assay. Letters a, b and c indicate homogeneous subsets according to the result of Tukey's pairwise comparison ($p \leq 0.001$). Initial values (samples from day 0) were not included in the Tukey's test. Error bars are one standard error from the mean, n=3.

Table 3.4: Results of two factorial ANOVA of chlorophyll a data from the light level assay. Factor light had the levels 90 and 25 % surface irradiance and factor macronutrients had the levels C, N, P and PN. Initial values (samples from day 0) were not included in the analysis.

Source	MS	F	R^2	p
light	2.90	1.86	0.01	0.191
macronutrients	50.11	32.19	0.23	<0.001
light*macronutrients	163.06	104.76	0.73	<0.001

3.3.2 Micronutrient assay

Cyanobacterial response *Aphanizomenon*, *Aphanocapsa* and *Chroococcus* responded with significantly increased growth in this assay (Figure 3.4 and Table 3.5). *Aphanizomenon* and *Chroococcus* biovolumes increased in the treatments containing trace metal additions (p=0.048 and p<0.001, respectively). There was a significant interaction (p=0.002) between macro- and micronutrient treatments for *Aphanocapsa*. The highest *Aphanocapsa* biovolumes were found in the N and the PN treatment with added trace metals solution. The growth response to those treatments was higher than the growth response to any of the other treatments and the control. *Anabaena* biovolumes increased in the P treatments with and without added trace metal solutions when compared with the control but this was not significant.

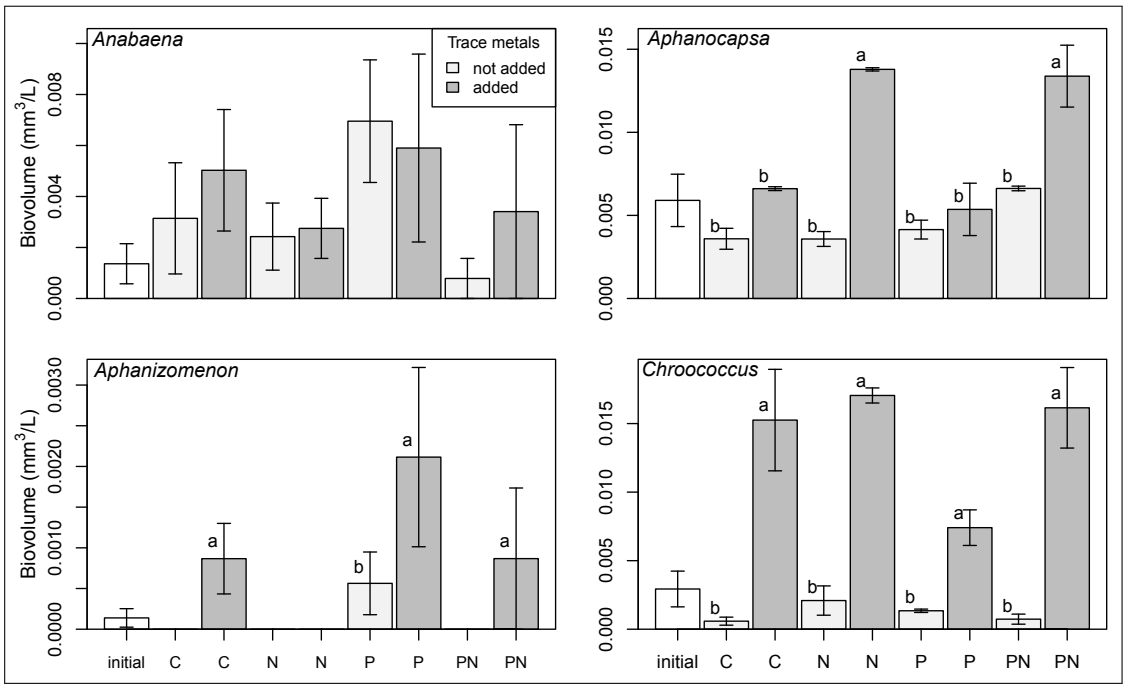

Figure 3.4: Mean biovolumes in the micronutrient assay. Letters a and b indicate homogeneous subsets according to the result of Tukey's pairwise comparison (p=0.029). Initial values (samples from day 0) were not included in the Tukey's test. Error bars are one standard error from the mean, n=3 (except for treatment "N trace metals added" where n=2).

Assemblage response According to the NMDS ordination plot, which represented a good ordination of site and species scores (stress=0.133), there was a

Table 3.5: Results of two factorial ANOVA of cyanobacterial biovolumes from the micronutrient assay. Factor micronutrients had the levels trace metals present and trace metals absent and factor macronutrients had the levels C, N, P and PN, n=3 (except for treatment "N trace metals added" where n=2). Initial values (samples from day 0) were not included in the analysis.

	Source	MS	F	p
Anabaena				
	micronutrients	$5.44*10^{-6}$	0.308	0.587
	macronutrients	$2.23*10^{-5}$	1.263	0.322
	micro*macronutrients	$4.00*10^{-5}$	0.226	0.877
Aphanocapsa				
	micronutrients	$1.37*10^{-5}$	4.949	0.042
	macronutrients	$6.30*10^{-6}$	2.282	0.121
	micro*macronutrients	$4.69*10^{-4}$	7.799	0.002
Aphanizomenon				
	micronutrients	$4.26*10^{-6}$	4.627	0.048
	macronutrients	$1.80*10^{-6}$	1.953	0.164
	micro*macronutrients	$5.35*10^{-6}$	0.581	0.637
Chroococcus				
	micronutrients	$9.14*10^{-4}$	89.64	<0.001
	macronutrients	$2.13*10^{-5}$	2.093	0.144
	micro*macronutrients	$2.97*10^{-5}$	2.913	0.069

change in phytoplankton assemblage structure due to macro- and micronutrient additions (Figure 3.5). Site scores of the PN treatments with and without added trace metal solution were placed apart from each other and also apart from site scores of the remaining treatments, indicating that their genera composition was different to the one in the other treatments and also different from each other. Site scores of the P treatment without added trace metal solution were close to each other and closer to the PN treatment without added trace metal solution than to any of the other treatments, indicating similarity of their genera compositions. In contrast, large distances between site scores of the P treatment with added trace metal solution and their placements among site score of other treatments suggested that their genera composition was not similar. Site scores of controls and N treatments of both levels of micronutrients treatment were close together, i.e. their genera compositions were similar.

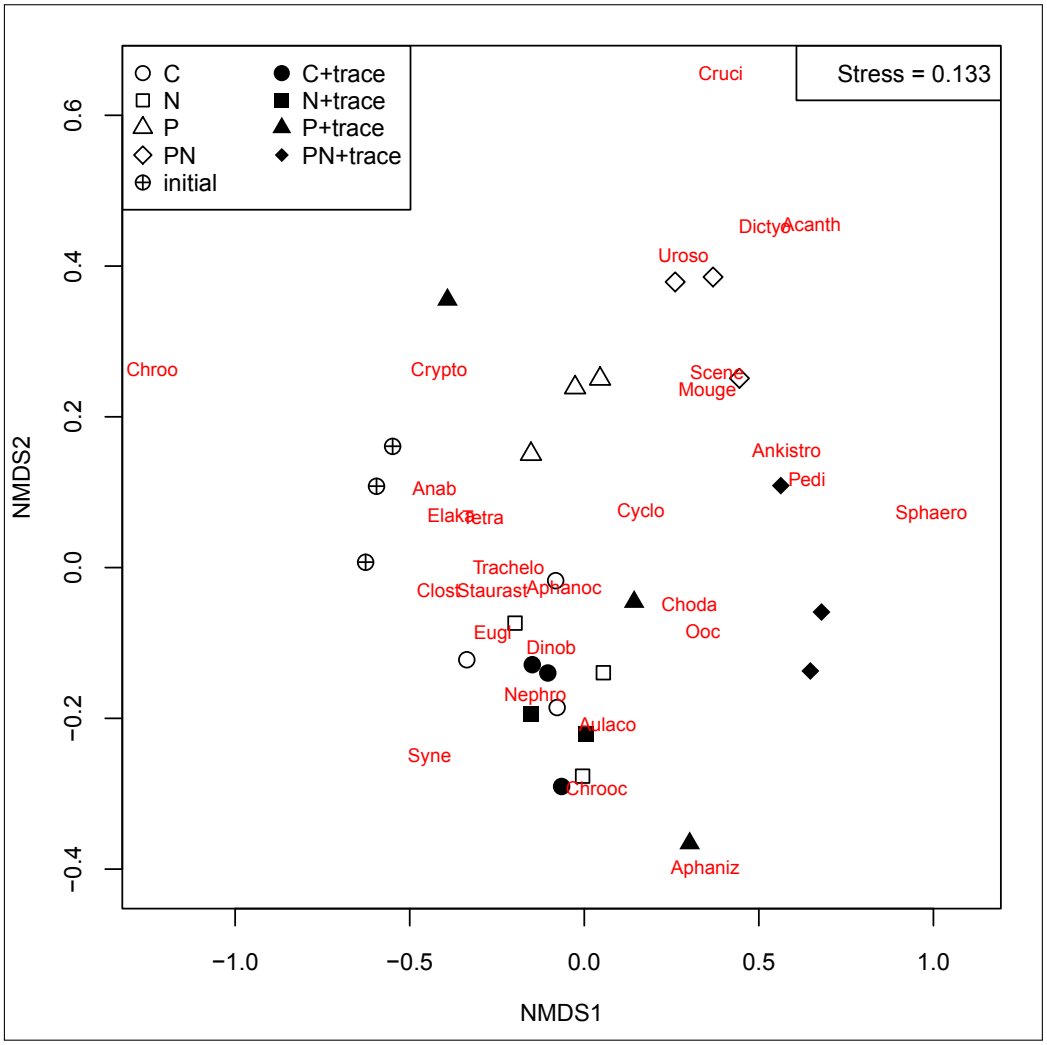

Figure 3.5: NMDS plot of the most abundant phytoplankton genera in the micronutrient assay. Species scores (phytoplankton genera) are weighted and expanded averages of site scores (treatments) which leads to species scores being close to the site scores they are relatively more associated with. Names of genera were abbreviated and stand for Anab = *Anabaena*, Aphaniz = *Aphanizomenon*, Aphanoc = *Aphanocapsa*, Chrooc = *Chroococcus*, Ankistro = *Ankistrodesmus*, Choda = *Chodatella*, Clos = *Closterium*, Cruci = *Crucigenia*, Dictyo = *Dictyosphaerium*, Elaka = *Elakatothrix*, Mouge = *Mougeotia*, Nephro = *Nephrocytium*, Ooc = *Oocystis*, Pedi = *Pediastrum*, Scene = *Scenedesmus*, Sphaero = *Sphaerocystis*, Staurast = *Staurastrum* , Tetra = *Tetraedron*, Acanth = *Acanthoceras*, Aulaco = *Aulacoseira*, Cyclo = *Cyclotella*, Syne = *Synedra*, Uroso = *Urosolenia*, Eugl = *Euglena*, Trachelo = *Trachelomonas*, Chroo = *Chroomonas*, Crypto = *Cryptomonas* and Dinob = *Dinobryon*. n=3, except for treatment "N trace metals added" where n=2.

Species scores in the NMDS plot showed that *Aphanizomenon* and *Chroococcus* were relatively more associated with treatments containing trace metal solution than treatments without trace metal solution. The scores of *Anabaena* and *Aphanocapsa* were placed relatively close to the P treatment without added trace metal solution and the N treatment with added trace metal solution. Species scores of the Chlorophyceae *Crucigenia*, *Dictyosphaerium*, *Mougeotia* and *Scenedesmus* and the Bacillariophyceae *Acanthoceras* and *Urosolenia* were close to site scores of the PN treatment without trace metal solution. Other Chlorophyceae (*Ankistrodesmus*, *Pediastrum* and *Sphaerocystis*) were associated with the PN treatment with trace metal solution added as indicated by their species scores being closer to the site scores of this treatment.

The interaction between macro- and micronutrient additions suggested by the NMDS ordination was significant according to the PERMANOVA result (p=0.014) (Table 3.6).

Table 3.6: Results of PERMANOVA of the micronutrient assay. Factor micronutrient had the levels trace metals present and trace metals absent and factor macronutrient had the levels C, N, P and PN. Number of permutations was 999, n=3 (except for treatment "N trace metals added" where n=2). Initial values (samples from day 0) were not included in the analysis.

Source	MS	F	R^2	p
micronutrient	0.0358	7.544	0.147	0.001
macronutrient	0.0350	7.371	0.432	0.001
micro*macronutrient	0.0103	2.169	0.127	0.014

Biomass response Chlorophyll a concentrations indicated that there was a growth response of the phytoplankton assemblage to additions of macro- and micronutrients (Figure 3.6). There was a significant interaction between macronutrient and micronutrient additions (Table 3.7 and Figure 3.6). The highest chlorophyll a concentration was found in the PN treatment with trace metal solution added. This growth response was significantly higher than the second highest one which was found in the PN treatment without additional trace metal solution ($p \leq 0.001$). Chlorophyll a concentrations in both PN treatments were significantly higher ($p \leq 0.001$) than concentrations in all other treatments and in the

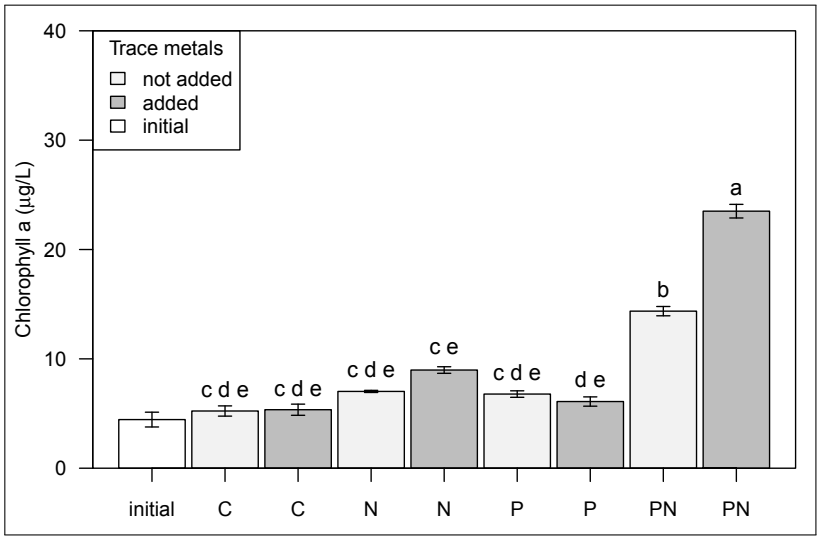

Figure 3.6: Mean chlorophyll a concentration in the micronutrient assay. Letters a, b, c, d and e indicate homogeneous subsets according to the result of Tukey's pairwise comparison ($p \leq 0.012$). Initial values (samples from day 0) were not included in the Tukey's test. Error bars are one standard error from the mean, n=3 (except for treatment "N trace metals added" where n=2).

controls. The N treatment with added trace metal solution contained significantly higher chlorophyll a concentrations than the P treatment with trace metal solution ($p \leq 0.012$). Neither treatment was significantly higher or lower than the controls and the remaining N or P treatments.

Table 3.7: Results of two factorial ANOVA of chlorophyll a data from the micronutrient assay. Factor micronutrients had the levels trace metals present and trace metals absent and factor macronutrients had the levels C, N, P and PN, n=3 (except for treatment "N trace metals added" where n=2). Initial values (samples from day 0) were not included in the analysis.

Source	MS	F	R^2	p
micronutrients	0.02	0.04	<0.01	0.847
macronutrients	50.11	92.27	0.61	<0.001
micro*macronutrients	30.04	55.31	0.36	<0.001

3.4 Discussion

The resources light and micronutrients in combination with macronutrients influenced cyanobacterial growth and affected phytoplankton assemblage structure

and biomass in Grahamstown Dam. The four most abundant cyanobacteria during the assays responded with increased growth to different light levels and trace metal additions; sometimes in unexpected ways.

Growth of diazotrophic and potentially toxic cyanobacteria *Anabaena* and *Aphanizomenon* seemed to be stimulated by additions of P. Their biovolumes, especially of *Aphanizomenon*, were low, leading to large standard errors which was probably the reason why their responses to P additions were not significant. However, responses were consistent in both assays. Responding to P additions suggests that these genera may have fixed atmospheric N to acquire sufficient amounts of the nutrient to accumulate biomass.

Anabaena growth was significantly enhanced by the lower light level treatment and *Aphanizomenon* also appeared to respond to this treatment. These results are in contrast to De Tezanos Pinto and Litchman (2010b)'s study that found diazotrophic cyanobacteria accumulated more biovolume under the relatively high light intensity treatment. The high light treatment in the respective experiment was much lower (100 μmol $m^{-2}s^{-1}$) than the light intensity measured at 25 % surface irradiance in Grahamstown Dam (approximately 540 μmol $m^{-2}s^{-1}$). This suggests that even lower light levels than 25 % surface irradiance would be more conducive to *Anabaena*. It is also possible that the light spectrum at the 25 % surface irradiance was more beneficial for *Ananbaena* then the spectrum at 95 %. This highlights the difficulties of comparing results from field and laboratory experiments. Surface irradiance is highly variable and the light spectrum is depth dependent in the field while these parameters are usually kept constant in the laboratory.

The growth of *Aphanizomenon* and *Chroococcus* was significantly enhanced by micronutrient additions and in the case of *Chroococcus*, not by any of the macronutrients. Growth limitation by a micronutrient (Fe) has been observed in *Microcystis* (Xu et al., 2013). In contrast to *Chroococcus*, which was micronutrient limited only, Fe was the primary limiting nutrient and P the secondary nutrient for *Microcystis*. *Aphanizomenon* growth may have been colimited by micronutrients and P, but low biovolumes prevented a distinct result.

Aphanocapsa was colimited by micronutrients and N in combination, as its sig-

nificant response to treatments containing N and micronutrients showed. While Fe and nitrate colimitation have often been observed in marine phytoplankton (Price et al., 1991; Hutchins and Bruland, 1998), it has been demonstrated that Fe can also facilitate the uptake of nitrate in freshwater phytoplankton (North et al., 2007). One would expect this response of the diazotrophic genera, reflecting the need of trace metals for nitrogenase activity (Zhao et al., 2006). As *Aphanocapsa* does not fix N, micronutrients would have played a different role in N utilization in this case.

Anabaena was the only genus of the four most abundant cyanobacteria that did not respond to micronutrient additions. This suggests that micronutrients are not likely to limit or colimit *Anabaena* growth in Grahamstown Dam. It has been shown that even tightly bound Fe can be available to *A. flos-aquae* (Gress et al., 2004) so it is possible that *Anabaena* would become micronutrient limited at lower concentrations than the other cyanobacteria. However, it is also possible that *Anabaena* abundance was too low to show an effect of the trace metal enrichment.

Although cyanobacterial growth was stimulated in both assays the response of cyanobacteria was not reflected by biomass increase of the phytoplankton assemblage. This was likely due to low cyanobacterial biovolumes in combination with a relatively short experimental duration. Biomass increase measured as chlorophyll a concentration was largest in the PN treatments at 90 % surface irradiance and the PN treatments with added micronutrients. Chlorophyceae and Bacillariophyceae usually make up the largest proportion of phytoplankton in Grahamstown Dam and members of both taxonomic groups responded to the treatments that the highest biomass increase was measured in. This suggests that the biomass increase was caused by members of the Chlorophyceae and Bacillariophyceae in both assays.

In conclusion, it is unlikely that thermal stratification may cause rapid growth of the Chlorophyceae and Bacillariophyceae by giving them access to surface light intensities unless this coincides with increased levels of dissolved inorganic N and P. Further, it has been shown that Bacillariophyceae may not benefit from higher surface light intensities during thermal stratification events as they tend to sink out of the euphotic zone (Sherman et al., 1998; McCausland et al., 2002).

Thermal stratification may be benficial for *Anabaena* and in contrast to the Chlorophyceae and Bacillariophyceae, it may be enough for *Anabaena* growth to increase, if thermal stratification coincides with increased concentration of dissolved inorganic P only.

It may be useful to determine which ones of the individual trace metal components were conducive to cyanobacterial growth and also determine the minimum concentration which they have an effect at. In particular, knowing what stimulates *Aphanizomenon* and *Aphanocapsa* would be of interest for the management of Grahamstown Dam as the former is potentially toxic and the latter a potential cause for taste and odour events in the reservoir. Further, identifying sources of the micronutrient stimulants, such as the sediments or inflows from the catchment, may be helpful in preventing excessive growth of the two cyanobacteria in future.

Chapter 4

Assessing the importance of the nutrients N and P for the growth of *Anabaena circinalis*

4.1 Introduction

In Australia, cyanobacterial blooms are often dominated by *Anabaena circinalis* and blooms of the cyanobacterium are frequent in many inland waters. For example, *A. circinalis* constituted the main part of two extreme mass occurrences that extended for 1000 km and more in the Barwon-Darling River in 1991 (Bowling and Baker, 1996) and in the Murray River in 2009 (Al-Tebrineh et al., 2012), threatening livestock and recreational use. Australian strains of *A. circinalis* are capable of producing saxitoxins, a group of neurotoxins known to cause paralytic shellfish poisoning (Humpage et al., 1994; Baker and Humpage, 1994; Beltran and Neilan, 2000) and also compounds that affect taste and odour, such as geosmin. The closure of a drinking water supply due to a cyanobacterial bloom dominated by *A. circinalis* in Lake Cargelligo, NSW, in 1991 (Bowling, 1994), further illustrates the issue and points out the importance of managing growth conditions of the cyanobacterium in drinking water reservoirs.

Generally, it has been shown that cyanobacteria can be favoured when N:P ratios are low (Schindler, 1977; Smith, 1983). TN:TP ratios below 30 (by weight) (Smith, 1983; Nõges et al., 2008) have been found to be conducive to cyanobacteria in a phytoplankton assemblage while at higher ratios cyanobacterial abun-

dance decreases. Correlations between biomass of cyanobacteria with TP in lakes (Smith, 1985) has been demonstrated. Recently, Carvalho et al. (2013) showed that cyanobacteria responded in a nonlinear fashion to increased TP concentrations with highest abundances in a range from 20 to 100 μg TP/L. P seems to be critical in particular for N fixing cyanobacteria as suggested by correlations between N fixation and P concentrations (Howarth et al., 1988) and stimulation of N fixation rates under increased availability of P (Piehler et al., 2009). Prolific growth and dominance by cyanobacteria capable of fixing atmospheric N under low N:P ratios (0 to 20 by weight) has been experimentally shown (Vrede et al., 2009). Aside from P, high light availability may be important for the growth of N fixing cyanobacteria as N fixation is an energy intensive process and increases with light availability (Mugidde et al., 2003). Many *A. circinalis* blooms in Australia were associated with low flows resulting in thermal stratification of the water column (Mitrovic et al., 2003, 2011). This also stabilises the light regime towards high light near the surface and thus, is likely to promote *A. circinalis* growth conditions (Mitrovic et al., 2001b).

While flow management can be employed to disrupt those conditions and moderate *A. circinalis* blooms (Mitrovic et al., 2011), more detailed knowledge of species specific optimal growth conditions may provide further useful information for the management of the species. This is particularly of importance in lakes and reservoirs, where flow management is not always feasible, but nutrient concentrations may be controlled. Studies have investigated the effect of different nutrient regimes on the morphology of different strains of several *Anabaena* species (Zapomělová et al., 2010), the effect of different sources of N on *A. circinalis* toxicity (Velzeboer et al., 2001) and the effect of light availability on ecophysiological responses of three N fixing *Anabaena* and *Aphanizomenon* species (De Tezanos Pinto and Litchman, 2010a). However, there is little information on growth of the N fixing cyanobacterium *A. circinalis* under different nutrient supply and light regimes.

The genus *Anabaena* has been detected in the phytoplankton of Grahamstown Dam since phytoplankton monitoring started at the beginning of 1992 (Hunter Water monitoring data). Cell counts are usually low, i.e. below 1000 cells/ml,

but have increased to 20 000 on occasion. In particular, growth of *A. circinalis* is of concern as there are currently no facilities in place to treat the drinking water supplied from the lake for saxitoxins. In order to better understand and manage *A. circinalis* growth in Grahamstown Dam, it is important to identify conditions that are conducive to the cyanobacterium.

The aims of this chapter were to determine how different nutrient ratios and concentrations affect growth rates and abundances of *A. circinalis* and if different light levels affect the response. It was hypothesised that external P supply was more important than external N supply for the N fixing cyanobacterium and that absolute concentrations were not as important as nutrient ratios. Thus, *A. circinalis* growth rates would be higher under low N:P ratios irrespective of nutrient concentrations. Also, more energy for N fixation should promote this effect. Thus, high light availability would further increase *A. circinalis* growth rates under low N:P ratios.

4.2 Methods

Laboratory cultured *A. circinalis* was used to determine the influence of the nutrients N and P in different concentrations and ratios as well as the influence of different light intensities on its growth in two batch experiments. Cell counts were used to quantify the growth response in the first batch experiment, while measurements of optical density were used in the second one. The relationship between cell counts and optical density of *A. circinalis* was examined prior to the experiments and was found to be linear with a coefficient of determination (R^2) of 0.95 (Figure C.1).

4.2.1 *A. circinalis* cultures

Stock cultures of *A. circinalis* (strain C8-337/01 ACBU01 MLA 6/10/09) were maintained axenically in acid-washed Erlenmeyer flasks (250 ml) containing 100 ml modified Swedish Standard (SIS) *Lemna* growth medium, including MOPS buffer (OECD, 2006) with a pH of 6.6 and conductivity of 270±10 μS/cm. The medium was prepared according to the method presented in appendix C and

filter sterilised (0.2 μm filter) before use for *A. circinalis* stock cultures. Stock cultures were kept in environmental cabinets at 21 °C, an average light intensity of 10 μmolm^{-2}s^{-1} (cool white fluorescent light) and a 16:8 hour light-dark cycle. Stock cultures were renewed approximately every two weeks by sterile transfer of culture to give a slightly cloudy solution.

Preparation of *Lemna* base medium (LM-N&P)

A base mixture concentrate of nutrients was prepared from stock solutions used for the SIS medium, omitting the Na_2CO_3 solution (Solution IV) as the carbonate precipitated in the concentrate (for amounts of stock solutions see Table C.2). *Lemna* base medium without N or P (LM-N&P) was prepared by adding 34 ml of base mixture concentrate and 10 ml of 4 g/L Na_2CO_3 solution (Solution IV) to approximately 1800 ml of ultrapure water. The solution was then made up to 2000 ml. The pH was adjusted to 7.5 with 0.1 M NaOH.

Preparation of a seeding suspension for batch experiments

A. circinalis cultures in exponential growth phase were pooled and 45 ml were poured into each of four 50 ml Falcon tubes. To remove the high nutrient SIS medium, tubes were centrifuged in an Eppendorf 5810R centrifuge at 1000$*$g, 15 °C, for 10 minutes. The supernatant was removed and the cells resuspended in LM-N&P medium at room temperature. Centrifuging and resuspending was repeated once.

For the first batch experiment (testing nutrient concentrations and ratios), two samples of the cell suspension were taken for enumeration in a Sedgwick-Rafter chamber. The cell suspension was then diluted to give a density of 500 000 cells/ml and used for seeding.

For the second batch experiment (testing the influence of light levels), optical density (absorbance) of the washed cell suspension was measured at 560 nm using a Varian Cary 50 Bio UV spectrophotometer and 1 cm polystyrene cuvettes. At this wavelength the cyanobacterial's pigmentation did not interfere with absorbance according to the spectrum (400 nm to 700 nm) of a concentrated sample of *A. circinalis* suspension. The undiluted washed suspension had an optical den-

sity of 0.0867, corresponding to approximately 40 000 cells/ml.

4.2.2 Batch experiments

1. Testing the influence of different nutrient ratios at different concentrations

A. circinalis growth was determined in 16 nutrient treatments over a period of 14 days. Treatments were single additions and combinations of N and P in concentrations of 50, 100 and 250 μg N/L and 5, 20 and 50 μg P/L. The experimental design, including all nutrient combinations, is shown in Table 4.1.

Table 4.1: Experimental design of the first batch experiment. All treatments and the control (C) were prepared in quadruplicate.

Treatment:	N3P1	N3P2	N3P3	N2P1	N2P2	N2P3	N1P1	N1P2	N1P3	N1	N2	N3	P1	P2	P3	C
N (μg/L)	250	250	250	100	100	100	50	50	50	50	100	250	0	0	0	0
P (μg/L)	5	20	50	5	20	50	5	20	50	0	0	0	5	20	50	0
mass N:P ratio	50	12.5	5	20	5	2	10	2.5	1	–	–	–	–	–	–	–

The experiment was performed in acid-washed Erlenmeyer flasks (250 ml), stoppered with cotton wool plugs and autoclaved before use. All test flasks were rinsed with LM-N&P medium before use. Then 200 ml aliquots of non sterilised LM-N&P medium were dispensed into each flask and aliquots of $NaNO_3$ solution (280.2 mg N/L) and KH_2PO_4 solution (61.0 mg P/L) were added to make up the correct concentration for each treatment. Duplicate surrogates were prepared for each treatment in order to measure physicochemistry.

An aliquot of 200 μl *A. circinalis* seeding suspension was added to all flasks to establish a final concentration of 500 cells/ml. After thorough mixing, 2.5-ml aliquots were removed and preserved with Lugol's iodine for cell counts. In order to test if the high nutrient SIS medium had been successfully removed from the seeding suspension, 40 ml of LM-N&P medium was collected from the N1, N2, N3, P1, P2 and P3 surrogates before and after seeding for nutrient analysis. The remaining 160 ml in the surrogates were seeded with 200 μL of *A. circinalis* suspension.

Flasks were incubated in environmental cabinets as described in section 4.2.1. Samples for cell counts (2.5 ml) were taken daily from day 0 (13/5/2011) until day 14 (28/5/2011) and preserved with Lugol's iodine. Nutrient samples were

collected on day 0 from the surrogates, and on every second day thereafter from all flasks in two replicates (10 ml from each replicate), with replicates being rotated to maintain an equal volume change in all flasks. Temperature, pH, conductivity and dissolved oxygen were measured in one set of surrogate solutions on day 0 and in all flasks at the termination.

Cell counts were performed using a Sedgwick-Rafter counting chamber at 200 times magnification, counting columns of 20 grids at random. Individual trichomes were counted until 200 were reached or until 25 columns of the chamber were completed. Numbers of trichomes were multiplied by the averaged cell count of the first 30 trichomes. Heterocysts were counted for each trichome.

2. Testing the influence of different light levels and nutrient ratios

A. circinalis growth was determined at three different light levels in combination with four nutrient treatments over a period of 12 days. Light levels were 12, 40 and 95 μmolm^{-2}s^{-1}. Nutrient treatments contained N at concentrations of 0, 100 or 600 μg/L and P at concentrations of 0 or 50 μg/L. Combinations of nutrient concentrations and their ratios as well as treatment names are shown in Table 4.2.

Table 4.2: Nutrient concentrations in treatments in the second batch experiment. Nutrient treatments were tested at three different light levels with irradiances of 12, 40 and 95 μmolm^{-2}s^{-1}. All treatments were prepared in triplicate.

Treatment:	N2P	N1P	N1	P
N (μg/L)	600	100	100	0
P (μg/L)	50	50	0	50
mass N:P ratio	12	2	–	–

The experiment was performed in acid-washed Erlenmeyer flasks (250 ml), stoppered with cotton wool plugs and autoclaved before use. All test flasks were rinsed with LM-N&P medium before use. Then 200 ml aliquots of non sterilised LM-N&P medium were dispensed into each flask and aliquots of $NaNO_3$ solution (100 mg N/L) and KH_2PO_4 solution (100 mg P/L) were added to make up the correct concentration for each treatment. Surrogates were prepared for each treatment in order to measure physicochemistry and to sample for nutrients on day 0. Undiluted washed *A. circinalis* suspension was added to achieve a concen-

tration of approximately 200 cells/ml.

Flasks were incubated in a controlled temperature room at 22.5 °C and a 16:8 hour light-dark cycle. Designated light intensities (daylight fluorescent light) were realised by adjusting the distance between light source and test containers for the 95 and 40 μmolm^{-2}s^{-1} treatments and attaching shade cloth to the light source for the 12 μmolm^{-2}s^{-1} treatment. According to measurements of water temperature in a surrogate beaker under each treatment light source, there was no difference in water temperature between treatments despite different distances to the light source. The spatial light distribution under each light was measured with a Licor light meter using a quantum sensor for measurement in air and test containers were placed in areas varying between 12 and 17, 40 and 45 and 95 and 100 μmolm^{-2}s^{-1} respective of treatment. The positions of test containers were randomised after sampling.

The *A. circinalis* suspension of each replicate was sampled for determination of optical density on day 0 (10/10/2012), 1, 2, 3, 5, 6, 7, 8, 9 and 12. After measuring optical density, samples were preserved in Lugol's iodine. Nutrients were sampled on day 0 before and after additions of *A. circinalis* in surrogates and in the test flasks on the last day of the experiment. Water quality (pH, dissolved oxygen and conductivity) was measured on day 0 in surrogates and in the test flasks on day 12 after sampling. Concentration of *A. circinalis* was measured as optical density at 560 nm.

Analysis of nutrient samples

All nutrient samples were filtered with 0.45 μm syringe filters and stored frozen at -18°C. Nutrient concentrations were determined photometrically with a QuikChem 8500 Lachat nutrient analyser. Soluble reactive phosphorus (srP) was measured with the molybdate blue method using ascorbic acid as reductant. Nitrate and nitrite (NO_x) were analysed as surrogate of nitrate with the sulphanilamide method after reduction by a cadmium column (APHA, 1995).

4.2.3 Data analysis

For the determination of growth rates, cell counts and optical density were plotted semilogarithmically over time. A linear regression curve was fitted to at least four values within the exponential growth phase (usually on days 0, 2, 4 and 6 in the batch experiment testing the influence of different nutrient ratios and days 0, 2, 3, 5 and 6 in the batch experiment testing the influence of different light levels) of each replicate. The growth rate per day during the exponential phase was then given by the slope of the linear regression curve. Growth rates were compared with one-way ANOVA (batch experiment testing the influence of different nutrient ratios) and two-way ANOVA (batch experiment testing the influence of different light levels).

Cell yield was defined as the maximum cell concentration in each replicate during the course of the experiment in the first batch experiment. Similarly, maximum optical density was defined as the highest optical density measured in each replicate during the course of the experiment in the second batch experiment. Yields were compared with one-way ANOVA and maximum optical densities with two-way ANOVA.

Homogeneity of variance of growth rates, yield data and maximum optical density was tested with the Levene statistic. The software SigmaPlot 10.0 was used for the linear regression in the first batch experiment and ANOVAs and Tukey's tests were carried out using the General Linear Model function of the statistics software Minitab 15. Analyses were carried out with the statistics software R, version 2.13.0, (R Development Core Team, 2011), using the functions lm, LeveneTest (with median as the centre), glm and Anova (with type III model and F-test) from the packages MASS and car for the second batch experiment. All graphs were prepared with R.

4.3 Results

1. Testing the influence of different nutrient ratios at different concentrations

Positive growth of *A. circinalis* was observed in all treatments except in the P only ones where cell counts remained below those of the controls. Growth curves of

combined N and P treatments and the control are shown in Figure 4.1. Nutrient treatments had a significant effect on growth rates (p=0.002) and cell yield (p≤0.001) (Table 4.3).

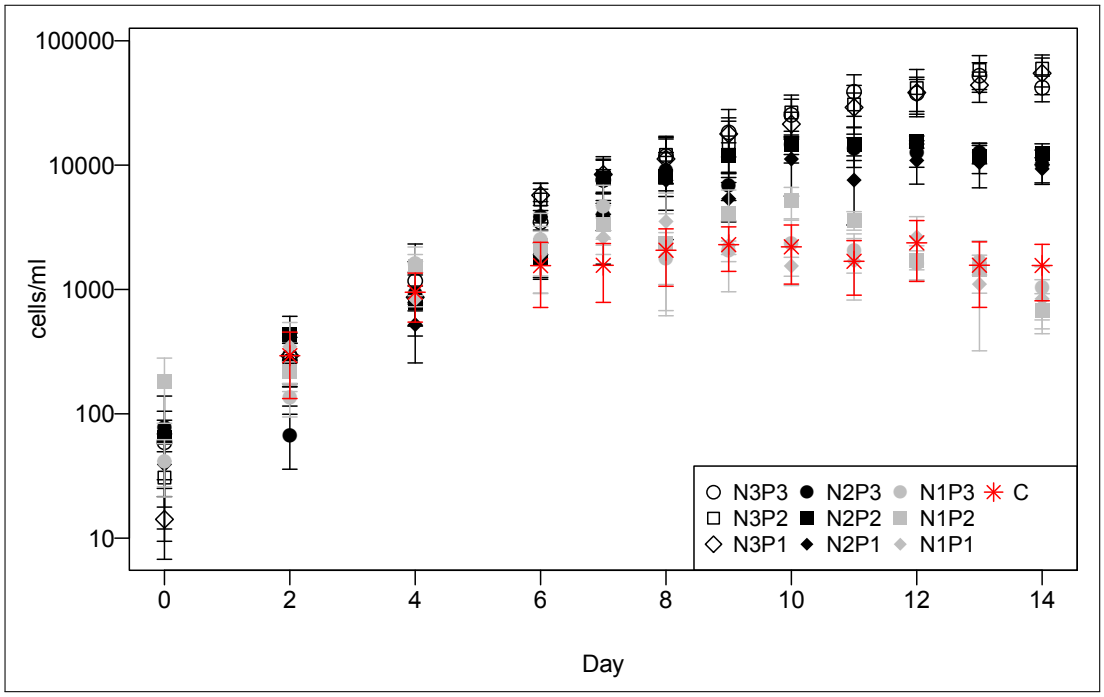

Figure 4.1: Growth of *A. circinalis* in the first batch experiment. Note that the y-axis has a logarithmic scale. Error bars are one standard error from the mean, n=3.

Table 4.3: Results of ANOVA of growth rate and yield from the first batch experiment. Factor nutrients had 14 levels (N3P1, N3P2, N3P3, N2P1, N2P2, N2P3, N1P1, N1P2, N1P3, N1, N2, N3 and C).

	Source	MS	F	p
Growth rate:	nutrients	0.014	3.82	0.002
Yield:	nutrients	$1.32*10^9$	8.19	≤0.001

The highest growth rate, 0.36±0.07 day^{-1} (mean±standard error), was found in the N3P2 treatment (Figure 4.2). It was significantly higher than the growth rate in the control (0.17±0.07 day^{-1}, p=0.034) and the N2 (0.18±0.01 day^{-1}, p=0.046) and N3 treatments (0.14±0.01 day^{-1}, p=0.009). The second fastest growth occurred in treatment N3P1 (0.34±0.05 day^{-1}). This was significantly faster than growth in treatment N3 (p=0.0249). The remaining growth rates did not vary statistically from each other.

Cell yield in the N3P1, N3P2 and N3P3 treatments was 54757±17887, 60643

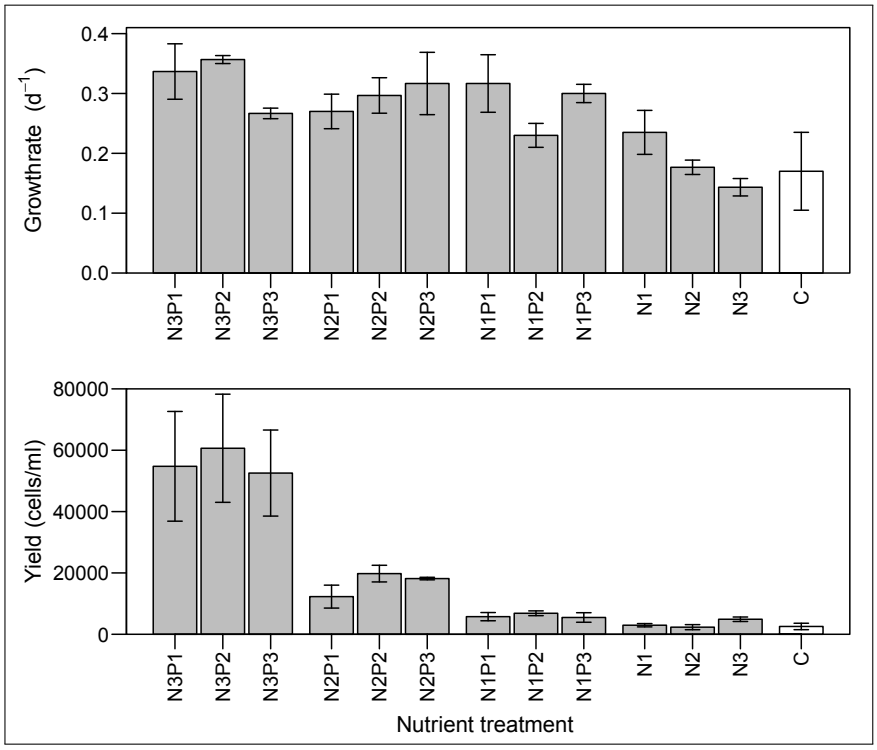

Figure 4.2: *A. circinalis* growth rate per day and yield (cells/ml) for all nutrient treatments with positive growth and the control (C) in the first batch experiment. Error bars are one standard error from the mean, n=3.

±17629 and 52556±14026 cells/ml and was significantly higher than in most other treatments ($p \leq 0.032$) and the control (2550±1046 cells/ml, $p \leq 0.003$). The comparison of treatment N3P1 with N2P3 and N2P2 and also N3P3 with N2P3 and N2P2 showed no significant difference (Figure 4.2). This is most likely due to large standard errors in the N3P1, N3P2 and N3P3 treatments. Although yield in treatment N3P2 was higher than in N3P1 and N3P3 this was not significant. Cell yield in N2P1, N2P2 and N2P3 (12278±3736, 19777±2714 and 18161±409 cells/ml) was higher than in N1P1, N1P2 and N1P3 (5749±1341, 6849±781 and 5478±1554 cells/ml), this was also not significant.

Abundance of heterocysts was variable within the first four days of the experiment (Figure 4.3). It increased with abundance of vegetative cells and independent of treatment as indicated by similar ratios of heterocysts to vegetative cells in all treatments containing N and P throughout the experiment (Figure 4.3).

Nutrient uptake was observed in all combined N and P treatments as indicated by the nutrient measurements on day 14 (Table 4.4). P was not detected in the following treatments: N3P1, N3P2, N2P1 and N1P1 and it was noticeably decreased in treatments N3P3, N2P2, N2P3, N1P2 and N1P3 compared with initial concentrations. N was depleted in N2P1, N2P2, N2P3 and N1P2 and decreased in all other treatments except N1 and N3.

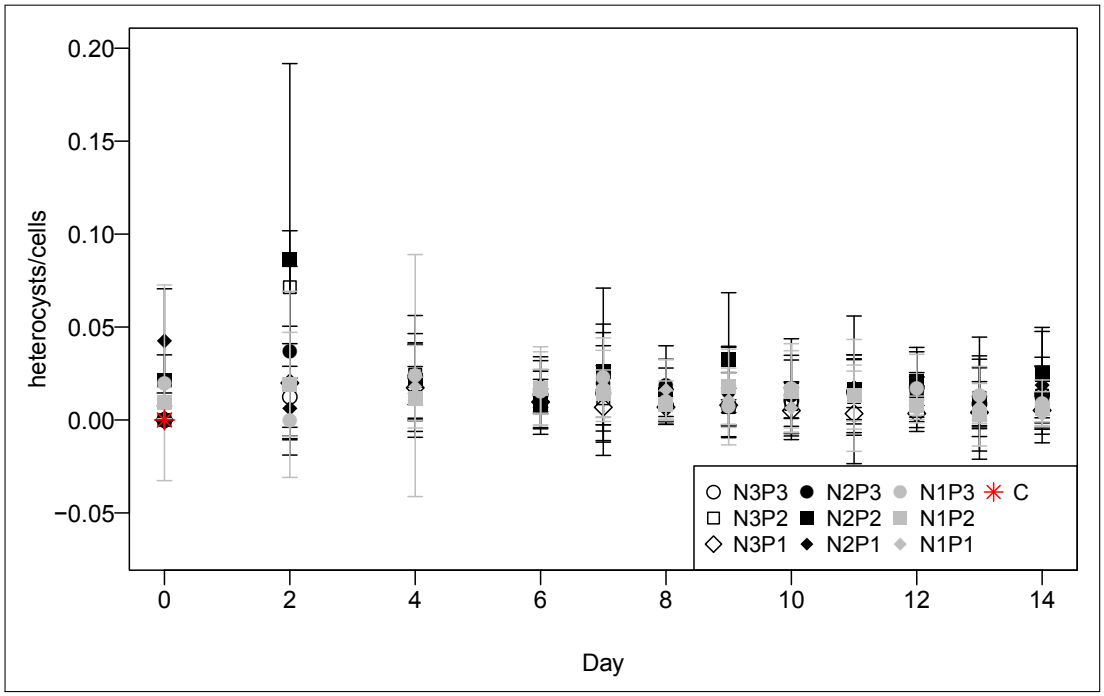

Figure 4.3: Ratio of heterocysts to vegetative cells of *A. circinalis* in the first batch experiment. Error bars are one standard error from the mean, n=3.

Table 4.4 shows that nutrient levels were amended to levels close to the target concentrations and that high nutrient SIS medium was removed from the seeding suspension.

Table 4.4: Nutrient concentrations of test medium at the end of the first batch experiment (day 14), intended concentration for each treatment on day 0 and concentration in the surrogates after seeding on d 0.

	Treatment:	N3P1	N3P2	N3P3	N2P1	N2P2	N2P3	N1P1	N1P2	N1P3	N1	N2	N3	P1	P2	P3	C
day 14	N (μg/L)	52.0	35.3	9.3	0.0	0.0	0.0	2.9	0.0	0.5	43.7	80.3	251.5	0.0	0.0	0.0	4.0
	P (μg/L)	0.0	0.0	21.4	0.0	2.7	32.4	0.0	8.2	40.4	4.7	1.2	0.2	4.3	13.8	46.3	0.0
day 0	N (μg/L)	250	250	250	100	100	100	50	50	50	50	100	250	0	0	0	0
intended	P (μg/L)	5	20	50	5	20	50	5	20	50	0	0	0	5	20	50	0
day 0	N (μg/L)	-	-	-	-	-	-	-	-	-	46.2	94.9	257.5	0.0	0.0	0.0	0.0
surrogates	P (μg/L)	-	-	-	-	-	-	-	-	-	0.0	0.0	0.0	4.7	14.0	45.6	0.0

2. Testing the influence of different light levels and nutrient ratios

Light availability had an effect on *A. circinalis* growth rate and maximum optical density in the N2P treatments only (Figure 4.4). There were significant interactions between nutrient and light treatments, affecting growth rates (p=0.004) and maximum optical densities (p=0.033) (Table 4.5).

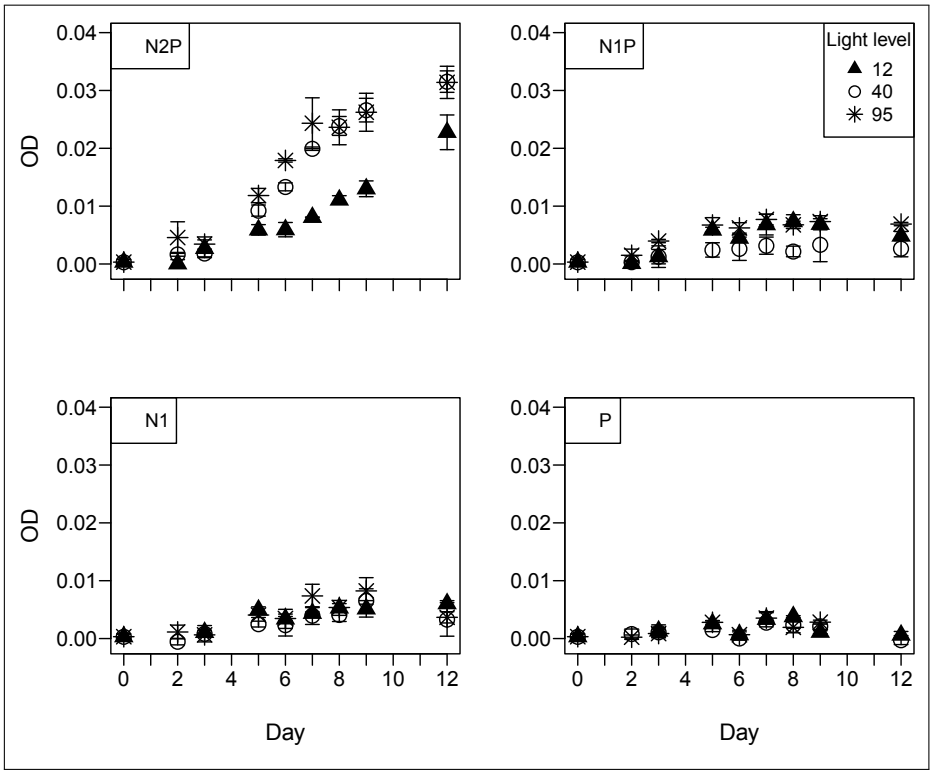

Figure 4.4: Growth of *A. circinalis* measured as optical density (OD) in the second batch experiment. Error bars are one standard error from the mean, n=3.

The highest growth rate was 0.76±0.07 day^{-1} (mean±standard error) and was recorded in the N2P treatment incubated at 95 μmolm^{-2}s^{-1}, followed by 0.60±0.02 day^{-1} in the N2P treatment incubated at 40 μmolm^{-2}s^{-1} (Figure 4.5). Growth rates in the N2P treatment incubated at 12 μmolm^{-2}s^{-1} and the N1P treatment at all three light levels remained below 0.36±0.02 day^{-1}. Optical densities increased irregularly in the replicates of the N1 and the P treatments and it was not possible to fit a significant linear regression with R^2 higher than 0.5 to replicates of these treatments.

The highest maximum optical density, a surrogate measure for the yield, 0.0315 ±0.0019 (approximately 115 500 cells/ml), was found in the N2P treatment incubated at 40 μmolm^{-2}s^{-1} (Figure 4.6). The maximum optical density in this treatment was not significantly different from the N2P treatment incubated at 95 μmolm^{-2}s^{-1} according to Tukey's pairwise comparison. Both treatments, i.e. N2P incubated at 40 and 95 μmolm^{-2}s^{-1}, had significantly higher ($p \leq 0.047$) maximum optical densities than the N2P treatment incubated at 12 μmolm^{-2}s^{-1} (0.0224 ±0.0027, approximately 72 500 cells/ml). Maximum optical densities in all three

Table 4.5: Results of ANOVA of growth rate and maximum optical density (OD) in the second batch experiment. For the growth rate, factor light had the levels 95, 40 and 12 μmolm^{-2}s^{-1} and factor nutrients had the levels N2P and N1P. For maximum OD, light had the same levels as for the growth rate but nutrients had the levels N2P, N1P, N1 and P.

	Source	MS	F	p
Growth rate				
	light	1.55*10^{-3}	0.206	0.817
	nutrients	6.00*10^{-4}	0.079	0.783
	light*nutrients	7.36*10^{-2}	9.782	0.004
Maximum OD				
	light	2.92*10^{-6}	0.318	0.731
	nutrients	5.99*10^{-4}	21.74	$\leq$0.001
	light*nutrients	2.57*10^{-5}	2.797	0.033

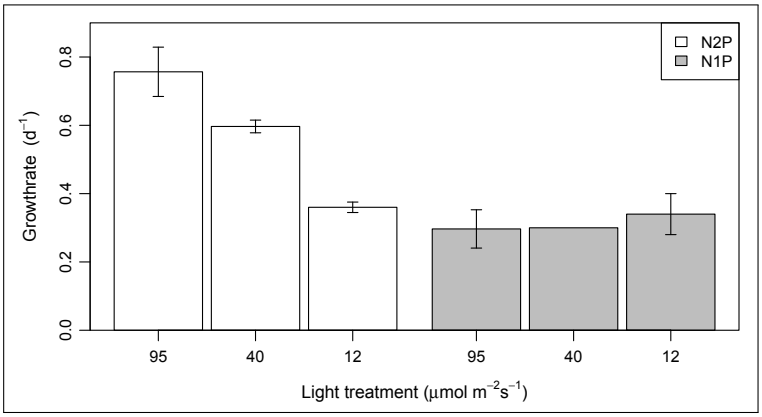

Figure 4.5: Mean growth rates in the second batch experiment. Error bars are one standard error from the mean, n=3 (except for treatment 40 N1P where n=1).

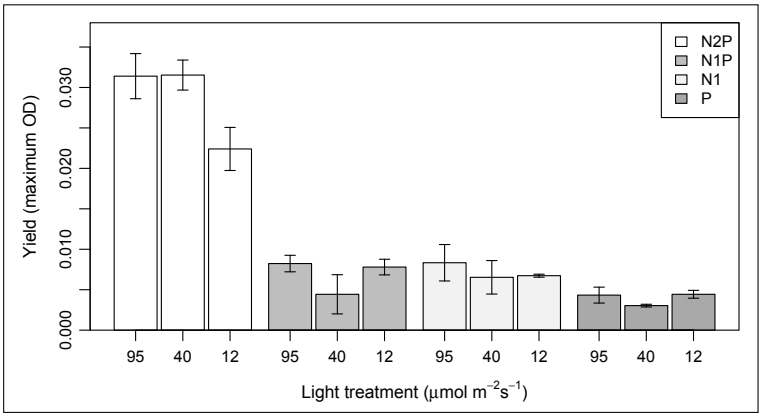

Figure 4.6: Mean yield measured as maximum optical density (OD) in the second batch experiment. Error bars are one standard error from the mean, n=3.

N2P treatments were significantly higher than in the remaining treatments which had a maximum optical density of $\leq$0.0083 (approximately 5700 cells/ml) and were, according to Tukey's pairwise comparison, not different from each other.

Instead of 200 ml of medium, 220 ml were accidentally added to test flasks. Accordingly, nutrient concentrations were approximately 10 % lower than intended (Table 4.6). High nutrient SIS medium was removed from the seeding suspension (Table 4.6).

Table 4.6: Nutrient concentrations and standard errors (s.e.) in surrogate treatments in the second batch experiment on day 0 before and after seeding with washed cell suspension. All treatments were prepared in triplicate.

	Treatment:	N2P	s.e.	N1P	s.e.	N1	s.e.	P	s.e.
before seeding	N (μg/L)	460	16	64	11	83	1	0	0
	P (μg/L)	31	6	41	2	2	0	35	3
after seeding	N (μg/L)	457	3	77	2	76	1	0	0
	P (μg/L)	38	1	37	0	0	0	35	3

Uptake of N and P occurred in all treatments (Table 4.7). Uptake was highest in the N2P treatment, nearly depleting P and depleting N at all light levels on day 12. N was also depleted in the N1P treatments across all light levels on day 12, while P concentrations were diminished but not entirely used up.

Table 4.7: Nutrient concentrations and standard errors (s.e.)in the second batch experiment on day 12. All treatments were prepared in triplicate.

Light level:	12								40								95							
Treatment:	N2P	s.e.	N1P	s.e.	N1	s.e.	P	s.e.	N2P	s.e.	N1P	s.e.	N1	s.e.	P	s.e.	N2P	s.e.	N1P	s.e.	N1	s.e.	P	s.e.
N (μg/L)	0	0	0	0	0	0	0	0	0	0	46	24	22	25	0	2	0	0	0	0	0	0	0	0
P (μg/L)	2	1	32	0	1	0	43	0	2	0	37	5	1	0	41	1	1	0	28	2	1	0	38	4

4.4 Discussion

The growth response of *A. circinalis* to different N:P ratios at different concentrations did not depend on the amount of P available but rather on the amount of N in the two batch experiments. This was indicated by the significant increase in yield in the treatments with high N concentrations in combination with any concentration of P in both batch experiments. Additionally, there was no positive

growth in the P only treatments in the first batch experiment and little growth in the second, while there was some growth in the N only treatments in both experiments.

Nutrient ratios did not have an effect on growth rate or yield in the first batch experiment. There was no difference between growth rates of *A. circinalis* under low and high N:P ratios at different nutrient concentrations. Yield was highest in the treatments with high N concentrations in combination with any of the three concentrations of P (5, 20 or 50 μg P/L). However, there was no difference between those three treatments, indicating that nutrient ratios of 50, 12.5 or 5 were not important for yield.

Increased light availability was expected to enhance the growth response to additions of P as it would provide more energy for N fixation which would compensate for the relative lack of N. However, this was not observed. In contrast, higher light availability promoted the increase in yield (measured as optical density) and growth rates in the treatments containing high concentrations of N in combination with P (600 μg N/L, 50 μg P/L). This did not occur in treatments with a lower amount of N (100 μg N/L, 50 μg P/L) and thus, a lower N:P ratio.

Heterocysts, the sites of N fixation in many filamentous cyanobacteria, were present in *A. circinalis* in both experiments. Ratios of heterocysts to vegetative cells remained similar in all treatments of combined N and P throughout the first batch experiment, suggesting that N availability had no effect on heterocyst differentiation. Low external concentrations of fixed N, such as ammonium and nitrate, usually induce heterocyst differentiation in *Anabaena* (Zhang et al., 2006) but this is not always the case and *Anabaena* strains have been observed to form heterocysts when concentrations of external fixed N were high (Thiel and Leone, 1986; Buikema and Haselkorn, 1991). However, some strains of *Anabaena* are not capable of fixing atmospheric N in their heterocysts (Thiel and Leone, 1986). Whether the strain that was used in the experiments described here falls into this category is not known. Inability to fix atmospheric N in heterocysts could be a possible, albeit speculative, explanation for the results observed.

A lack of the trace metals Fe and molybdenum (Mo) can limit nitrogenase activity as the trace metals are essential parts of the enzyme (Zerkle et al., 2006).

Velzeboer et al. (2001) provided 0.004 mg/L Mo to encourage N fixation in N free cultures of *Anabaena* in which N fixation occurred. The same amount of Mo was part of the LM-N&P medium used in the two batch experiments and would have been expected to be sufficient for nitrogenase activity.

It is possible that irradiance levels in both batch experiments were not high enough to stimulate N fixation. Zapomělová et al. (2010) reported that the growth optimum for strains of the *A. circinalis* and *A. crassa* complex lay at light intensities between 220-360 μmolm^{-2}s^{-1} for temperatures between 17.5 and 22.5 °C. Compared with these values, *A. circinalis* would have been light limited in both batch experiments which in turn suggests that there may not have been enough energy to facilitate N fixation. On the other hand, it has been shown that although *A. circinalis* had high light requirements, as indicated by its compensation irradiance of 13 μmolm^{-2}s^{-1}, its flotation rate decreased at irradiances of more than 100 μmolm^{-2}s^{-1}, suggesting that higher irradiances are not conducive to the cyanobacterium (McCausland et al., 2005). Further, experiments have shown that *A. circinalis* grown in N free medium at 50 μmolm^{-2}s^{-1} has similar growth rates to *A. circinalis* grown in the same medium with added nitrate at the same irradiance (Velzeboer et al., 2001). This suggests that at least the higher level of irradiance in the second batch experiment would have been enough to facilitate N fixation. Thus, it is likely that the *A. circinalis* strain used in the two batch experiments is capable of forming heterocysts but that N fixation within the heterocysts may be repressed. However, this may not be a common phenomenon and whether it occurs in many *A. circinalis* strains is not known.

Cell yields and growth rates were higher in the N only treatments than in the P only ones. As cyanobacteria are capable of storing P in the form of polyphosphates (Kromkamp, 1987), it may be possible that cells transferred from the high nutrient culture medium utilised their P stores for growth once transferred to the N only test medium. Removing the high nutrient SIS medium seemed to be successful as the nutrient concentrations in the surrogate flasks suggested. However, traces of P may have contributed to growth in the N only treatments as P concentrations in those treatments, shown in Table 4.4, indicate.

In the first batch experiment growth rates in treatments containing N and P

were more than two times lower than maximum growth rates for *A. circinalis* reported in the literature (0.86 day^{-1} (Bruggeman, 2011); 0.7 day^{-1} (McCausland et al., 2005). Growth rates under increased irradiance, i.e. 95 $\mu molm^{-2}s^{-1}$, in the second batch experiment were closer to reported maximum growth rates, suggesting that *A. circinalis* growth rates were primarily limited by light in the first batch experiment (10 $\mu molm^{-2}s^{-1}$) and in the lower light treatments (12 and 40 $\mu molm^{-2}s^{-1}$) in the second one. Blooms of *A. circinalis* still occur and reach high densities in Australian waterways when growth rates are not close to reported maximum growth rates. Mitrovic et al. (2003) observed a growth rate of 0.4 d^{-1} in the Darling River during the development of an *A. circinalis* bloom that reached a concentration of more than 100 000 cells/ml.

It is generally accepted that N fixing cyanobacteria compensate for low concentrations of dissolved inorganic N through N fixation. N fixing cyanobacteria have been observed to thrive under low N:P nutrient supply ratios and to outcompete other phytoplankton assemblage members (Schindler, 1977; Vrede et al., 2009; De Tezanos Pinto and Litchman, 2010b). Thus, *A. circinalis* was expected to compensate for the lack of dissolved inorganic N in the test medium by fixing atmospheric N and achieve higher growth rates and yields with increasing P availability. However, N fixation did not seem to be a major activity and P availability did not control its growth rates or yield.

The results in this chapter suggest that, from a management perspective, P inputs to Grahamstown Dam may be less important than N inputs in the formation of *Anabaena* blooms. The current levels of N in the lake, described in chapter 2, are mostly below the concentration that facilitated the highest increase of biomass in this experiment. However, there were periods in recent years when dissolved inorganic N concentrations reached more than 200 $\mu g/L$, while srP concentrations were 5 $\mu g/L$ or more, particularly at Site 3. These temporarily increased nutrient levels may lead to *Anabaena* densities close to those reached in this experiment although other factors such as light, grazing and competition need to be considered.

Managing N may help to reduce the risk of blooms in Grahamstown Dam. However the cyanobacterium's ability to fix atmospheric N under favourable

light conditions cannot be completely ignored. Results from both batch experiments were unexpected and appear to be unusual. It may not be advisable to base management decisions on these before they can be clarified further. In order to confirm that lack of compensation for N deficiency was dependent on the particular *A. circinalis* strain used, the experiments could be repeated with a different strain. Using a wild strain isolated from Grahamstown Dam would also contribute to realism and add validity to the results for this particular site. Moreover, N fixation rates of the current strain could be measured to confirm that the ability to fix N is absent.

Chapter 5

Nutrient release from the sediments in Grahamstown Dam

5.1 Introduction

Lake sediments often act as storage for nutrients in waterways, in particular for P (Boström et al., 1988; Granéli, 1999; Hupfer and Lewandowski, 2008). However, release of P from the sediments may occur under some conditions and can impair or prevent the effect of restoration measures such as reduction of external P input (Søndergaard et al., 2001, 2003) as flux of P from lake sediments can be equivalent to (Moore et al., 1998), or larger than, external inputs (Burger et al., 2007; Pollman and James, 2011). Similar to P retention, N retention depends on the amount of N input to a lake (Saunders and Kalff, 2001). Most N enters aquatic systems as nitrate (Golterman, 2004) and although sediments play an important role as a nitrate sink through denitrification (Saunders and Kalff, 2001), they can also be a source of ammonium, with release rates sometimes being much higher than external input (Burger et al., 2007). Therefore, sediments can become important sources of nutrients for phytoplankton growth.

Nutrients generally reach the sediments in the form of particulate organic matter and to a lesser extent, bound in or adsorbed to mineral particles (Golterman, 2004). In the sediments, microbially mediated transformations and chemical sorption and dissolution processes lead to the release of dissolved inorganic nutrients. These processes depend on environmental conditions such as dissolved oxygen concentration, redox potential, pH and temperature (Mortimer,

1971; Boström et al., 1988; Jensen and Andersen, 1992; Penn et al., 2000). In particular, oxygen availability has been observed to affect the release of nutrients from the sediments. In anoxic conditions, release rates of P (Andersen and Ring, 1999; Malecki et al., 2004; Nowlin et al., 2005), N (Malecki et al., 2004; Beutel et al., 2008) and Fe (Andersen and Ring, 1999) can be significantly higher compared to oxic conditions. The conventional mechanism for the increased release of P and Fe is the reduction of Fe(III) oxyhydroxides in anoxic conditions. Fe(III) oxyhydroxides are a main binding partner for orthophosphates in the sediments (Lijklema, 1980; Baldwin et al., 2002; Perrone et al., 2008) and when Fe(III) oxyhydroxides are reduced, orthophosphates that were bound to them become soluble again. This process may be microbially meditated as Fe(III) serves as an electron acceptor for microbial metal reduction during anoxia (Lovley and Philips, 1986; Weber et al., 2006). In contrast, the transport of inorganic dissolved P out of the sediments can be hindered if the surface layer of the sediments is oxidised (Penn et al., 2000; Christophoridis and Fytianos, 2006).

Although the water column in shallow lakes is often well mixed and thus oxygenated, there may be periods when the water at the sediment interface becomes anoxic. Stable weather conditions, i.e. low wind speeds, may lead to persistent thermal stratification of the water column during hotter periods. Organic matter mineralisation can consume the dissolved oxygen in bottom waters and result in anoxia and increased nutrient release. These periods of stratification can lead to accumulation of dissolved inorganic nutrients in bottom waters followed by large nutrient pulses into the euphotic zone and intensified phytoplankton growth (Wilhelm and Adrian, 2008).

The large surface area of Grahamstown Dam in combination with prevailing windy coastal weather patterns are assumed to lead to a well mixed water column at most times. However, limited data suggests that periods of thermal stratification may occur for several days, mainly in summer (Cole and Williams, 2011) and anoxia of bottom waters may accompany these periods. In terms of phytoplankton management, it will be useful to assess whether thermal stratification does occur and may lead to an increase in bioavailable nutrients released from the sediments.

Experiments in this chapter were designed to determine which types of major algal nutrients are released. The hypothesis that sediments release higher amounts of nutrients, in particular of P, in anoxic conditions than oxic conditions in Grahamstown Dam was tested in two sediment incubation experiments. To examine the nature of thermal stratification in the lake, longterm data were collected using temperature data loggers.

5.2 Methods

5.2.1 Sediment incubation experiments

Nutrient release experiments were conducted in winter 2010 and in summer 2011, each one lasting for 28 days. Sediment was collected from the three sites in Grahamstown Dam described in section 2.2.1 on day 0 (17/6/2010 and 10/2/2011, respectively). Treatments included incubation under anoxic and oxic conditions with five replicates per treatment. The release of TN, TP, srP, NO_x (nitrate and nitrite), ammonium (NH_4^+) and Fe(II) was monitored on days 0, 1, 4, 7, 11, 15, 21 and 28.

Plastic containers (2 L) with a lid that allowed airtight sealing, a sampling port (a butyl rubber septum) that allowed sampling with a syringe and hypodermic needle and a device for pressure equalisation (constructed according to Baldwin et al. (2005) and shown in Figure 5.1) served as incubation chambers for anoxic treatments. Oxic treatment chambers were similar but did not have the pressure equalisation device, were aerated for the duration of the experiment and only sealed airtight for transport to the laboratory. Oxic chambers were kept in water up to their lids and anoxic chambers fully under water in a dark temperature controlled environment at 16 °C in winter and 23 °C in summer.

Sediments were collected from Grahamstown Dam using an Ekman grab sampler. An amount of 500 ml of sediment was immediately transferred to each incubation chamber and topped up with ultrapure water. Before sealing, pH, temperature and dissolved oxygen were measured. The conductivity of the ultrapure water had been previously adjusted to match lake water conductivity of ∼200 μS/cm by adding a few millilitres of a concentrated sodium chloride solution.

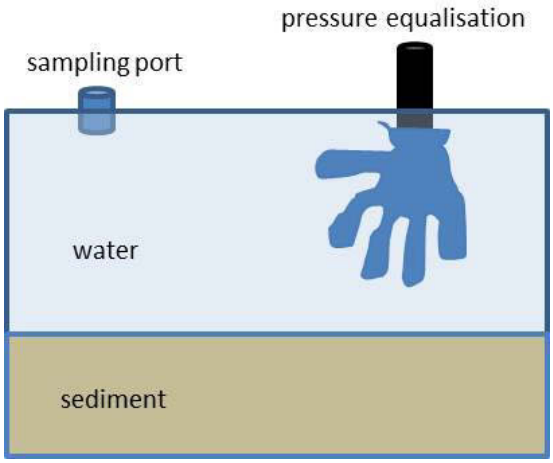

Figure 5.1: Diagram of a sediment incubation chamber (used for the anoxic treatments) consisting of an airtight plastic box fitted with a butyl rubber septum as a sampling port and a nitrile glove connected to the outside of the box for pressure equalisation during sampling.

Ultrapure water used for anoxic treatments had also been degassed by bubbling helium gas through it until dissolved oxygen concentrations reached 2.0 mg/L. Degassing was omitted in the summer experiment as dissolved oxygen concentrations decreased from 8.5 mg/L to below 1.0 mg/L in the supernatant after addition to the incubation chambers of the oxic treatments.

Dissolved nutrient samples and Fe(II) samples were filtered (0.45 μm) and Fe(II) samples were preserved in 10 % ultrapure concentrated hydrochloric acid (0.1 ml per 1 ml sample). Dissolved oxygen, pH and temperature were measured in the anoxic treatments in a freshly taken sample and on day 28 after opening the chambers. Oxic treatments were monitored on the sampling days.

Concentrations of srP, NO_x, NH_4^+, TN and TP were determined photometrically (QuikChem 8500 Lachat nutrient analyser) following APHA (1995) methods. Fe(II) was analysed photometrically (Varian Cary 50 Bio UV spectrophotometre) using the ferrozine method (Stookey, 1970; Lovley and Philips, 1986).

5.2.2 Thermal stratification

Temperature data loggers (TidbiT v2 Water Temperature Data Logger, onset) were deployed at each of the three sites. Data loggers were positioned at approximately 0.5 metres above the sediment surface and at one to two metre intervals throughout the water column for 17 months (September 2011 – Feburary 2013). Water temperatures were recorded at 30 minute intervals.

5.2.3 Data analysis

Analysis of Similarities (ANOSIM) and non metric multidimensional scaling (NMDS) was performed to illustrate qualitative similarities in nutrient release between the three different sites for both assays. As there were four replicates for srP in the winter asssay, it was not included in ANOSIM. The analysis was carried out with the statistics software R (version 2.13.0, R Development Core Team (2011)) using the functions anosim and metaMDS (with Euclidean distances) from the vegan package. Thermal stratification data was smoothed with a rolling mean using the function rollmean of R's zoo package before plotting. All graphs were prepared with R.

5.3 Results

5.3.1 Thermal stratification

Several periods of persistent thermal stratification occurred at the three sites throughout the 17 month measuring period (see figures in appendix D). Events of persistent thermal stratification were observed mostly during the spring and summer months, while the water column was well mixed in winter. The longest and most pronounced period of stratification was recorded in November 2011. It was most obvious at Site 2 (Figure 5.2, graphs for Site 1 and 3 are in appendix D) with temperature differences of up to 7.80 °C between surface (0.5 m) and bottom water (9 m) lasting for up to 23 days. Diurnal stratification affecting the surface to a depth of about 3 m occurred frequently, sometimes temperature differences between warm surface layer and colder bottom layer were up to 4.64 °C.

5.3.2 Environmental conditions in the incubation chambers

Dissolved oxygen concentrations were 0.4±0.0 mg/L (mean±one standard error) in the supernatant of the oxic treatments and 0.3±0.0 mg/L in the anoxic treatments within a few minutes after addition of the ultrapure water on day 0 of the winter experiment. In the summer experiment, concentrations decreased rapidly from 8.8 mg/L to 4.0±0.3 mg/L in the supernatant of the oxic treatments and to

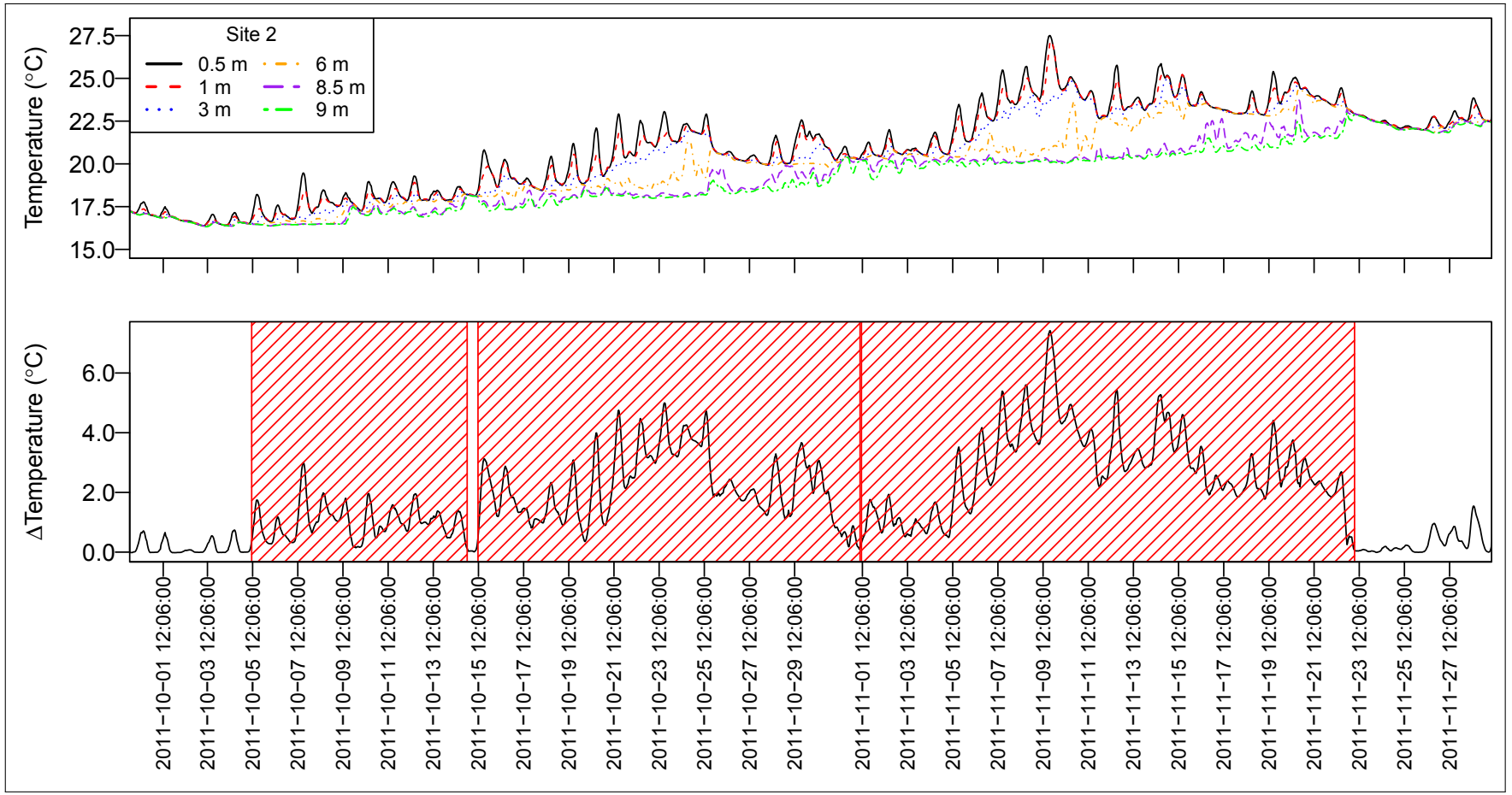

Figure 5.2: Temperature profile of the water column at Site 2 in Grahamstown Dam and temperature difference between surface and 9 m during the period of the most pronounced stratification between September 2011 and February 2013. Shaded areas highlight temperature differences >0.15 °C (according to Sherman et al. (1998), persistent thermal stratification occurs when the difference between water surface and bottom water is >0 °) and lasting for more than 24 hours. Data was smoothed with a rolling mean with a window of 10 observations.

3.2±0.5 mg/L in the anoxic treatments.

On day 28, dissolved oxygen concentrations were 10.0±0.0 mg/L in the oxic and 0.9±0.1 mg/L in the anoxic treatments in the winter experiment. In the summer experiment, concentrations were 9.2±0.2 mg/L in the oxic and 0.4±0.1 mg/L in the anoxic treatments.

At the time of sediment collection, the water temperature was 15 °C in June 2010 and 27 °C in February 2011. Temperatures of the supernatant water remained between 16 and 17 °C in both treatments in the incubation chambers in the winter experiment and between 22 and 24 °C in the summer experiment. The pH was 7.2 in the oxic treatments and 6.7 in the anoxic treatments on day 0 in the winter experiment. In the summer experiment, the pH was higher in the anoxic treatments compared with the oxic ones, particularly on day 28 when it decreased to 4.3 while it was 6.0 in the anoxic treatment.

5.3.3 Nutrient release from the sediments

The presence of oxygen had a large impact on the type and amount of nutrients released in both experiments. Sediments from all three sites released N in both experiments and there was a distinct difference in the release of N species from oxic and anoxic treatments (Figures 5.3 and 5.4). TP concentrations decreased independently from oxygen presence during the course of both experiments (Figures 5.3 and 5.4). A release of srP and Fe(II) was detected at all three sites in the summer experiment (Figure 5.4).

ANOSIM indicated that site location exerted small influences on nutrient release in both experiments (winter experiment: global R=0.0140, p=0.014; summer experiment: global R=0.121, p=0.001). The NMDS ordination plots illustrate the similarities between sites (Figure 5.5).

As site did not have a big impact on nutrient release, concentrations of nutrients were averaged over the three sites and figures in the following text are the mean±standard error of all three sites (n=15, except srP in the winter experiment where n=12).

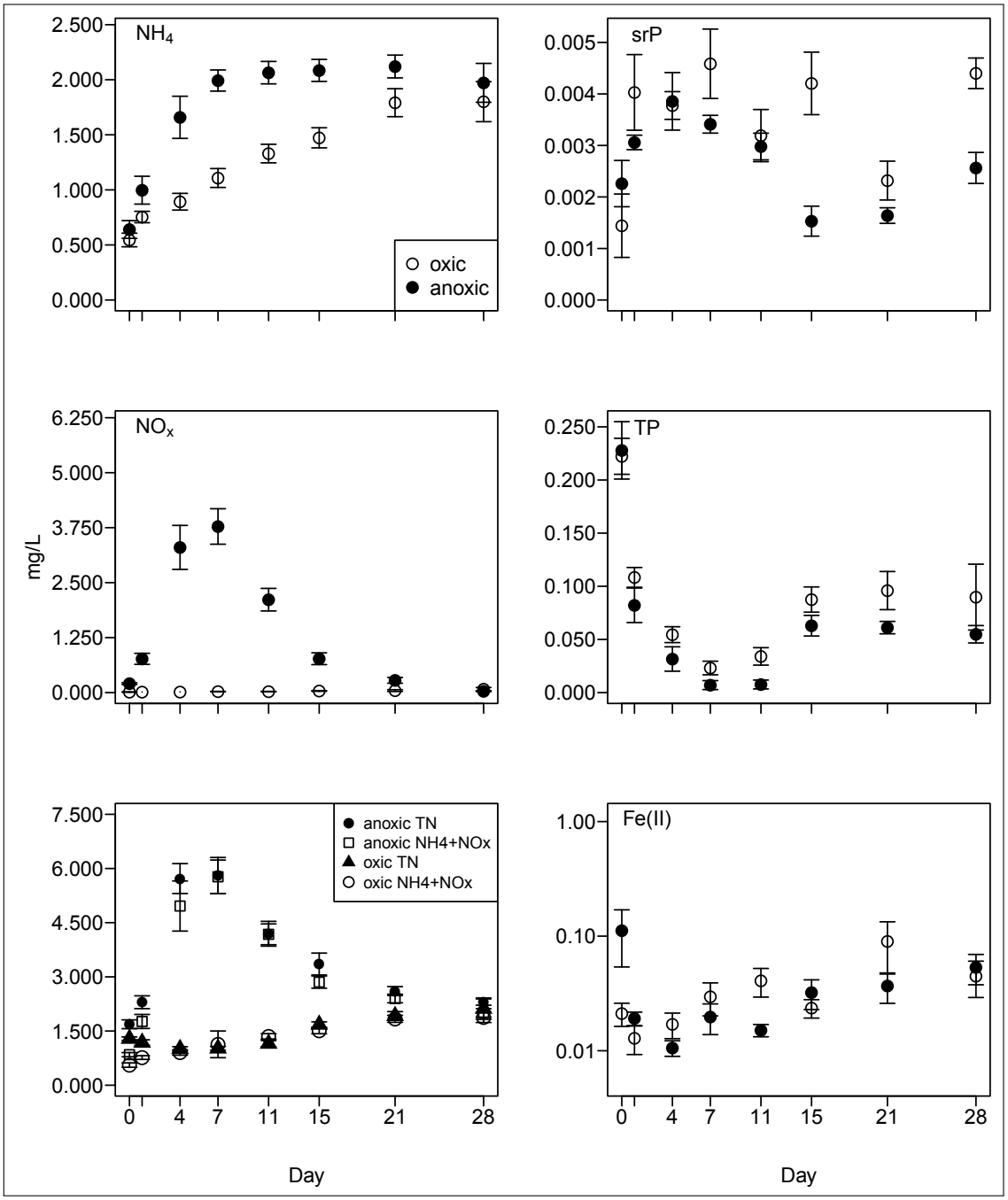

Figure 5.3: N and P species and Fe(II) concentration in both treatments during the sediment release experiment in winter 2010. Concentrations are averages of five replicates from the three sites. Note that the graph for Fe(II) has a logarithmic y-axis. Error bars are one standard error from the mean, n=15, except for srP where n=12.

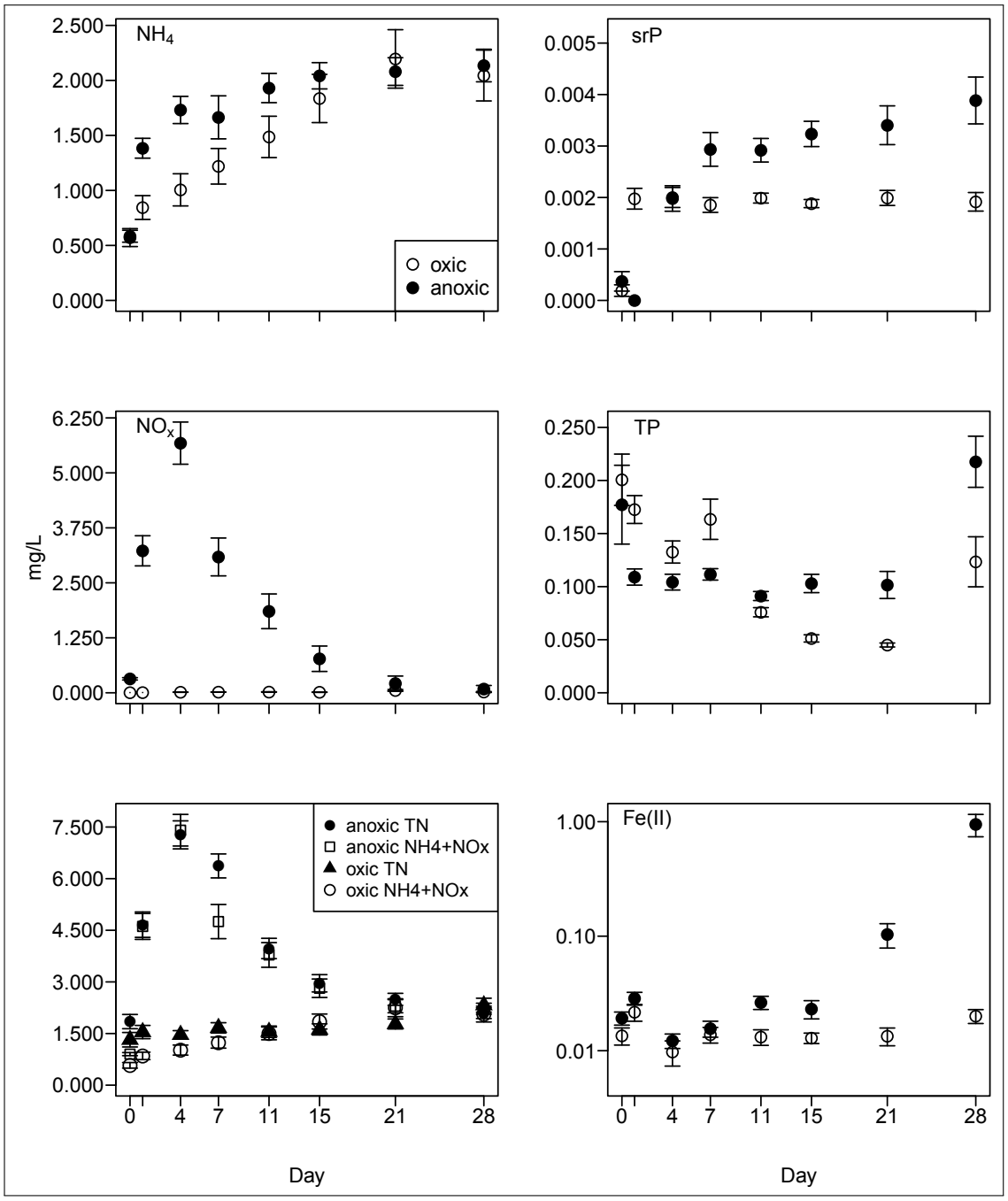

Figure 5.4: N and P species and Fe(II) concentration in both treatments during the sediment release experiment in summer 2011. Concentrations are averages of five replicates from the three sites. Note that the graph for Fe(II) has a logarithmic y-axis. Error bars are one standard error from the mean, n=15.

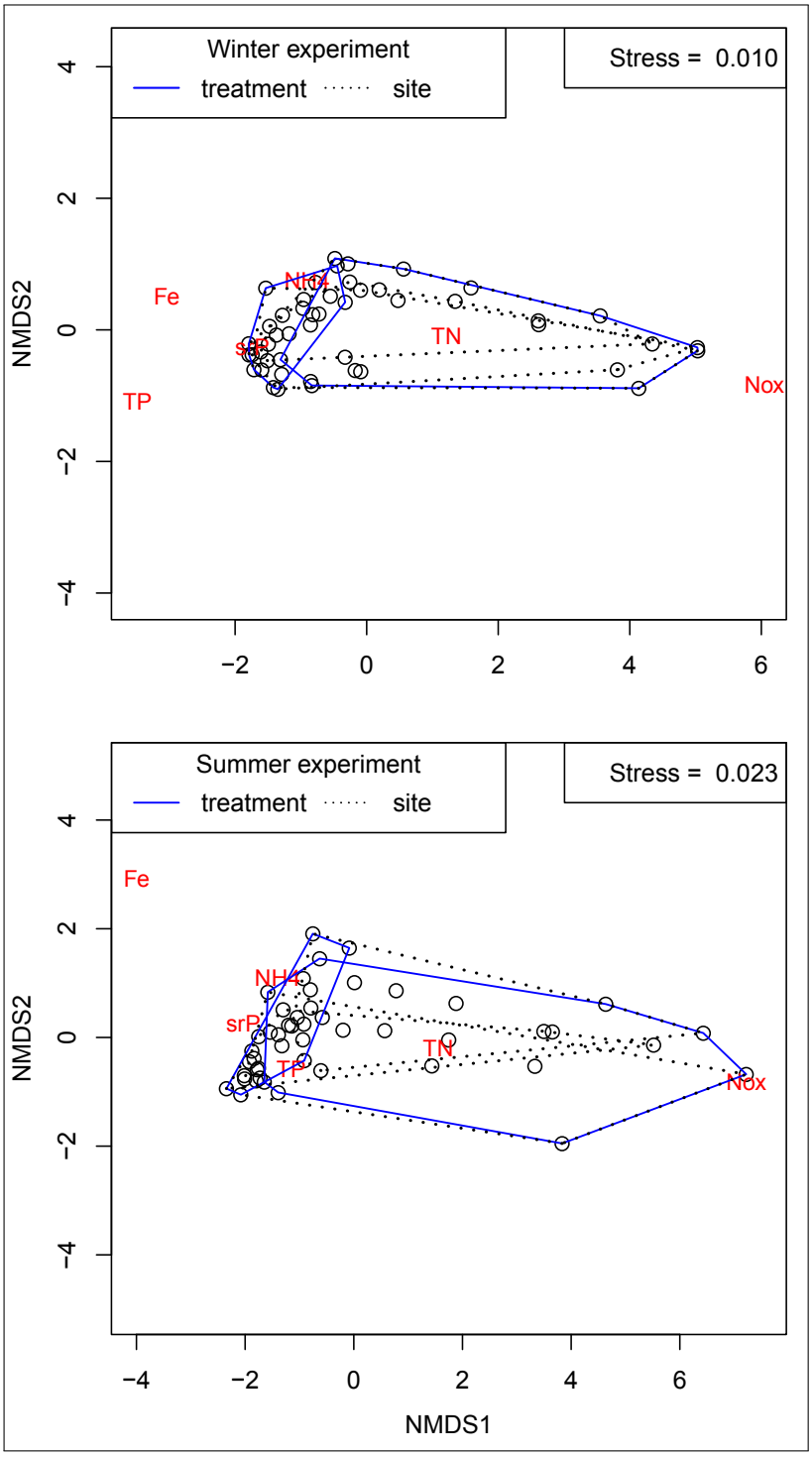

Figure 5.5: NMDS ordination plots for all nutrient data in the sediment nutrient release experiments conducted in winter and summer. Species scores (NH_4^+=NH4, NO_x=Nox, TN, srP, TP, Fe) are weighted and expanded averages of sample scores (species and sample scores have equal variances) which leads to species scores being close to the sample scores they are relatively more associated with. Convex hulls indicate sample scores belonging to the three sites and the anoxic (extended to the right) and oxic (on the left) treatments.

NH_4^+ release NH_4^+ was released rapidly until day 7 in the anoxic treatments in the winter experiment and day 4 in the anoxic treatments in the summer experiment. Subsequently, release rates slowed down and concentrations continued to increase slowly until the end of the experiment. In the winter experiment, concentration of NH_4^+ increased from 0.636±0.057 mg N/L on day 0 by 1.590±0.031 mg N/L on day 28. In the summer experiment, concentrations of NH_4^+ increased from 0.583±0.054 mg N/L on day 0 by 1.564±0.204 mg N/L on day 28.

In the oxic treatments, NH_4^+ was released more slowly and nearly linearly during the course of both experiments reaching its maximum net increase of 1.411±0.050 mg N/L on day 28 in the winter experiment. The concentration on day 0 was 0.545±0.062 mg N/L. In the summer experiment, the maximum net NH_4^+ increase was 1.624±0.452 mg N/L on day 21 and the concentration on day 0 was 0.571±0.082 mg N/L.

NO_x release In the anoxic treatments, NO_x was released within the first seven days in the winter experiment and the first four days in the summer experiment. Subsequently, its concentrations declined until almost initial values were reached. NO_x concentration increased by 3.765±0.221 mg N/L in the anoxic treatments between day 0 and day 7 in the winter experiment. The concentration on day 0 was 0.205±0.020 mg N/L. In the summer experiment, maximum concentrations were reached between day 0 and day 4. The maximum net increase was 5.672±0.331 mg N/L. The concentration on day 0 was 0.318±0.026 mg N/L.

In the oxic treatments, NO_x release was several orders of magnitude smaller and also slower compared with the anoxic treatments. Maximum concentrations were reached on day 28 in the winter experiment and day 21 in the summer experiment. In the winter experiment, NO_x concentration was 0.012±0.005 mg N/L in the oxic treatments at the start of the experiment and increased by less than 0.067±0.051 mg N/L by day 28. In the summer experiment, NO_x concentration was 0.004±0.001 at the start of the summer experiment. It increased by 0.050±0.080 mg N/L by day 21.

TN release The release of TN followed a pattern similar to the release of NO_x in both treatments albeit TN concentrations being higher. When added up, con-

centrations of NO_x and NH_4^+ were nearly the same as TN concentrations in both treatments and both experiments (Figures 5.3 and 5.4).

In the winter experiment, TN concentration was 1.687±0.122 mg N/L on day 0. TN concentration increased by 4.517±0.482 mg N/L in the anoxic treatments between day 0 and day 28. In the summer experiment, the maximum net TN increase was 5.952±0.331 mg N/L in the anoxic treatments on day 4. On day 0, TN concentration was 1.847±0.207mg N/L.

Similar to the release of NO_x, the release TN was much slower and smaller in the oxic treatment than in the anoxic ones. At the beginning of the winter experiment, TN concentration was 1.288±0.049 mg N/L. It increased slowly by 0.830±0.080 mg N/L until day 28. In the summer experiment, TN concentration was 1.320±0.210 mg N/L on day 0. TN concentration increased slowly by 1.003±0.482 mg N/L until day 28.

P release Concentrations of srP varied within a range of less than 0.001 and 0.003 mg P/L but neither a clear increase nor decrease was discernible in any of the treatments in the winter experiment. During the summer experiment, concentrations of srP were less than 0.001 mg P/L and increased by 0.004±0.001 mg P/L in the anoxic treatments and by 0.002 mg P/L in the oxic treatments.

Concentration of TP decreased in both treatments at all sites and in both experiments. The decrease was fastest during the first 7 days of the experiment in winter and the first 4 days of the experiment in summer. In the winter experiment, TP decreased by 0.173±0.040 mg P/L in the anoxic treatments and 0.132±0.028 mg P/L in the oxic ones. Concentrations at the beginning were 0.228±0.027 and 0.222±0.017 mg P/L, respectively. In the summer experiment, the decrease was 0.081±0.040 mg P/L in the anoxic treatments and 0.077±0.028 mg P/L in the oxic treatments, starting concentrations were 0.223±0.038 and 0.200±0.024 mg P/L, respectively.

Fe(II) release In the winter experiment, Fe(II) concentrations varied within a range of 0.01 and 0.27 mg Fe/L but neither a clear increase nor decrease was discernible in either treatment. Fe(II) was released between day 15 and 28 in the anoxic treatments in the summer experiments. Concentrations increased from

0.02±0.003 by 0.95±0.36 mg Fe/L at the end of the experiment. In the oxic treatments, the concentration change of Fe was less than 0.01±0.005 mg Fe/L.

5.4 Discussion

The results presented in this chapter show that sediments of Grahamstown Dam were a source of dissolved inorganic N, particularly when the overlying water column was depleted of oxygen. In fact, lack of oxygen was decisive for the release of NO_x and also Fe. Release rates of NH_4^+ were also higher in the absence of oxygen. In contrast, TP concentrations decreased independently from oxygen presence in the overlying water during the course of both experiments. Summer temperatures led to a higher amount of N released and an acceleration in N flux. As microbial activity is dependent on temperature, it was not unexpected that nutrient release was subject to seasonal temperature differences. There was no difference in the pattern of concentration change over time and also little difference in the net release of each nutrient between the three sites, suggesting that sediments and nutrient cycling processes within the lake were relatively homogeneous.

It is questionable, if anoxia, i.e. the complete absence of oxygen, occurred in the anoxic treatment chambers, especially in the summer experiment. However, the rapid consumption of oxygen in the oxygenated water used as supernatant alludes to a high oxygen demand of the sediments and dissolved oxygen concentrations at the end of both experiments were close to true anoxic conditions. Thus, lack of oxygen was most likely responsible for the difference between oxic and anoxic treatments in both experiments. Also, it is likely that this would have occurred for the entire experimental period. The observed patterns of the release of nutrients from oxic and anoxic treatments were similar in both experiments, substantiating that not degassing the supernatant for the anoxic chambers prior to the experiment in February 2011 did not affect the processes involved in the release of nutrients. Ideally, oxygen concentrations would have been monitored inside at least one replicate of the anoxic treatments during the experiment to ensure that conditions close to anoxia were maintained.

N release Detecting a large release of NO_x in the absence of oxygen, while comparatively little was released in the aerated treatments, was unexpected. Nitrate is usually the product of nitrification in the oxygenated part of sediments and it is possible that the NO_x release in the anoxic treatments consisted mainly of nitrite. Speculative explanations for the N fluxes in the anoxic and oxic treatments may be found in the interaction of several processes. When the sediments were first collected from the lake, it is likely that they were, at least partly, oxygenated as the water column was well mixed at the time of sampling. This means nitrification and denitrification would both have taken place. The covering with oxygen depleted polished water of the anoxic treatment sediments would have slowed down and eventually stopped the nitrification process. Nitrite might have accumulated as oxygen became increasingly limiting and could have caused the distinct increase of NO_x. Once the denitrifying bacteria adapted to these new conditions, i.e. a greater supply of nitrite and increasing anoxia, NO_x concentrations would have decreased again.

However, the decrease in NO_x concentrations may have been also due to anaerobic ammonium oxidation (anammox) or a combination of denitrification and anammox. Anammox bacteria use nitrite as an electron acceptor to oxidise NH_4^+ with dinitrogen gas being the product (Jetten et al., 1999). This process occurs under suboxic and anoxic conditions in marine (Thamdrup and Dalsgaard, 2002; Francis et al., 2007), terrestrial (Humbert et al., 2010) and lacustrine (Schubert et al., 2006; Hamersley et al., 2009) habitats. Decreasing NO_x concentration in combination with decreasing NH_4^+ release rates after four to seven days suggest that anammox would have been more likely to have occurred in the anoxic treatments than denitrification. Denitrification would not have had an influence on the NH_4^+ concentration.

As denitrifying bacteria are heterotrophic, it is possible that denitrification was limited by the type or amount of organic matter available as substrate. Denitrification has been shown to be inhibited by carbon availability in marine sediments (Brettar and Rheinheimer, 1992), groundwater and river sediments (Bradley et al., 1992, 1995). Wang et al. (2007) demonstrated that additions of different types of carbon stimulated either nitrate reduction to nitrite or nitrate reduction

to NH_4^+ in sediments of a eutrophic lake.

The lack of NO_x in the oxic treatments may have been due to denitrification offseting nitrification. Denitrification rates are higher than nitrification rates in many sediments (Seitzinger, 1988) and denitrification has also been observed in aerobic conditions (Davies et al., 1989; Lloyd, 1993; Chen et al., 2006). Thus, it is not unlikely that nitrification and denitrification were balanced to the extent that free NO_x was not detectable. In order to confirm the occurrence of denitrification, dinitrogen and nitrous oxide gases would need to be monitored in the oxic treatments.

The release of NH_4^+ under oxic and anoxic conditions in the present study is not unusual because organic N can be mineralised under both conditions. Similar release rates of NH_4^+ from oxic and anoxic sediments have been reported previously (Nowlin et al., 2005). The flux of organic N from the sediment was comparatively small as the concentrations and release patterns of the three N species (TN, NO_x and NH_4^+) suggested. TN mainly consisted of dissolved inorganic N, i.e. the type of N that can be utilised by phytoplankton.

P and Fe release The results of both nutrient release experiments have shown that contrary to the generally accepted paradigm, absence of oxygen did not lead to the release of large amounts of P from the sediments.

Although anoxia is thought to be the main driver of P regeneration from the sediments, and the release of srP from the sediments due to anaerobic conditions has often been reported (Andersen and Ring, 1999; Penn et al., 2000; Beutel et al., 2008; Wilhelm and Adrian, 2008), some studies have found P release to be independent from the availability of oxygen (Burger et al., 2007). Moreover, the release of P from aerobic sediments has also been observed (Jensen and Andersen, 1992).

Cycling of P and Fe are often observed simultaneously under anoxic conditions (Andersen and Ring, 1999; Beutel et al., 2008). However, Fe release is not necessarily coupled with P release. Baldwin and Williams (2007) detected Fe reduction with a release of up to 0.95 mg/L Fe(II) while srP was not released to a great extent in a sediment incubation study similar to the one presented here.

The lack of srP release from the sediments in the anoxic treatments observed in the sediment incubation experiments could be due to the presence of nitrate. Although this explanation is speculative in the case of this experiments described here, nitrate has been found to inhibit P release under anoxic conditions in laboratory incubation and field studies (Andersen, 1982; Beutel et al., 2008; Hemond and Lin, 2010). It has been suggested that the increased redox potential created by nitrate suppresses the release of P. Andersen (1982) reports that nitrate concentrations above 0.5 mg N/L inhibited the release of phosphate from the sediments of Danish lakes without summer stratification and Jensen and Andersen (1992) found that a decrease of nitrate from 2.52 to 0.098 mg N/L enhanced srP release in two shallow lakes. The concentrations of NO_x measured in summer and winter experiments were higher than 0.5 mg N/L in most replicates in the anoxic treatments between day 1 and day 15, thus it is possible that nitrate suppressed anoxic P release. However, it has also been found that some lake sediments do not release any P whether nitrate is present or not (Boström and Pettersson, 1982).

The presence of nitrate could also explain the lack of Fe release. Fe can be used as an electron acceptor in the oxidation of organic matter (Lovley, 1991). However, if nitrate is available, it is preferred over Fe as an electron acceptor (Nealson and Saffarini, 1994). This may explain the increasing Fe release from day 15 in the summer experiment coinciding with decreasing NO_x concentrations.

The absence of biologically available carbon and sulfate may limit the release of P. Mitchell and Baldwin (1998) demonstrated that if sulfate and organic carbon are available, sulfate reducing bacteria may form hydrogen sulfide in anoxic sediments. This in turn reduced Fe minerals, or other sulfide reducible minerals P may be bound to, leading to the release of P. Although the role of sulfate was not examined in their study, Watts (2000) found enhanced release of P under anaerobic conditions after the addition of carbon in the form of macrophyte material. Whether carbon and (or) sulfate availability had an influence on the P release in the study described here is not clear.

The decrease of TP within the first four to seven days of the experiment may be due to microbial mineralisation of organic P and incorporation of P into bacterial cells. Gächter and Meyer (1993) suggest that net P release was controlled

by bacterial demand for P. They support this with the observation that settling detritus did not release inorganic P as would be expected in the mineralisation process. If bacteria are taking up P, the sediments may be a sink rather than a source for P. An alternative explanation for the TP flux in the supernatant may be the following. Adding the ultrapure water to the incubation chambers might have mixed some particles containing TP into the supernatant. These particles would have settled over time and TP concentrations would have decreased simultaneously.

Dissolved inorganic N released from the sediments may contribute to phytoplankton development in Grahamstown Dam. Periods of persistent thermal stratification of up to 23 days occur in the lake (Figures 5.2, D.4 and D.5) and it is likely that bottom waters will become oxygen depleted during those events. The subsequent breakdown of stratification may mix dissolved inorganic N into the euphotic zone where it would be available for algal growth. It may be useful to measure the profile of NO_x and NH_4^+ concentrations in the water column during and after thermal stratification in the lake in order to confirm this scenario. According to Cole and Williams (2011), the average depth of the euphotic zone is usually around 4 m while the average depth of the lake is 7 m. However, the euphotic zone had comprised the entire water column on some occasions since 1993. Coinciding with thermal stratification and oxygen depletion of bottom waters an event like that would make large amounts of NO_x available for algal growth.

Moreover, sediment resuspension due to lake mixing is another mechanism for dissolved inorganic nutrients to reach the euphotic zone in shallow lakes (Dzialowski et al., 2008; Reddy et al., 1996). It is not known, if sediment resuspension due to weather conditions occurs in Grahamstown Dam. However, sediment resuspension has been observed in the shallower parts of the lake near the inflow of Balickera Canal after extraction of water from the Williams River. It may be helpful for management purposes to examine if this process makes sediment nutrients available to phytoplankton growth.

The mechanistic interpretations of the N and Fe(II) fluxes as well as the interpretations of the lack of P cycling in Grahamstown Dam are speculative and further research would be necessary to confirm them. This could include deter-

mining the carbon content of the sediments and examination of carbon limitation of microbiological processes. In particular, it would be useful to examine if the microbial cycling of P is limited by availability of organic carbon in Grahamstown Dam. Investigating the content of TP and the type of Fe and P minerals present in the sediments would allow further insight into how the sediments contribute to nutrient availability to phytoplankton.

Chapter 6

Effects of organic carbon on sediment nutrient release and sediment characteristics

6.1 Introduction

Results from previous experiments (chapter 5) showed unusual patterns of nutrient release which were difficult to explain with common nutrient release models in the literature. In order to better understand nutrient cycling processes and nutrient release from the sediments, further experiments were performed and sediment properties were determined.

Microbial mineralisation plays a major role in nutrient cycling processes in lake sediments. Nutrients mostly reach the sediments incorporated in particulate organic matter where microbial mineralisation can lead to the release of dissolved forms of inorganic N and P. Particulate organic matter is decomposed by heterotrophic bacteria in several steps. Extracellular bacterial enzymes hydrolyse organic polymers into monosaccharides and amino acids which are further degraded into alcohols, short-chain aliphatic carboxylic acids, ammonia, hydrogen and carbon dioxide by fermenting bacteria (Sigee, 2005). While the first step is performed by aerobic and facultative anaerobic bacteria, fermenters are obligate anaerobic, i.e. their metabolism requires the absence of oxygen. Different functional groups within the microbial community use different species of fermentation products as electron donors in catalysing redox reactions to gain en-

ergy. To complete the reaction, electron acceptors such as Fe(III) and Mn(IV) minerals (Lovley, 1991), ionic and gaseous nitrogen oxides (NO_3^-, NO_2^-, NO, N_2O) (Knowles, 1982), sulfate (Laanbroek and Pfennig, 1981) or humic substances (Lovley et al., 1996) are used.

Generally, the amount of organic matter deposited in the sediments determines bacterial abundance (Schallenberg and Kalff, 1993) and bacterial activity has been shown to be stimulated by increased organic matter deposits (Goedkoop et al., 1997; Törnblom and Rydin, 1998). This in turn can increase the release of inorganic nutrients in the form of NH_4^+ and orthophosphate from the sediments (Watts, 2000; Autio et al., 2003).

Specifically, the availability of organic substrate type affects the bacterial community composition (Mitchell, 2002; Findlay et al., 2003; Torres et al., 2011) and can control rates and pathways of nutrient cycles in the sediments (Mitchell, 2002). For example, the additions of glucose (Bradley et al., 1992, 1995; Wang et al., 2007), acetate (Wang et al., 2007), starch (Wang et al., 2007) and propionate (Mitchell, 2002) can stimulate denitrification. Processes that release inorganic nutrients from the sediments can also be limited by carbon availability. Watts (2000) found that additional macrophyte material enhanced the release of P from lake sediments and Mitchell (2002) found P release limited by formate availability.

Nutrient cycling processes in Grahamstown Dam may be limited by the availability of substrate in the form of organic carbon (C) compounds released in the mineralisation process. The lake's catchment is dominated by sandstone and sand may be a major constituent of the sediments. The previous nutrient release experiments (chapter 5) have shown that P was not released from the sediments of Grahamstown Dam under oxic or anoxic conditions, both in summer and winter experiments. A low sediment P content could be responsible for a lack of release of larger amounts of P but it is also possible that microbial processes mediating the release of P were limited by the availability of organic electron donors. Denitrification might also be limited by organic C availability as the release of NO_x in the anoxic sediment chambers in previous experiments indicated. The accumulation of nitrate, nitrite or both suggests that activity of nitrate or nitrite reducing bacteria was limited.

In order to confirm the potential reasons for the lack of P release and release of NO_x in anoxic conditions, it is necessary to determine the content of total P, potential P binding partners and organic matter in the sediments as well as to investigate the effect of organic substrate on nutrient release. This would give insight into the interactions of the cycling of carbon and inorganic nutrients in the lake. To test the two hypotheses (1) anaerobic nutrient cycling processes, i.e. denitrification and the release of P, are limited by availability of organic substrate and (2) P concentrations in the sediments are low and thus, at least partly responsible for the lack of P release in anoxic conditions, a sediment incubation experiment was conducted and sediment properties were examined to elucidate organic matter, N, P and mineral composition.

6.2 Methods

6.2.1 Carbon limitation experiment

The previous nutrient release experiments (chapter 5) showed that there was no difference in nutrient type released or nutrient concentration between the three sites in Grahamstown Dam. Thus, only sediments from Site 1 (described in section 2.2.1) were used in this study. However, while sampling for this experiment, it was discovered that some areas at Site 1 had patches of very coarse, sandy sediments. According to Hunter Water, this sandy area was due to construction work that had been carried out on the dam wall and was not necessarily natural. Sediments from these patches were not used in this experiment.

Sediments were collected on day 0 (27/10/2011) and the methods described in section 5.2.1 were applied for collection, filling of incubation chambers, measurement of water quality parameters and incubation. The airtight incubation chambers used for the anoxic treatment in chapter 5 were used in this experiment. Sediments were incubated at 20 °C for 28 days.

Solutions of electron donors in the form of short-chain aliphatic carboxylic acids and glucose in ultrapure water were added to each chamber after filling with sediment. The four treatments were acetate, formate, propionate and glucose (n=4). One control contained ultrapure water and sediment (n=4). Solutions

were made from reagent grade chemicals to achieve a concentration of 120 mg C/L, i.e. 410 mg/L acetate, 680 mg/L formate, 320 mg/L propionate and 300 mg/L glucose, and degassed. Environmental concentrations are usually lower than 120 mg C/L (5 - 12 mg/L of total organic carbon at Site 1 according to Hunter Water monitoring data) but the concentration was chosen to ensure levels of carbon were high enough to be able to record a response to carbon additions in a batch experiment.

The release of TN, TP, srP, NO_x, NH_4^+ and Fe(II) was monitored on days 0, 1, 4, 7, 11, 14, 21 and 28. Nutrient concentrations were determined photometrically (QuikChem 8500 Lachat nutrient analyser) following APHA (1995) methods. Fe(II) was analysed photometrically (Varian Cary 50 Bio UV spectrophotometer) using the ferrozine method (Stookey, 1970; Lovley and Philips, 1986).

Graphs were prepared with the statistics software R, version 2.13.0 (R Development Core Team, 2011).

6.2.2 Sediment composition

Sediment was collected from Site 1, 2 and 3 (described in section 2.2.1) using an Ekman grab. Three grabs were taken at each site for each analysis unless otherwise mentioned. Fresh samples were stored at -20 °C until further processing unless mentioned otherwise. Sediments for loss on ignition (LOI) and pore water analysis were collected on day 0 (27/10/2011) of the C limitation experiment. Analysis of total C (TC), TN and TP was conducted on sediments collected on 8/04/2013 and also on sediments that had been used in the oxic treatments of the nutrient release experiment in June 2010 (chapter 5) and stored at -20 °C until analysis in July 2013. Sediments for X-ray fluorescence spectrometry (XRF) were collected on 2/11/2012.

LOI Loss on ignition (LOI) was carried out to determine the organic matter content of the sediments. Samples were stored in the refrigerator until processing on the 21/03/2012, then dried at 110 °C for 24 hours. Subsamples of 22 to 38 g of dried sediment were taken and combusted at 550 °C for three hours in a muffle furnace. After cooling in a desiccator, they were weighed again. LOI was

calculated as the percentage of the dry weight of the sediment before combustion.

Pore water nutrients Concentrations of pore water nutrients in comparison with concentrations in the overlying water column are an indicator for the diffusion gradient for nutrient release into the water column. The focus was in particular on N species to elucidate details of the N cycle in Grahamstown Dam. Samples of about 50 ml were collected in 50 ml PE centrifuge tubes from each grab. Samples were centrifuged at 3500 rpm for at least 10 minutes on the day of sampling. The supernatant was filtered (0.45 μm) and stored at -10 °C until analysis. NO_x, NH_4^+, Fe(II) and srP were analysed as described above. As there was not enough supernatant from some samples to measure all nutrients, srP was only measured in three samples.

TC, TN and TP content TC indicates the amount of C that is potentially available as electron donor. TN and TP content indicate the amount of these nutrients that could potentially be remobilised. Three grabs were taken at Site 2, two grabs were taken at Site 3 due to windy conditions and two grabs were taken at Site 1. Samples were dried at 110 °C and ground up before analysis. Analysis of TC and TN was carried out with a TruSpec CN Determinator (Leco Corporation) at UTS. TP was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after acid digestion by Envirolab Services Pty Ltd, Sydney, Australia.

XRF XRF was used to gain insight into concentrations of the major elements. Three grabs were taken at Site 1 and 2 and one grab was taken at Site 3 due to windy conditions. Samples were dried at 90 °C for 15 hours on the day of their collection and ground up before analysis. The analysis was carried out by the Advanced Analytical Centre at James Cook University, Townsville, Australia.

6.3 Results

6.3.1 Environmental conditions in the incubation chambers

After degassing, dissolved oxygen concentrations in all of the C solutions were below 0.9 mg/L. Dissolved oxygen concentrations were below 1.0 mg/L in the

supernatant in the incubation chambers after addition of the degassed C solutions. On day 28, incubation chambers of all treatments had less than 0.6 mg/L dissolved oxygen in the supernatant water. At the time of sediment collection, the water temperature was 20 °C and temperatures of the supernatant water remained between 18 and 21 °C in all treatments during the experiment. The pH was between 6.5 and 7.0 in all treatments on day 0. Subsequently, pH varied between 5.5 and 7.5 in the different treatments. The glucose treatment had the lowest pH, followed by the control and the acetate and propionate treatments. The highest pH of up to 7.5 was found in the formate treatment.

6.3.2 Nutrient release from the sediments

Addition of different C sources increased the release of Fe(II) and srP from the sediments, while the release of NH_4^+ and NO_x was not stimulated by the additional C (Figure 6.1). Concentrations in the following represent the mean of four replicates ± one standard error for each treatment.

Fe(II) release The concentration of Fe(II) increased in all treatments and the control from day 0 until the end of the experiment. The highest increase was stimulated by additions of glucose, followed by propionate, acetate, formate and the control. In the glucose treatment, Fe(II) concentration increased from 0.03±0.03 Fe mg/L on day 0 by 25.59±4.28 mg Fe/L on day 21. In the control, concentrations increased from 0.05±0.05 mg Fe/L on day 0 by 2.55±1.68 mg Fe/L on day 28.

srP release The concentration of srP increased in all treatments from day 0 until day 14. The highest increase was stimulated by the additions of formate. Concentrations on day 0 were ≤0.002 mg P/L in all treatments and increased by 0.027±0.015 mg P/L in the formate treatment, by 0.011±0.003 mg P/L in the acetate treatment and by ≤0.008±0.003 mg P/L in the remaining treatments by day 14. After day 14, the concentration of srP decreased in all treatments and the control except the formate treatment where it remained stable until the end of the experiment.

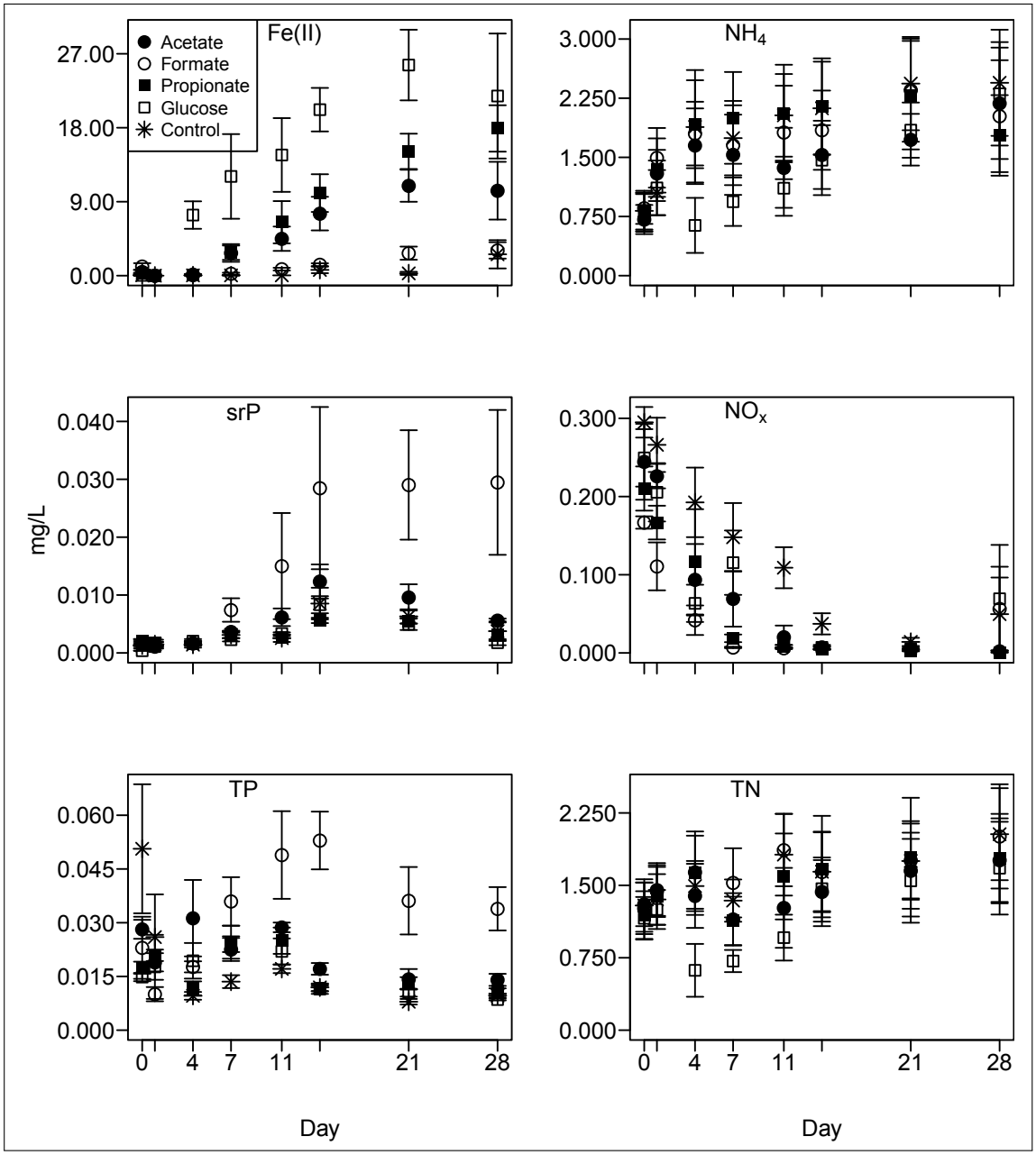

Figure 6.1: Nutrients released over the 28 day C limitation experiment. Error bars are one standard error from the mean, n=4.

TP release Concentrations of TP were 0.027±0.006 mg P/L in all treatments and the control on day 0. After day 1, the concentrations of TP increased in the formate treatment by 0.043±0.009 mg P/L on day 14. Subsequently, it decreased to 0.034±0.006 mg P/L on day 28. After day 11, TP concentrations decreased in the acetate, propionate and glucose treatments and in the control to 0.011±0.001 mg P/L on day 28.

NH_4^+ release The concentration of NH_4^+ increased from 0.800±0.024 mg N/L by 1.013±0.042 mg N/L in the treatments with acetate, formate and propionate additions and in the control between day 0 and day 4. After day 4, NH_4^+ release slowed down in the control and the propionate and formate treatments while the concentration of NH_4^+ decreased in the acetate treatment until day 11. NH_4^+ release in the glucose treatment started after day 4 and increased steadily until day 28 when NH_4^+ concentrations in all treatments and controls were similar (2.147±0.116 mg N/L).

NO_x release The concentration of NO_x decreased from 0.233±0.021 mg N/L on day 0 to 0.002±0.001 mg N/L until day 28 in all treatments and the control.

TN release The concentration of TN was 1.242±0.028 mg N/L in all treatments on day 0 and increased in the control and all treatments except the one with glucose addition by 0.274±0.079 mg N/L on day 4. Similar to the release of NH_4^+, TN was released steadily after day 4 until the end of the experiment in the glucose treatment. TN concentration in all treatments and the control were lower than NH_4^+ concentrations between day 4 and 28.

6.3.3 Sediment composition

Sediments at Site 1 were patchy and consisted of either sand or silty grey brown material interspersed with some small roots and other plant matter. The silty sediments were visually similar to the sediments found at the other two sites.

Content of TC, TP and TN, different minerals and organic matter in the sediments as well as concentrations of NH_4^+, NO_x, Fe(II) and srP in the pore water are summarized for all three sites in Table 6.1.

Table 6.1: Proportion and standard error (s.e.) in mg analyte per gram sediment based on dry weight. Pore water nutrient concentrations are in mg/L. TC_{ox}, TN_{ox} and TP_{ox} were measured in sediments collected from oxic incubation chambers after the winter nutrient release experiment (chapter 5). TC, TN and TP were measured in sediments collected in April 2013. LOI was measured as part of the XRF analysis (LOI_{XRF}) and by dry combustion at 550 °C in samples from October 2011 (LOI). Elemental proportions of Al, Fe, Ca and P were calculated from Al_2O_3, Fe_2O_3T, CaO and P_2O_5 values.

		Site 1	s.e.	n	Site 2	s.e.	n	Site 3	s.e.	n
Total nutrients	TC_{ox}	74.9	1.2	5	97.2	1.3	5	60.7	3.2	4
	TC	48.1	40.8	2	74.4	0.8	3	62.5	5.6	2
	TN_{ox}	5.37	0.17	5	6.73	0.18	5	4.27	0.19	4
	TN	3.13	3.01	2	6.36	0.52	3	5.40	0.32	2
	TP_{ox}	0.42	0.01	5	0.53	0.02	5	0.41	0.01	4
	TP	0.22	0.20	2	0.70	0.02	3	0.50	0.03	2
Minerals	SiO_2	618	31	3	472	15	3	575	–	1
	Al_2O_3	73.5	6.1	3	141	1.5	3	147	–	1
	Al	38.7	3.3	3	74.7	0.7	3	78.0	–	1
	Fe_2O_3T	40.4	6.9	3	66.3	8.4	3	48.0	–	1
	FeT	28.3	5.0	3	46.7	5.8	3	34.0	–	1
	TiO_2	5.8	0.1	3	6.3	0.2	3	8.0	–	1
	CaO	4.9	0.3	3	4.3	0.4	3	5.0	–	1
	Ca	3.5	0.2	3	3.1	0.3	3	3.6	–	1
	K_2O	3.2	0.4	3	5.2	0.6	3	12	–	1
	MgO	2.9	0.4	3	3.9	0.2	3	6.2	–	1
	Na_2O	1.7	0.5	3	2.5	0.3	3	7.0	–	1
	SO_3	1.3	0.03	3	1.8	0.7	3	0.7	–	1
	P_2O_5	0.9	0.1	3	1.9	0.2	3	1.3	–	1
	P	0.4	0.03	3	0.9	0.09	3	0.6	–	1
	MnO	0.4	0.06	3	0.5	0.06	3	0.3	–	1
Organic matter	LOI_{XRF}	240	25	3	285	26	3	178	–	1
	LOI	84	27	3	185	7	3	161	5	3
Pore water	NH_4^+	1.653	0.514	3	0.472	0.080	3	0.818	0.063	3
	NO_x	0.007	0.004	3	<0.001	<0.001	3	<0.001	<0.001	3
	srP	0.011	–	1	0.014	0.001	2	–	–	0
	Fe(II)	2.91	1.27	3	6.50	1.29	3	1.17	0.19	3

The main constituent of sediments from all three sites was SiO_2, followed by organic matter (measured by LOI_{XRF}), Al_2O_3, TC and Fe_2O_3T. Pore water contained up to 6.50±1.29 mg/L Fe(II) and 1.653±0.514 mg N/L in the form of NH_4^+, while concentrations of NO_x and srP were below 0.014 mg P/L and 0.007±0.004 mg N/L. Concentrations of N species in the pore water and proportions of SiO_2 were higher and proportions of Al_2O_3 were lower at Site 1 compared with the other two sites.

6.4 Discussion

Availability of different organic C sources stimulated the release of inorganic nutrients from Grahamstown Dam sediments. Fe and P release were stimulated by additional organic C while N release remained largely unchanged. Limitation of Fe and P release by bioavailable C indicates that P and Fe cycling was controlled by microbial processes and not solely by lack of oxygen resulting from microbial activity. This result supports the notion that mechanisms involved in P cycling are more complex than the absence of oxygen and resulting reducive conditions (Hupfer and Lewandowski, 2008) but that bacteria play an important role in actively controlling the cycling of P (Gächter and Meyer, 1993; Davelaar, 1993).

Moreover, the results presented here indicate that P release was not caused by the reduction of Fe minerals and concurrent dissolution of orthophosphate as often suggested as a mechanism of anoxic P release (Boström et al., 1988; Amirbahman et al., 2003; Christophoridis and Fytianos, 2006; Loh et al., 2013) but rather that cycling of Fe and P occurs independently in the sediments of Grahamstown Dam. Each added C source, except formate, enhanced the release of Fe and glucose caused by far the highest release. In contrast, P release was mostly stimulated by formate additions and to a small extent by acetate additions. While Fe was released until day 21 or 28 in some treatments, P release stopped after day 14 and appeared to be taken up by the sediments. Fermenting bacteria have been shown to use Fe(III) as an electron acceptor (Lovley, 1991). As glucose is a main substrate for fermenters, fermentation would explain the increased release of Fe in the glucose treatment.

Sediment pore water concentrations of Fe were exceeded by release of Fe in the glucose, propionate and acetate treatments. Similarly, srP concentrations released in the formate treatment but not the other treatments or the control were higher than pore water concentrations. This substantiates the claim that Fe and P release were due to additional bioavailable C. Had the concentration of Fe or P increased in the supernatant solely due to diffusion of Fe and P dissolute in the pore water prior to the experiment, concentrations in the supernatant in the incubation chambers would not have exceeded pore water concentrations.

The results for P and Fe release in the C limitation experiment suggesting independent microbial release mechanisms for Fe and P are in accordance with the results from a previous experiment. Mitchell (2002) examined the effect of different C sources (acetate, formate, lactate, propionate and glucose) on microbial communities and microbial metabolite cycling in wetland sediments. It was observed that glucose stimulated the highest Fe release (6.72 mg per one litre microcosm) while formate stimulated the highest P release (0.93 mg per one litre microcosm) from the sediments. Acetate additions also lead to a small release of P (0.31 mg per one litre microcosm). Moreover, an inverse pattern was also recorded: formate led to Fe uptake and glucose to P uptake by the sediments.

While there were differences in the amount of metabolites released – the maximum amount of Fe release was approximately four times higher in the C limitation experiment (25.59$\pm$4.28 mg/l) and the maximum amount of P release (0.027 $\pm$0.015 mg/L) was approximately 30 times lower than in Mitchell (2002)'s experiment – the finding that Fe and srP releases were stimulated by the same C sources, i.e. glucose and formate, respectively, indicates that sediment composition and also microbial communities in both sediments may have been similar.

Sediments used in Mitchell (2002)'s experiment came from Norman's Billabong, a shallow, well vegetated, oxbow lake on the Murray River in NSW. Their composition resembled sediments collected from Grahamstown Dam (Table 6.1, note that values are given in mg/g) with SiO_2 making up the largest part (59.13 %) followed by organic matter (20.53 % by LOI), Al_2O_3 (12.76 %) and Fe_2O_3T (3.18 %). Content of P_2O_5 was 0.13 %.

Content of organic matter in sediments often increases with increasing trophic

state and in eutrophic lakes more than 20 % (by LOI) are common (Dean and Gorham, 1998). The organic matter content in Grahamstown Dam constituted the second highest proportion of sediment components (Table 6.1) and is similar to that in eutrophic lake sediments. However, heterotrophic decomposition not only relies on the amount but also on the type of organic matter supplied. Often, autochthonous C, e.g. deriving from phytoplankton blooms, increases bacterial activity, while allochthonous C, deriving from mainly terrestrial, vascular plants, is resistant to mineralisation and is buried in the sediments (Gudasz et al., 2012; Keuskamp et al., 2013). The ratio of organic C:N in sediments can be used to distinguish between both types of C. Values lower than 10 are typical for autochthonous material while allochthonous C typically has ratios between 20 to 30 (Meyers and Ishiwatari, 1993). Sediment C content was measured as TC which includes inorganic C (carbonates) and organic C. According to the elemental composition at Site 1, carbonate C (based on the assumption that Ca was originally present as $CaCO_3$ and using Ca from the amount of CaO determined in the XRF analysis as the base of calculations) would have been less than 3 % of TC. Assuming an error of 3 %, the C:N ratio would be 11 ± 0.33 to 15 ± 0.45 in Grahamstown Dam sediments (depending on site and sample), suggesting a mixture of organic matter from both sources. Also, the fact that P and Fe release were enhanced by mineralisation products suggests that a large proportion of the C in the sediments of Grahamstown Dam is not bioavailable and hence, possibly not mineralised to a great extent.

Microbial pathways of P remobilisation other than the dissolution of P by catalysing the reduction of metal oxyhydroxides, have been described: bacteria may release cellular P, stored during oxic conditions as polyphosphates, in periods of anoxic stress (Hupfer et al., 2007) or P may derive from the utilisation of phosphatase enzymes to overcome C limitation (Steenbergh et al., 2011). These processes cannot explain the P release observed in the carbon limitation experiment. If anoxic stress was responsible, P release should have been observed in all treatments and also in the anoxic treatments in previous experiments (chapter 5). Additional substrate increased P release which does not support phosphatase activity due to C limitation. Increased phosphatase activity could however have

led to P release. Relief of C limitation would have increased the energy available for bacterial growth which in turn would have increased bacterial demand for P. Phosphatase activity could have led to the increase of P concentration in the overlying water column in the sediment incubation chambers. The stagnation of P release after 14 days could be explained by satisfaction of bacterial P needs, depletion of the C source or abiotic P sorption onto the sediments.

The amount of P the sediments released, seems to be comparatively small. The highest net release of srP was 0.027 mg/L in the formate treatment after 14 days of incubation. Amounts of srP recorded by Mitchell (2002) were approximately 30 times higher, for example. Even without additional bioavailable C, P release from the sediments in anoxic conditions is often much higher than measured in this study. Burger et al. (2007) reported srP concentrations between 0.2 and 0.6 mg/L after 24 to 48 hours of in situ sediment incubations in Lake Rotorua, and Malecki et al. (2004) found concentrations of srP release between 0.5 and 2.0 mg/L in sediment cores from the St. Johns River in 25 day incubation experiments.

Obviously, the size of the P pool in the sediments influences how much P can potentially be remobilised. Accordingly, sediments from the St. Johns River and Lake Rotorua sediments contained two to three times as much TP (between 0.88 and 1.34 g/kg (Malecki et al., 2004) and around 1.1 to 1.7 g/kg (Hickey and Gibbs, 2009), respectively) than the ones at Site 1 in Grahamstown Dam (0.42$\pm$0.01 g/kg, Table 6.1). Content of P_20_5 was 0.13 % in the sediments examined by Mitchell (2002) while 0.09 % were found in Grahamstown Dam at Site 1 (Table 6.1). In contrast to Lake Rotorua or the St. Johns River, Grahamstown Dam has not received high external nutrient loads in the past decades and thus, it is not surprising that the P content in its sediments is lower.

Aside from the amount of P in the sediments, it is possible that the form it is bound in might have inhibited a greater release. Other than Fe oxyhydroxides which are a main binding partner in the classical Fe and P cycling model, P may adsorb to Al, Mn or calcium minerals or be present in organic form. Al hydroxide has the capacity to adsorb remobilised P (Kopáček et al., 2007; Rydin, 2000) and P has been observed to bond with Al rather than Fe if both metals and P are precipitated (Ulrich and Pöthig, 2000). In contrast to Fe oxyhydroxides, Al hydroxide

is not redox sensitive, i.e. P precipitated with Al hydroxides will not dissolute in anoxic conditions (Ulrich and Pöthig, 2000). It has been shown that molar ratios of Al:Fe larger than three or Al:P larger than 25 prevent the release of P from the sediments (Kopáček et al., 2005; Lake et al., 2007). Ratios of Al:Fe were smaller than 3 for all three sites sampled in Grahamstown Dam but Al:P were 105, 86 and 130 for Site 1, 2, and 3, respectively, indicating that Al precipitates may inhibit the release of P in Grahamstown Dam. In conclusion, a combination of low P content, high Al content and C limitation of bacterial P regeneration processes is likely to be responsible for the small amount of P release from Grahamstown Dam sediments.

In contrast to Fe and P, NH_4^+ and NO_x cycling did not seem to be influenced by C additions to a large extent. Only glucose seemed to have a slowing effect on NH_4^+ release. Similarly, Mitchell (2002) found that glucose additions caused the highest uptake and lowest release of NH_4^+ from wetland sediments. In contrast, she found that NH_4^+ was first taken up by the sediments in all treatments and released again after three days, however, final values were smaller than initial ones.

Initial concentrations of NH_4^+ were at least four times higher (3.5 mg N per one litre microcosm) than in the C limitation experiment (0.800$\pm$0.024 mg N/L) while final concentrations were similar in both experiments (approximately 2.1 mg N/L). These differences may be due to different oxygen concentrations near the sediment surface. The wetland sediments were observed to be anaerobic most of the time (Mitchell, 2002) which may lead to high NH_4^+ concentrations in the pore water in the surface layer of the highly organic sediments. In contrast, Grahamstown Dam is subject to frequent mixing of the water column which would keep the surface layer of the sediments better oxygenated leading to potentially lower concentrations of NH_4^+ in the pore water. According to the temperature profile in Figure 5.2, the sediments for the C limitation experiment were collected just after a mixing event (27/10/2011) which may have oxygenated the surface sediments. Thus, the sediments used in the C limitation experiment may initially not have contained as much NH_4^+ in the pore water as the ones used in Mitchell (2002)'s experiment, resulting in lower NH_4^+ concentrations on day 0.

The pattern of NH_4^+ release in the C limitation experiment was also very similar to the one observed in previous experiments (chapter 5). Pore water concentrations of NH_4^+ reflected the concentration of NH_4^+ released into the supernatant in the incubation chambers. This further supports that NH_4^+ cycling was not affected by bacterial processes relying on availability of organic C substrate and was more likely due to mineralisation.

While NO_x accumulated in the anoxic treatment in previous experiments, this was only recorded in one replicate of the control in the C limitation experiment. The decrease of initially present NO_x in the supernatant in the incubation chambers could have been due to denitrification. Mitchell (2002) observed similar patterns in nitrate release in her experiment (nitrite was mostly below the detection limit): a preexisting amount of nitrate (0.035 to 0.056 mg N per one litre microcosm) was taken up by the sediments during the course of the experiment in all treatments except the glucose one. Initial concentrations of nitrate in Mitchell (2002)'s experiment were four times lower than initial NO_x concentrations in the C limitation experiment. This again may be due to oxygenation of the sediments from Grahamstown Dam. As nitrate is quickly reduced in anaerobic conditions one would expect to find higher nitrate concentrations released from the potentially better oxygenated sediments from Grahamstown Dam.

Pore water concentrations of NO_x were lower than initial concentrations in the supernatant in the C limitation experiment, indicating that denitrification took place in lower parts of the sediments, possibly unaffected by organic C availability. Thus, it is possible that additional organic substrate could have stimulated denitrification at the sediment surface in the incubation chambers (as speculated in section 5.4). This would not have affected the control and according to results in chapter 5, a release of NO_x in the control was expected. However, as the latter was not observed, results regarding the stimulation of denitrification by additional C sources in the C limitation experiment are inconclusive.

Concentrations of dissolved TN could not be used as an indicator for presence of organic and inorganic N in this experiment as they were lower than concentrations of NH_4^+ in the supernatant in the incubation chambers. This was due to problems with the persulfate digestion of samples. The check standard used for

the process (urea) indicated that recovery was about 80 % of original concentrations.

Mineral content, low release rates only during external stimulation of microbiological processes and low srP concentrations in the sediment pore water (Table 6.1) indicate that the sediments are not usually a source of P to the water column. It is conceivable that the opposite may be the case, i.e. that the sediments act as a P sink. Grahamstown Dam is a comparably young reservoir, constructed solely for the purpose of drinking water supply at the site of a former wetland between 1956 and 1965 (Cole and Williams, 2011). The lake's catchment is largely forested and not densely populated. Nutrient loading would have been much smaller compared with many other lakes that store large amounts of P in their sediments as they received urban or agricultural run off or were used as receptacles for sewage. Thus, it is possible that the potential of the sediments to retain P, e.g. through microbial demand or mineral adsorption, exceeds their tendency to release P.

The potential of the sediments to adsorb P could be examined in adsorption isotherm experiments. These experiments have been frequently used to determine suitability of clay minerals to remove P in waste water treatment (Yan et al., 2010; Shanableh and Elsergany, 2013). Sorption capacities of clay minerals for P are determined by exposing the minerals to different concentrations of dissolved P and measuring the P concentrations in the solution over time. The adsorption isotherms demonstrate the relationship between the concentration of P in solution and the amount of P sorbed onto the clay at equilibrium for different concentrations of P. Different models, e.g. Langmuir or Freundlich equations (Duff et al., 1988; Potgieter, 1991), can be fitted to the data to estimate maximum adsorption capacity of the minerals tested.

Chapter 7

General discussion and conclusion

7.1 Summary and discussion

The aim of this thesis was to gain a detailed understanding of the role of the main algal nutrients N and P for phytoplankton growth and in particular, cyanobacterial growth, in Grahamstown Dam. From this understanding, suggestions for improvement of management practices of nutrients and phytoplankton were to be derived.

The aim was approached by nutrient enrichment assays on two different spatial and temporal scales to satisfy the need to find the limiting nutrient for algal and cyanobacterial growth in the lake. The results of these investigations led to the examination of the interaction of nutrient enrichment and other factors that affect algal growth: light availability and trace metal nutrients. Some growth characteristics of the potentially toxic cyanobacterium *A. circinalis* which is present in Grahamstown Dam and which often dominates toxic blooms in Australia were determined in the laboratory. As a potential source of nutrients, the lake sediments were examined for nutrient flux under conditions that are likely to occur during periods of persistent thermal stratification. The outcome of those experiments inspired the examination of the effects of organic C on microbial nutrient cycling within the sediments.

The main findings of this thesis are summarised in the following:

Chapter 2:

- Phytoplankton biomass was colimited by N and P in Grahamstown Dam at

most times except on some occasions in winter. Genera of the Chlorophyceae and Bacillariophyceae, which represented a major part of the phytoplankton assemblage, and some non toxic colonial cyanobacteria were particularly stimulated by additions of both nutrients.

- Time played an important role in the development of a growth response by individual genera. Extending the monitoring time after nutrient enrichment was crucial in detecting a significant response to either P on its own or P and N in combination for the slow growing, potentially toxic cyanobacteria *Anabaena* and *Aphanizomenon*.

Chapter 3:

- High levels of surface irradiance (90 %) as well as trace metal additions interacted with nutrient additions at the phytoplankton assemblage level. Both high irradiance levels and increased trace metal availability combined with additions of N and P resulted in a significant increase in phytoplankton biomass.
- Responses of cyanobacterial genera to irradiance levels and trace metal additions were more differentiated than assemblage responses. For some genera, trace metal and nutrient additions interacted and led to an increased growth response (e.g. *Aphanocapsa*), while for others trace metal additions were more important than additional N or P (e.g. *Aphanizomenon*).
- Lower levels of irradiance (25 %) enhanced the growth of *Anabaena* while there was no apparent effect of different levels of light availability on other cyanobacteria.

Chapter 4:

- Growth of *A. circinalis* depended on a high external N supply in combination with any concentration of P offered and, contrary to expectations, did not depend on the N:P supply ratio.
- The cyanobacterium's abundance increased significantly due to high N concentrations in combination with any concentration of P while its growth rates were not affected.
- Higher irradiance levels had an exacerbating effect on this result. Yield and growth rate increased significantly due to high light availability and high

supply of N and some P.

Chapter 5 and 6:

- Periods of persistent stratification occurred frequently during spring and summer lasting for up to 23 days.
- The sediments were identified as sources of nutrients in particular under conditions that are likely to occur during persistent thermal stratification, i.e. low oxygen availability. The type and amount of nutrient released depended on the presence of oxygen but also on the availability of organic C.
- N was released especially during the absence of oxygen. Absence of oxygen was a prerequisite for the release of Fe but Fe release was also highly limited by the availability of organic C.
- There was hardly any flux of P from the sediments under oxic or anoxic conditions. This was probably due to the limitation of microbial processes by organic C and the relatively small sedimentary pool of P.
- P and Fe were not released concomitantly and thus, were unlikely to be bound in the same compound in the sediments.

Results of chapters 2 and 3 imply that it is difficult to link potential prolific cyanobacterial growth to a pulse of nutrient enrichment alone in Grahamstown Dam. Although cyanobacterial growth was stimulated by nutrient additions in some of the in situ assays, abundances of potentially toxic cyanobacteria remained low. *Anabaena* and *Aphanizomenon* responded first to additions of P and N in combination and later to P only in the mesocosm experiments conducted in chapter 2. While there was a distinct response by both genera, they did not achieve dominance of the phytoplankton assemblage. Biovolumes of *Anabaena* remained below the notification level (1.5 mm^3/L) suggested by the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011) throughout the experiments.

These results do not mean that nutrient enrichment will not induce cyanobacterial blooms in the lake. It is still possible that a more gradual increase in nutrient loading (e.g. over months or years) would change the phytoplankton assemblage towards cyanobacterial dominance. In particular, the responses of

Anabaena and *Aphanizomenon*, recorded at the end of the mesocosm experiment, hint at that scenario.

Persistent stratification is associated with toxic cyanobacterial blooms in Australia, especially blooms of *A. circinalis*. For example, Mitrovic et al. (2003) found that extended periods of persistent thermal stratification promoted *A. circinalis* growth. *Anabaena* and *Aphanizomenon* biovolumes increased in the mesocosm experiments after 12 days. Further, *Aphanizomenon* was stimulated by high irradiance levels as would occur during stratified conditions (chapter 3). This suggests that these usually slow growing genera may need prolonged periods of thermal stratification in combination with nutrient enrichment to gain a competitive advantage over other phytoplankton in the lake.

Periods of persistent stratification occur for up to 23 days in Grahamstown Dam (chapter 5). *Anabaena* and *Aphanizomenon* growth rates periodically reached up to 0.85 ± 0.19 day^{-1} (mean$\pm$ standard error) and 1.22 ± 0.28 day^{-1}, respectively, in the P treatment in the January assay. Assuming an initial biovolume of 0.001937 mm^3/L (equivalent to 10 cells/ml for *Anabaena*) at a time when growth conditions were ideal, a growth rate of 0.85 day^{-1} would lead to an increase of more than 1.6 mm^3/L within 11 days and more than 5.7 mm^3/L within 14 days, exceeding the notification level (1.5 mm^3/L) and alert level (5 mm^3/L) recommended by the Australian Drinking Water Guidelines (NHMRC and NRMMC, 2011) for *A. circinalis*. Thus, the observed period of persistent thermal stratification is relevant for *Anabaena* and *Aphanizomenon* growth in Grahamstown Dam and may lead to cyanobacterial growth that would require management action.

Although mesocosm assays in chapter 2 covered a relevant time period, they did not address conditions that occur during persistent stratification (i.e. mixing occurred within the mesocosm bags). While chapter 3 examined improved light climate during stratification events, it did not extend to a time period that may be relevant for *Anabaena* and *Aphanizomenon*. In order to confirm that persistent thermal stratification for extended periods (weeks) in combination with increased nutrient availability would lead to excessive *Anabaena* and *Aphanizomenon* growth, further research would need to be conducted.

Chapter 5 revealed that N release was highest within the first four to seven

days of reduced oxygen availability to the sediments, implying that short periods of thermal stratification would be enough to increase nutrient concentration in the overlying water column. Further, sediment organic matter content was high (chapter 6) and oxygen consumption of the sediments used in oxic treatments (chapter 5, section 5.2.1) occurred rapidly (within minutes), suggesting that anoxic conditions would set in soon after thermal stratification became stable. Periods of persistent thermal stratification were frequently interrupted by mixing of the entire water column. This may increase nutrient availability in the euphotic zone and may promote cyanobacterial growth during a period of prolonged, i.e. longer than 12 days, thermal stratification. Thermal stratification mainly occurs during the phytoplankton growing season, i.e. spring and summer, which may be a further contributing factor to cyanobacterial growth.

Supply of P was important for *Anabaena* and *Aphanizomenon* growth in the mesocosm assays and availability of organic C stimulated the release of P from the sediments during anaerobic conditions. Concentrations of srP increased up to 14 times due to organic C additions (the net increase was 0.027 ± 0.015 mg P/L). Thus, enhanced availability of organic C to the sediments may lead to increased P release and potentially promote *Anabaena* and *Aphanizomenon* growth.

From a management perspective any mechanisms that may deposit organic C or increase the availability of C sources and thus stimulate the P release from the sediments should be considered. A potential source of organic C may be water pumped into the lake from the Williams River weir pool. High inputs of dissolved organic C, as commonly associated with high river flows and floods (Buffam et al., 2001; Westhorpe and Mitrovic, 2012), may accumulate in the weir pool due to flooding upstream in the Williams River. If pumped across into the reservoir, these C sources might stimulate P release from the sediments. A similar effect may be expected from increased suspended solids and nutrient concentrations in the weir pool which may enhance phytoplankton growth. As phytoplankton derived dissolved organic C can stimulate bacterial activity in the sediments (Goedkoop et al., 1997; Autio et al., 2003), the organic C derived from algal blooms in the weir pool may also stimulate P release. Both scenarios would require further investigation.

Although this thesis holds some limitations, as described above, new insights into phytoplankton and nutrient dynamics were obtained.

Firstly, P on its own was not the limiting nutrient for phytoplankton growth in Grahamstown Dam. This finding is not in accordance with the long lasting paradigm of P limitation of freshwater systems (Hecky and Kilham, 1988). Instead, it supports criticism of the paradigm and recent suggestions to revise it (Elser et al., 2007; Sterner, 2008; Lewis and Wurtsbaugh, 2008). It has been proposed that nutrient limitation is strongly dependent on timescale and, more specifically, that in the longterm phytoplankton biomass would be limited by P (Davies et al., 2004, 2010). Experiments in chapter 2 could not confirm this hypothesis. However, it is still possible that if experimental timescale is increased beyond that of the mesocosm assays, i.e. to months or years, P might turn out to be the limiting nutrient.

The strong dominance of colimitation of phytoplankton biomass and especially of individual genera does not conform to an even older paradigm, Liebig's law of the minimum. Despite being originally conceived to describe growth of monocultured crop plants (as described by de Baar (1994)), the law is now often applied to phytoplankton assemblages. One physiological explanation for nutrient colimitation at the genus level may be found in physiological plasticity which widens the range of N:P ratio an organism can grow in (Schade et al., 2005). If the individual range of N:P ratios is broad and nutrient additions fall within this range, colimitation may be observed. Whether this mechanism is responsible for the response of individual algal genera in Grahamstown Dam could be tested in further nutrient limitation experiments, applying a range of N:P ratios.

Davies et al. (2004) argue that colimitation reflected the immediate nutrient status of phytoplankton and the term nutrient codeficiency would be more appropriate than colimitation. It is possible that the frequent mixing in a shallow lake and thus, frequently changing conditions within the water column could lead to this status of codeficiency, especially if nutrient levels are moderate to low. Examining other shallow lakes with similar conditions might give insight into this phenomenon.

Regarding cyanobacterial growth, specifically the growth of N fixing poten-

tially toxic *A. circinalis*, results were not as expected. The amount of external N supplied determined the cyanobacterium's abundance under different light regimes. This is somewhat in contrast to outcomes of the field experiments conducted in chapter 2 and 3 where the biovolume of two N fixing cyanobacterial genera was more likely to be determined by P than by N. A comparison of the outcome of these two types of experiment may not be appropriate for several reasons. Conditions at the start of field (chapter 2 and 3) and laboratory experiments (chapter 4) were inversed: while the natural phytoplankton assemblage was exposed to an increase in supplied nutrients at the beginning of the field experiments, cultures of *A. circinalis* were placed from the high nutrient culture medium into the test medium with much lower, albeit environmentally realistic, concentrations. Moreover, *A. circinalis* was not exposed to competition in the laboratory assays and scale and environmental conditions were quite different in both types of assay.

New findings were also obtained in the examination of nutrient release processes in the sediments of Grahamstown Dam. In particular, a better understanding of P and Fe cycling was achieved. The most popular explanation for anoxic P release in lake sediments, inspired by Mortimer (1941), states that P and Fe are bound to the same compounds in lake sediments that, when reduced in anoxic conditions, will release P and Fe. Its simplicity and logic make this explanation attractive which possibly led to it being reiterated in the literature. However, chapters 5 and 6 showed that anoxic release of P and Fe were not linked and P and Fe were not likely to be bound to the same compounds in the sediments of Grahamstown Dam. Previous studies have confirmed that Fe and P release can be independent from each other (Mitchell, 2002; Baldwin and Williams, 2007) which shows that the reality is rather more complicated.

In contrast to other shallow lakes, the amount of P in the sediments was comparatively small (Malecki et al., 2004; Christophoridis and Fytianos, 2006; Hickey and Gibbs, 2009) indicating that the sediments are not a main source of P and management of external P sources may be sufficient in preventing algal blooms.

7.1.1 Recommendations for future research

The findings and limitations of this thesis, summarised and discussed in the previous sections, elicit further research questions. Addressing those questions would deepen the understanding of the phytoplankton ecology in Grahamstown Dam and further assist with management of cyanobacteria. Explicit suggestions for future research are:

Further examination of timescale dependence of nutrient limitation:

- test the effect of a slowly increasing nutrient load over time instead of one single pulse on phytoplankton nutrient limitation and
- extend the experimental period (months instead of weeks) to test if P will become the limiting nutrient.

Closer examination of colimitation:

- test if colimitation is the result of physiological plasticity of individual genera as suggested by Schade et al. (2005) and thus evident over a range of N:P ratios,
- examine if nutrient colimitation can occur across all phytoplankton genera if nutrient concentrations fall within their individual range of N:P ratios and
- investigate if colimitation is typical for shallow, i.e. well mixed, lakes with low to moderate nutrient levels.

Closer examination of *Anabaena* and *Aphanizomenon* growth:

- test the effect of thermal stratification, nutrient enrichment and time on the growth of *Anabaena* and *Aphanizomenon* in Grahamstown Dam and
- determine which trace metals in particular stimulate *Aphanizomenon* growth.

Further sediment examinations:

- determine if the sediments are a sink for P in adsorption isotherm experiments,
- determine if nutrients released from the sediments during periods of thermal stratification reach the euphotic zone,
- examine the effect of water extraction from the Williams River on sediment resuspension and nutrient release.

Define evidence based nutrient thresholds for water extraction:

- determine the minimum concentrations of a pulse of N and P that can in-

crease phytoplankton growth or that increases phytoplankton growth to undesired levels and

- determine dilution rates of water pumped into the lake from the Williams River.

7.1.2 Implications for management of algal and cyanobacterial growth in Grahamstown Dam

The outcome of experiments in this thesis suggest that in order to prevent excessive algal growth and minimise the risk of cyanobacterial blooms in Grahamstown Dam, certain management strategies would be advisable.

Results from chapter 2, 3 and 4 imply that N is equally or in some cases even more important than P for algal and cyanobacterial growth. Moreover, the sediments were found to be a source of N to the water column, in particular during anoxia which is likely to occur during persistent thermal stratification. Thus, N released from the sediments may become available to the phytoplankton. However, the current management plan for water quality and water extraction from the Williams River is based on P and cyanobacterial abundances (Cole and Williams, 2011). As a preventative measure, it would be recommendable to include N in the plan as well. Periods for water extraction from the Williams River and volumes of water taken, may need to be adjusted according to N concentrations as well as P concentrations. For this purpose, it would be useful to experimentally define threshold concentrations of N and P species that are bioavailable for phytoplankton growth (e.g. nitrate and orthophosphate) as well as concentrations of total nutrient.

It is not known if nutrients, in particular N, released from the sediments during persistent thermal stratification reach the euphotic zone. As a precautionary measure, it would be advisable to monitor water column temperature in order to identify thermal stratification events and monitor nutrient release from the sediments, in particular the release of N, during these events. In turn this implies that investigating the nutrient dynamics within the water column during thermal stratification and subsequent mixing would be useful. Further, it would be sensible to reduce P input or at least maintain it at a current level to prevent its

accumulation in the sediments which currently contain little P.

As nutrient enrichment alone did not evoke excessive growth of the potentially toxic cyanobacteria that are present in the lake, it would be highly advisable to conduct further research into potential cofactors for cyanobacterial blooms in the lake. These include: examination of the combined effect of thermal stratification, nutrient enrichment and time (i.e. time frames that are relevant for thermal stratification and growth of *Anabaena* and *Aphanizomenon*); identification of the trace metal which affected *Aphanizomenon* and if there are any potential sources in the reservoir or its catchment that would require management; determination of the minimum amount of added N and P that leads to a phytoplankton growth response and investigation of the capacity of the sediments to bind P.

7.2 Conclusion

Most studies of shallow lakes have examined eutrophic inland lakes in Europe and North America that have often been subject to ongoing cultural eutrophication. Less information exists on shallow lakes that have not undergone excessive nutrient enrichment. In this thesis, a coastal, moderately nutrient enriched, shallow lake was examined in regards to phytoplankton nutrient limitation and sediment nutrient cycling.

Major findings were made: Phytoplankton colimitation prevailed in Grahamstown Dam and the growth response of potentially toxic cyanobacteria was slower than the response of other algae. Also, abundances of a prominent toxic cyanobacterium were determined by concentration of N and not by the nutrient ratio or by concentration of P in laboratory assays. Further, the sediments were a source of N, in particular during periods of low oxygen concentrations as can occur during thermal stratification. Thus, persistent thermal stratification is likely to affect the cyanobacterial response in a positive way by increasing the availability of N to the water column and by providing an improved light climate. The release of P from the sediments was due to microbial activity and thus, not solely controlled by absence of oxygen but by availability of dissolved organic C. Moreover, P release and Fe release from the sediments were independent from each other.

These findings confirm the need to investigate each water body individually for potential causes of algal and cyanobacterial blooms. Moreover, they contribute to the knowledge of phytoplankton and sediment dynamics in moderately enriched shallow coastal lakes and imply that further research on this type of lake is required.

Bibliography

Al-Tebrineh, J., C. Merrick, D. Ryan, A. Humpage, L. Bowling, and B. A. Neilan (2012). Community composition, toxigenicity, and environmental conditions during a cyanobacterial bloom occurring along 1,100 kilometers of the Murray River. *Applied and Environmental Microbiology 78*(1), 263–272. 2, 80

Amirbahman, A., A. R. Pearce, R. J. Bouchard, S. A. Norton, and J. S. Kahl (2003). Relationship between hypolimnetic phosphorus and iron release from eleven lakes in Maine, USA. *Biogeochemistry 65*, 369–386. 125

Andersen, F. Ø. and P. Ring (1999). Comparison of phosphorus release from littoral and profundal sediments in a shallow, eutrophic lake. *Hydrobiologia 408*, 175–183. 99, 112

Andersen, J. M. (1982). Effect of nitrate concentration in lake water on phosphate release from the sediment. *Water Research 16*, 1119–1126. 113

APHA (Ed.) (1995). *Standard Methods for the Examination of Water and Wastewater* (19 ed.). American Public Health Association/American Water Works Association/Water Pollution Control Federation: Washington, DC. 42, 86, 101, 119

Autio, L., K. Mäkelä, K. K. Lethonen, J. Kuparinen, and H. Haahti (2003). Effects of algal sedimentation and *Monoporeia affinis* on nutrient fluxes, pore water profiles and denitrification in sediment microcosms. *Boreal Environment Research 8*, 229–243. 117, 136

Azevedo, S. M. F. O., N. Chernoff, I. R. Falconer, M. Gage, E. D. Hilborn, M. J. Hooth, K. Jensen, R. MacPhail, E. Rogers, G. R. Shaw, and I. Stewart (2008). Chapter 28: Cyanobacterial poisoning in livestock, wild mammals and birds - an overview. In *International Sym-*

posium on Cyanobacterial Algal Blooms - State of the Science and Research Needs, http://www.epa.gov/cyanohabssymposium/monograph.html, accessed 7/7/2010. 8

Backer, L. C., S. V. McNeel, T. Barber, B. Kirkpatrick, C. Williams, M. Irvin, Y. Zhou, T. B. Johnson, K. Nierenberg, M. Aubel, R. LePrell, A. Chapman, A. Foss, S. Corum, V. R. Hill, S. M. Kieszak, and Y.-S. Cheng (2010). Recreational exposure to microcystins during algal blooms in two California lakes. *Toxicon 55*, 909–921. 7

Baker, P. D. and A. R. Humpage (1994). Toxicity associated with commonly occurring cyanobacteria in surface waters of the Murray-Darling Basin, Australia. *Australian Journal of Marine and Freshwater Research 45*, 773–786. 80

Baldwin, D. S., A. M. Mitchell, and J. M. Olley (2002). *Agriculture, Hydrology and Water Quality*, Chapter 12. CABI International Press, Wallingford. 20, 21, 22, 23, 99

Baldwin, D. S., G. N. Rees, A. M. Mitchell, and G. Watson (2005). Spatial and temporal variability of nitrogen dynamics in an upland stream before and after a drought. *Marine and Freshwater Research 56*, 457–464. 100

Baldwin, D. S. and J. Williams (2007). Differential release of nitrogen and phosphorus from anoxic sediments. *Chemistry and Ecology 23*(3), 243–249. 23, 25, 112, 138

Beardall, J., E. Young, and S. Roberts (2001). Approaches for determining phytoplankton nutrient limitation. *Aquatic Sciences 63*, 44–69. 13, 15

Beltran, E. C. and B. A. Neilan (2000). Geographical segregation of the neurotoxin-producing cyanobacterium *Anabaena circinalis*. *Applied and Environmental Microbiology 66*(10), 4468–4474. 80

Berg, U., T. Neumann, D. Donnert, R. Nuesch, and D. Stuben (2004). Sediment capping in eutrophic lakes - efficiency of undisturbed calcite barriers to immobilize phosphorus. *Applied Geochemistry 19*(11), 1759–1771. 27

Bergström, A.-K., A. Jonsson, and M. Jansson (2008). Phytoplankton responses to nitrogen and phosphorus enrichment in unproductive Swedish lakes along a gradient of atmospheric nitrogen deposition. *Aquatic Biology 4*, 55–64. 35, 36, 59

Berman, T. (2001). The role of DON and the effect of N:P ratios on occurrence of cyanobacterial blooms: Implications from the outgrowth of *Aphanizomenon* in Lake Kinneret. *Limnology and Oceanography 46*(2), 443–447. 18

Beutel, M. W. (2006). Inhibition of ammonia release from anoxic profundal sediments in lakes using hypolimnetic oxygenation. *Ecological Engineering 28*(3), 271–279. 24, 25, 28

Beutel, M. W., A. J. Horne, W. D. Taylor, R. F. Losee, and R. D. Whitney (2008). Effects of oxygen and nitrate on nutrient release from profundal sediments of a large, oligo-mesotrophic reservoir, Lake Mathews, California. *Lake and Reservoir Management*. 23, 25, 99, 112, 113

Beutel, M. W., T. M. Leonard, S. R. Dent, and B. C. Moore (2008). Effects of aerobic and anaerobic conditions on P, N, Fe, Mn, and Hg accumulation in waters overlaying profundal sediments of an oligo-mesotrophic lake. *Water Research*. 112

Bianchi, T. S., E. Engelhaupt, P. Westman, T. Andren, C. Rolff, and R. Elmgren (2000). Cyanobacterial blooms in the Baltic Sea: Natural or human-induced. *Limnology and Oceanography 45*(3), 716–726. 5

Blomqvist, P., A. Pettersson, and P. Hyenstrand (1994). Ammonium-nitrogen - a key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems. *Archiv für Hydrobiologie 132*(2), 141–164. 18

Boers, P. C. M. (1991). The influence of pH on phosphate release from lake sediment. *Water Research 25*(3), 309–311. 21, 22

Bormans, M., P. W. Ford, and L. Fabbro (2005). Spatial and temporal variability in cyanobacterial populations controlled by physical processes. *Journal of Plankton Research 27*, 61–70. 19

Boström, B., J. M. Andersen, S. Fleischer, and M. Jansson (1988). Exchange of phosphate across the sediment-water interface. *Hydrobiologia 170*, 229–244. 20, 22, 24, 30, 98, 99, 125

Boström, B. and K. Pettersson (1982). Different patterns of phosphorus release from lake sediments in laboratory experiments. *Hydrobiologia 92*, 415–429. 113

Bowling, L. C. (1994). Occurrence and possible causes of a severe cyanobacterial bloom in Lake Cargelligo, New South Wales. *Australian Journal of Marine and Freshwater Research 45*, 737–745. 2, 5, 19, 35, 80

Bowling, L. C. and P. D. Baker (1996). Major cyanobacterial bloom in the Barwon-Darling River, Australia, in 1991, and underlying limnological conditions. *Marine and Freshwater Research 47*, 643–657. 1, 80

Bradley, P. M., J. M. Fernandez, and F. H. Chapelle (1992). Carbon limitation of denitrification rates in an anaerobic groundwater system. *Environmental Science and Technology 26*, 2377–2381. 111, 117

Bradley, P. M., P. B. McMahon, and F. H. Chapelle (1995). Effects of carbon and nitrate on denitrification in bottom sediments of an effluent-dominated river. *Water Resources Research 31*(4), 1063–1068. 111, 117

Brauer, V. S., M. Stomp, and J. Huisman (2012). The nutrient-load hypothesis: Patterns of resource limitation and community structure driven by competition for nutrients and light. *The American Naturalist 179*(6), 723–740. 62

Brettar, I. and G. Rheinheimer (1992). Influence of carbon availability on denitrification in the central Baltic Sea. *Limnology and Oceanography 6*(6), 1146–1163. 111

Brouwer, E. and J. G. M. Roelofs (2002). Oligotrophication of acidified, nitrogen-saturated softwater lakes after dredging and controlled supply of alkaline water. *Archiv für Hydrobiologie 155*(1), 83–97. 29

Bruggeman, J. (2011). A phylogenetic approach to the estimation of phytoplankton traits. *Journal of Phycology 47*(1), 52–65. 96

Buffam, I., J. N. Galloway, L. K. Blum, and K. J. McGlathery (2001). A stormflow/baseflow comparison of dissolved organic matter concentrations and bioavailability in an Appalachian stream. *Biogeochemistry 53*, 269–306. 136

Buikema, W. J. and R. Haselkorn (1991). Characterization of a gene controlling heterocyst differentiation in the cyanobacterium *Anabaena* 7120. *Genes & Development 5*, 321–330. 94

Bulgakov, N. G. and A. P. Levich (1999). The nitrogen:phosphorus ratio as a factor regulating phytoplankton community structure. *Archiv für Hydrobiologie 146*(1), 3–22. 15, 16

Burford, M. A., K. L. McNeale, and F. J. McKenzie-Smith (2006). The role of nitrogen in promoting the toxic cyanophyte *Cylindrospermopsis raciborskii* in a subtropical water reservoir. *Freshwater Biology 51*, 2143–2153. 18

Burger, D. F., D. P. Hamilton, J. A. Hall, and E. F. Ryan (2007). Phytoplankton nutrient limitation in a polymictic eutrophic lake: Community versus species-specific responses. *Archiv für Hydrobiologie*. 36, 54, 56

Burger, D. F., D. P. Hamilton, C. A. Pilditch, and M. M. Gibbs (2007). Benthic nutrient fluxes in a eutrophic, polymictic lake. *Hydrobiologia*. 98, 112, 128

Caraco, N. F., J. J. Cole, and G. E. Likens (1989). Evidence for sulphate-controlled phosphorus release from sediment of aquatic systems. *Nature 341*, 316–318. 23

Carignan, R. and D. Planas (1994). Recognition of nutrient and light limitation in turbid mixed layers: Three approaches compared in the Parank floodplain (Argentina). *Limnology and Oceanography 39*(3), 580–596. 37

Carmichael, W. (2008). Chapter 4: A world overview – one-hundredtwenty-seven years of research on toxic cyanobacteria – where do we go from here? In *International Symposium on Cyanobacterial Harmful Algal Blooms (ISOC-HAB)*, http://www.epa.gov/cyanohabssymposium/monograph.html, accessed 7/7/2010. 1, 5

Carmichael, W. W., S. M. F. O. Azevedo, J. S. An, R. J. R. Molica, E. M. Jochimsen, S. Lau, K. L. Rinehart, G. R. Shaw, and G. k. Eaglesham (2001). Human fatalities from cyanobacteria: Chemical and biological evidence for cyanotoxins. *Environmental Health Perspecitves 109*(7), 663–668. 6

Carpenter, S. R. (1996). Microcosm experiments have limited relevance for community and ecosystem ecology. *Ecology 77*(3), 677–680. 36

Carpenter, S. R. (2005). Eutrophication of aquatic ecosystems: Bistability and soil phosphorus. *Proceedings of the National Academy of Sciences 102*(29), 10002–10005. 10

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications 8*(3), 559–568. 10, 26

Carvalho, L., C. McDonald, C. de Hoyos, U. Mischke, G. Phillips, G. Borics, S. Poikane, B. Skjelbred, A. L. Solheim, J. V. Wichelen, and A. C. Cardoso (2013). Sustaining recreational quality of European lakes: Minimizing the health risks from algal blooms through phosphorus control. *Journal of Applied Ecology 50*, 315–323. 62, 81

Chen, F., Q. Xia, and L.-K. Ju (2006). Competition between oxygen and nitrate respirations in continuous culture of *Pseudomonas aeruginosa* performing aerobic denitrification. *Biotechnology and Bioengeneering 93*(6), 1069–1078. 112

Chen, J., H. Kong, D. Wu, X. Chen, D. Zhang, and Z. Sun (2007). Phosphate immobilization from aqueous solution by fly ashes in relation to their composition. *Journal of Hazardous Materials 139*, 293–300. 29

Christophoridis, C. and K. Fytianos (2006). Conditions affecting the release of phosphorus from surface lake sediments. *Journal of Environmental Quality 35*(4), 1181–1192. 99, 125, 138

Codd, G. A., J. Lindsay, F. M. Young, L. F. Morrison, and J. S. Metcalf (2005). *Harmful Cyanobacteria*, Volume 3, Chapter 1, pp. 1–23. Springer Netherlands. 1, 5

Codd, G. A., J. S. Metcalf, and K. A. Beattie (1999). Retention of *Microcystis aeruginosa* and microcystin by salad lettuce (*Lactuca sativa*) after spray irrigation with water containing cyanobacteria. *Toxicon 37*(8), 1181–1185. 7

Cohen, Y. and M. Gurevitz (2006). *The Prokaryotes - Volume 4: Bacteria: Firmicutes, Cyanobacteria*, Chapter 22. Springer New York. 4

Cole, B. and B. Williams (2011). *Grahamstown Dam Water Quality & Aquatic Ecological Functioning, A compilation of Scientific and Adaptive Management Studies*. 36 Honeysuckle Drive, Newcastle, NSW 2300: Hunter Water Corporation. 38, 61, 99, 114, 131, 140

Cooke, G. D. (2005). *Restoration and Management of Lakes and Reservoirs* (3 ed.). CRC Press. 25, 26, 27, 28, 29, 30

Correll, D. L. (2005). Principles of planning and establishment of buffer zones. *Ecological Engineering 24*, 433–439. 26

Cox, P. A., S. A. Banack, S. J. Murch, U. Rasmussen, G. Tien, R. R. Bidigare, J. S. Metcalf, L. F. Morrison, G. A. Codd, and B. Bergman (2005). Diverse taxa of cyanobacteria produce β-n-methylamino-l-alanine, a neurotoxic amino acid. *Proceedings of the National Academy of Sciences of the United States of America 102*, 5074–5078. 7

Crush, J. R., L. R. Briggs, J. M. Sprosen, and S. N. Nichols (2008). Effect of irrigation with lake water containing microcystins on microcystin content and growth of ryegrass, clover, rape, and lettuce. *Environmental Toxicology 23*(2), 246–52. 7

Danger, M., T. Daufresne, F. Lucas, S. Pissard, and G. Lacroix (2008). Does Liebig's law of the minimum scale up from species to communities? *Oikos 117*(11), 1741–1751. 56

Davelaar, D. (1993). Ecological significance of bacterial polyphosphate metabolism in sediments. *Hydrobiologia 253*, 179–192. 125

Davies, J.-M., W. H. Nowlin, B. Matthews, and A. Mazumder (2010). Temporal discontinuity of nutrient limitation in plankton communities. *Aquatic Sciences 72*, 393–402. 137

Davies, J.-M., W. H. Nowlin, and A. Mazumder (2004). Temporal changes in nitrogen and phosphorus codeficiency of plankton in lakes of coastal and interior British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences 61*(8), 1538–1551. 137

Davies, K. J. P., D. Lloyd, and L. Boddy (1989). The effect of oxygen on denitrification in *Paracoccus denitrificans* and *Pseudomonas aeruginosa*. *Journal of General Microbiology 135*, 2445–2451. 112

Davis, J. R. and K. Koop (2006). Eutrophication in Australian rivers, reservoirs and estuaries - a southern hemisphere perspective on the science and its implications. *Hydrobiologia 559*, 23–76. 10, 11, 26

de Baar, H. J. W. (1994). von Liebig's law of the minimum and plankton ecology (1899-1991). *Progress in Oceanography 33*, 347–386. 36, 56, 137

De Tezanos Pinto, P. and E. Litchman (2010a). Eco-physiological responses of nitrogen-fixing cyanobacteria to light. *Hydrobiologia*. 81

De Tezanos Pinto, P. and E. Litchman (2010b). Interactive effects of N:P ratios and light on nitrogen-fixer abundance. *Oikos*. 5, 17, 59, 63, 77, 96

De Vicente, I., A. Merino-Martos, L. Cruz-Pizarro, and J. de Vicente (2010). On the use of magnetic nano and microparticles for lake restoration. *Journal of Hazardous Materials 181*, 375–381. 29

De Wever, A., K. Muylaert, D. Langlet, L. Alleman, J.-P. Descy, L. Andre, C. Cocquyt, and W. Vyverman (2008). Differential response of phytoplankton to additions of nitrogen, phosphorus and iron in Lake Tanganyika. *Freshwater Biology 53*, 264–277. 63

Dean, W. E. and E. Gorham (1998). Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. *Geology 26*, 535–538. 127

Dodds, W., W. W. Bouska, J. L. Eitzmann, T. J. Pilger, K. L. Pitts, A. J. Riley, J. T. Schlosser, and D. J. Thornbrugh (2009). Eutrophication of U.S. freshwaters: Analysis of potential economic damages. *Environmental Science an& Technology 43*(1), 12–19. 1

Dokulil, M. T. and K. Teubner (2000). Cyanobacterial dominance in lakes. *Hydrobiologia 438*, 1–12. 2

Dokulil, M. T. and K. Teubner (2010). *Eutrophication and Climate Change: Present Situation and Future Scenarios*, Chapter 1, pp. 1–16. Springer Netherlands. 8

Downing, J. A. and E. McCauley (1992). The nitrogen:phosphorus relationship in lakes. *Limnology and Oceanography 37*(5), 936–945. 12

Downing, J. A., S. B. Watson, and E. McCauley (2001). Predicting cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences 58*, 1905–1908. 18

Downs, T. M., M. Schallenberg, and C. W. Burns (2008). Responses of lake phytoplankton to micronutrient enrichment: A study in two New Zealand lakes and an analysis of published data. *Aquatic Science 70*, 347–360. 63

Duff, D. G., S. M. C. Ross, and D. H. Vaughan (1988). Adsorption from solution – an experiment to illustrate the Langmuir adsorption isotherm. *Journal of Chemical Education 65*(9), 815–816. 131

Dzialowski, A. R., S.-H. Wang, N.-C. Lim, J. H. Beury, and D. G. Huggins (2008). Effects of sediment resuspension on nutrient concentrations and algal biomass in reservoirs of the Central Plains. *Lake and Reservoir Management 24*, 313–320. 114

Dzialowski, A. R., S.-H. Wang, N.-C. Lim, W. W. Spotts, and D. G. Huggins (2005). Nutrient limitation of phytoplankton growth in central plains reservoirs, USA. *Journal of Plankton Research 27*(6), 587–595. 56

Edlund, M. B., L. D. Triplett, M. D. Tomasek, and K. Bartilson (2009). From paleo to policy: Partitioning the historical point and nonpoint phosphorus loads to

the St. Croix River, Minnesota-Wisconsin, USA. *Journal of Paleolimnology 41*, 679–689. 10

Edmondson, W. T. and J. T. Lehman (1981). The effect of changes in the nutrient income on the condition of Lake Washington. *Limnology and Oceanography 26*(1), 1–29. 9

Eixler, S., U. Karstens, and U. Selig (2006). Phosphorus storage in *Chlorella vulgaris* (Trebouxiophyceae, Chlorophyta) cells and its dependence on phosphate supply. *Phycologia 45*, 53–60. 59

Elser, J. J., M. E. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters 10*, 1135–1142. 35, 137

Elser, J. J., E. R. Marzolf, and C. R. Goldman (1990). Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. *Canadian Journal of Fisheries and Aquatic Sciences 47*, 1468–1477. 12, 14

Entwistle, T. J., J. A. Sonneman, and S. H. Lewis (1997). *Freshwater Algae in Australia, A Guide to Conspicuous genera*. Sainty and Associates, Australia. 42

Falconer, I. R. (2005). *Cyanobacterial Toxins of Drinking Water Supplies*. Boca Raton, FL : CRC Press. 5

Falconer, I. R. and A. R. Humpage (2005). Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. *International Journal of Environmental Research and Public Health 2*(1), 45–50. 7

Ferber, L. R., S. N. Levine, A. Lini, and G. Livingston (2004). Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? *Freshwater Biology 49*, 690–108. 18

Findlay, S. E. G., R. L. Sinsabaugh, W. Sobczak, and M. Hoostal (2003). Metabolic and structural response of hyporheic microbial communities to variations in

supply of dissolved organic matter. *Limnology and Oceanography 48*(4), 1608–1617. 117

Francis, C. A., J. M. Beman, and M. M. M. Kuypers (2007). New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. *The ISME Journal 1*, 19–27. 111

Fraterrigo, J. M. and J. A. Downing (2008). The influence of land use on lake nutrients varies with watershed transport capacity. *Ecosystems 11*, 1021–1034. 10

Funari, E. and E. Testai (2008). Human health risk assessment related to cyanotoxins exposure. *Critical Reviews in Toxicology 38*, 97–125. 6, 7

Gächter, R. and J. S. Meyer (1993). The role of microorganisms in mobilization and fixation of phosphorus in sediments. *Hydrobiologia 253*, 103–121. 22, 113, 125

Gburek, W. J., A. N. Sharpley, L. Heathwaite, and G. J. Folmar (2000). Phosphorus management at the watershed scale: A modification of the phosphorus index. *Journal of Environmental Quality 29*(1), 130–144. 25, 26

Glass, J. B., F. Wolfe-Simon, J. J. Elser, and A. D. Anbar (2010). Molybdenum-nitrogen co-limitation in freshwater and coastal heterocystous cyanobacteria. *Limnology and Oceanography 55*(2), 667–676. 64

Goedkoop, W., K. R. Gullberg, R. K. Johnson, and I. Ahlgren (1997). Microbial response of a freshwater benthic community to a simulated diatom sedimentation event: Interactive effects of benthic fauna. *Microbial Ecology 34*, 131–143. 117, 136

Golterman, H. L. (2001). Phosphate release from anoxic sediments or 'What did Mortimer really write?'. *Hydrobiologia 450*, 99–106. 21, 23

Golterman, H. L. (2004). *Chemistry of Phosphate and Nitrogen Compounds in Sediments*. Dordrecht, Kluwer Academic. 98

Graham, L. E., J. M. Graham, and L. W. Wilcox (2009). *Algae* (2 ed.). Pearson Benjamin Cummings. 4, 17

Granéli, W. (1999). Internal phosphorus loading in Lake Ringsjön. *Hydrobiologia 404*, 19–26. 98

Gregor, J. and B. Maršálek (2004). Freshwater phytoplankton quantification by chlorophyll a: A comparative study of in vitro, in vivo and in situ methods. *Water Research 38*, 517–522. 41

Gress, C. D., R. G. Treble, C. J. Matz, and H. G. Weger (2004). Biological availability of iron to the freshwater cyanobacterium *Anabaena flos-aquae*. *Journal of Phycology 40*, 879–886. 78

Gudasz, C., D. Bastviken, K. Premke, K. Steger, and L. J. Tranvik (2012). Constrained microbial processing of allochthonous organic carbon in boreal lake sediments. *Limnology and Oceanography 57*(1), 163–175. 127

Haarhoff, J., O. Langenegger, and P. J. van der Merwe (1992). Practical aspects of water treatment plant design for a hypertrophic impoundment. *Water SA 18*(1), 27–36. 16

Hamersley, M. R., D. Woebkena, B. Boehrer, M. Schultze, G. Lavika, and M. M. M. Kuypers (2009). Water column anammox and denitrification in a temperate permanently stratified lake (Lake Rassnitzer, Germany). *Systematic and Applied Microbiology 32*, 571–582. 111

Haney, J. F. (1987). Field studies on zooplankton-cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research 21*, 467–475. 5

Harpole, W. S., J. T. Ngai, E. E. Cleland, E. W. Seabloom, E. T. Borer, M. E. Bracken, J. J. Elser, D. S. Gruner, H. Hillebrand, J. B. Shurin, and J. E. Smith (2011). Nutrient co-limitation of primary producer communities. *Ecology Letters 14*, 852–862. 35, 55, 56

Havens, K. E., R. T. James, T. L. East, and V. H. Smith (2003). N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by

non-point source nutrient pollution. *Environmental Pollution 122*(3), 379–390. 16, 17

Havens, K. E., E. J. Philips, M. F. Cichra, and B.-L. Li (1998). Light availability as a possible regulator of cyanobacteria species composition in a shallow subtropical lake. *Freshwater Biology 39*, 547–556. 62

Hecky, R. E. and P. Kilham (1988). Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnology and Oceanography 33*(4), 796–822. 11, 13, 14, 15, 137

Hemond, H. F. and K. Lin (2010). Nitrate suppresses internal phosphorus loading in an eutrophic lake. *Water Research 44*, 3645–3650. 113

Heo, W.-M. and B. Kim (2004). The effect of artificial destratification on phytoplankton in a reservoir. *Hydrobiologia 524*, 229–239. 30

Hernández, J. M., V. López-Rodas, and E. Costas (2009). Microcystins from tap water could be a risk factor for liver and colorectal cancer: A risk intensified by global change. *Medical Hypotheses 72*, 539–540. 7

Hickey, C. W. and M. M. Gibbs (2009). Lake sediment phosphorus release management - Decision support and risk assessment framework. *New Zealand Journal of Marine and Freshwater Research 43*, 819–856. 27, 28, 29, 30, 128, 138

Hill, A. R. (1996). Nitrate removal in stream riparian zones. *Journal of Environmental Quality 25*(4), 743–755. 26

Holtan, H., L. Kamp-Nielsen, and A. O. Stuanes (1988). Phosphorus in soil, water and sediment: An overview. *Hydrobiologia 170*, 19–34. 21

Horvatić, J., V. Peršić, and M. Mihaljević (2006). Bioassay method in evaluation of trophic conditions and nutrient limitation in the Danube wetland waters (1388-1426 r. km). *Hydrobiologia 563*, 453–463. 13

Howarth, R. and H. W. Paerl (2008). Coastal marine eutrophication: Control of both nitrogen and phosphorus is necessary. *PNAS 105*(49). 12

Howarth, R. W. (1988). Nutrient limitation of net primary production in marine ecosystems. *Annual Review of Ecology 19*, 89–110. 11, 13

Howarth, R. W., R. Marino, and J. J. Cole (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical control. *Limnology and Oceanography 33*(4), 688–701. 81

Hudnell, H. K., C. Jones, B. Labisi, V. Lucero, D. R. Hill, and J. Eilers (2010). Freshwater harmful algal bloom (FHAB) suppression with solar powered circulation (SPC). *Harmful Algae 9*, 208–217. 5, 30

Humbert, S., S. Tarnawski, N. Fromin, M.-P. Mallet, M. Aragno, and J. Zopfi (2010). Molecular detection of anammox bacteria in terrestrial ecosystems: Distribution and diversity. *The ISME Journal 4*, 450–454. 111

Humpage, A. R., J. Rositano, A. H. Bretag, R. Brown, P. D. Baker, B. C. Nicholson, and D. A. Steffensen (1994). Paralytic shellfish poisons from Australian cyanobacterial blooms. *Australian Journal of Marine and Freshwater Research 45*, 761–771. 80

Hunter Water (2011). *Catchment Management Plan Hunter Water's eight element plan for our catchments*. 36 Honeysuckle Drive, Newcastle, NSW 2300: Hunter Water Corporation. 3, 38

Hupfer, M., S. Gloess, and H.-P. Grossart (2007). Polyphosphate-accumulating microorganisms in aquatic sediments. *Aquatic Microbial Ecology 47*, 299–311. 127

Hupfer, M. and J. Lewandowski (2008). Oxygen controls the phosphorus release from lake sediments - a long-lasting paradigm in limnology. *International Review of Hydrobiology 93*(4), 415–432. 23, 98, 125

Hutchins, D. A. and K. W. Bruland (1998). Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. *Nature 393*, 561–564. 78

Hyenstrand, P., E. Rydin, and M. Gunnerhed (2000). Response of pelagic cyanobacteria to iron additions - enclosure experiments from Lake Erken. *Journal of Plankton Research 22*(6), 1113–1126. 63

Hyenstrand, P., E. Rydin, M. Gunnerhed, J. Linder, and P. Blomqvist (2001). Response of the cyanobacterium *Gloeotrichia echinulata* to iron and boron additions - an experiment from Lake Erken. *Freshwater Biology 46*, 735–741. 56, 63

Ibelings, B. W., L. R. Mur, and A. E. Walsby (1991). Diurnal changes in buyoancy and vertical distribution in populations of *Microcystis* in two shallow lakes. *Journal of Plankton Research 13*(2), 419–436. 30

Izydorczyk, K., A. Skowron, A. Wojtal, and T. Jurczak (2008). The stream inlet to a shallow bay of a drinking water reservoir, a 'hot-spot' for *Microcystis* blooms initiation. *International Review of Hydrobiology 93*(3), 257–268. 19

James, C., J. Fisher, and B. Moss (2003). Nitrogen driven lakes: The Shropshire and Cheshire Meres? *Archiv für Hydrobiologie 158*, 249–266. 12, 35

Jensen, H. S. and F. Ø. Andersen (1992). Importance of temperature, nitrate, and pH for phosphate release from aerobic sediments of four shallow, eutrophic lakes. *Limnology and Oceanography 37*(3), 577–589. 99, 112, 113

Jensen, J. P., E. Jeppesen, K. Olrik, and P. Kristensen (1994). Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Canadian Journal of Fisheries and Aquatic Sciences 51*(8), 1692–1699. 62

Jeppesen, E., M. Søndergaard, J. P. Jensen, K. E. Havens, O. Anneville, L. Carvalho, M. F. Coveney, R. Deneke, M. T. Dokulil, B. Foy, D. Gerdeaux, S. E. Hampton, S. Hilt, K. Kangur, J. Kohler, E. H. H. R. Lammens, T. L. Lauridsen, M. Manca, M. R. Miracle, B. Moss, P. Noges, G. Persson, G. Phillips, R. Portielje, S. Romo, C. L. Schelske, D. Straile, I. Tatrai, E. Willén, and M. Winder (2005). Lake responses to reduced nutrient loading - an analysis of contemporary long-term data from 35 case studies. *Freshwater Biology 50*, 1747–1771. 11, 35

Jetten, M. S., M. Strous, K. T. van de Pas-Schoonen, J. Schalk, U. G. J. M. van Dongen, A. A. van de Graaf, S. Logemann, G. Muyzer, M. C. M. van Loosdrecht, and J. G. Kuenen (1999). The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews 22*, 421–437. 111

Jin, X., X. Jiang, Y. Yao, L. Li, and F. Wu (2007). Effects of organisms on the release of phosphorus at the interface between sediment and water. *Water Environment Research 79*(11), 2253–2259. 22

Jochimsen, E. M., W. W. Carmichael, J. S. An, D. M. Cardo, S. T. Cookson, C. E. H. M. B. Antunes, D. A. de Melo Filho, T. M. Lyra, V. S. T. Barreto, S. M. Azevedo, and W. R. Jarvis (1998). Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. *The New England Journal of Medicine 338*, 873–878. 6

Jungo, E., P. M. Visser, J. Stroom, and L. R. Mur (2001). Artificial mixing to reduce growth of the blue-green alga *Microcystis* in Lake Nieuwe Meer, Amsterdam: An evaluation of 7 years of experience. *Water Science and Technology: Water Supply 1*(1), 17–23. 31

Keating, K. I. (1978). Blue-green algal inhibition of diatom growth: Transition from mesotrophic to eutrophic community structure. *199*, 971–973. 8

Keuskamp, J. A., H. Schmitt, H. J. Laanbroek, J. T. A. Verhoeven, and M. M. Hefting (2013). Nutrient amendment does not increase mineralisation of sequestered carbon during incubation of a nitrogen limited mangrove soil. *Soil Biology & Biochemistry 57*, 822–829. 127

Kim, G., W. Jeong, S. Choi, and J. Khim (2007). Sand capping for controlling phosphorus release from lake sediments. *Environmental Technology 28*, 381–389. 27

Kinnear, S. (2010). Cylindrospermopsin: A decade of progress on bioaccumulation research. *Marine Drugs 8*, 542–564. 7

Klapper, H. (2003). Technologies for lake restoration. *Journal of Limnology 62*, 73–90. 26, 29, 30

Kling, H. J. (2009). *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria): A brief historic overview and recent discovery in the Assiniboine River (Canada). *Fottea 1*, 45–47. 7

Knowles, R. (1982). Denitrification. *Microbiological Reviews 46*(1), 43–70. 24, 117

Kõiv, T. and K. Kangro (2005). Resource ratios and phytoplankton species composition in a strongly stratified lake. *Hydrobiologia 547*, 123–135. 17, 18

Kopáček, J., J. Borovec, J. Hejzlar, K.-U. Ulrich, S. Norton, and A. Amirbahman (2005). Aluminum control of phosphorus sorption by lake sediments. *Environmental Science & Technology 39*, 8784–8789. 129

Kopáček, J., M. Marešová, and J. Hejzlar (2007). Natural inactivation of phosphorus by aluminum in preindustrial lake sediments. *Limnology and Oceanography 52*(3), 1147–1155. 23, 128

Koski-Vähälä, J. and H. Hartikainen (2001). Assessment of the risk of phosphorus loading due to resuspended sediment. *Journal of Environmental Quality 30*(3), 960–966. 22

Kromkamp, J. (1987). Formation and functional significance of storage products in cyanobacteria. *New Zealand Journal of Marine and Freshwater Research 21*(3), 457–465. 95

Kuiper-Goodman, T., I. R. Falconer, and J. Fitzgerald (1999). *Toxic Cyanobacteria in Water*, Chapter 4, pp. 113–153. E & FN Spon, an imprint of Routledge, London. 6

Laanbroek, H. J. and N. Pfennig (1981). Oxidation of short-chain fatty acids by sulfate-reducing bacteria in freshwater and in marine sediments. *Archives of Microbiology 128*, 330–335. 117

Lake, B. A., K. M. Coolidge, S. A. Norton, and A. Amirbahman (2007). Factors contributing to the internal loading of phosphorus from anoxic sediments in six Maine, USA, lakes. *Science of the Total Environment 373*, 534–541. 129

Lampert, W. (1987). Laboratory studies on zooplankton-cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research 21*, 483–490. 5

Lee, R. E. (2008). *Phycology* (4 ed.). Cambridge University Press. 3

Levine, S. and D. Schindler (1999). Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplank-

ton species composition in the Experimental Lakes Area, Canada. *Canadian Journal of Fisheries and Aquatic Sciences 56*, 451–466. 14, 17

Lewis, W. M. and W. A. Wurtsbaugh (2008). Control of lacustrine phytoplankton by nutrients: Erosion of the phosphorus paradigm. *Interational Review of Hydrobiology 93*(4), 446–465. 11, 35, 137

Lijklema, L. (1980). Interaction of orthophosphate with iron(iii) and aluminum hydroxides. *American Chemical Society 14*(5), 537–541. 21, 99

Lindenschmidt, K. E. and I. Chorus (1997). The effect of aeration on stratification and phytoplankton populations in Lake Tegel, Berlin. *Archiv für Hydrobiologie 139*(3), 317–346. 31

Litchman, E. (2000). Growth rates of phytoplankton under fluctuating light. *Freshwater Biology 44*, 223–235. 37

Lloyd, D. (1993). Aerobic denitrification in soils and sediments: From fallacies to facts. *Trends in Ecology and Evolution 8*(10), 352–356. 112

Loh, P. S., L. A. Molot, G. K. Nurnberg, S. B. Watson, and B. Ginn (2013). Evaluating relationships between sediment chemistry and anoxic phosphorus and iron release across three different water bodies. *Inland Waters 3*(1), 105–118. 125

Łopata, M. and H. Gawrońska (2008). Phosphorus immobilization in Lake Głęboczek following treatment with polyaluminum chloride. *International Journal of Oceanography and Hydrobiology 37*(2), 99–105. 27

Lovley, D. R. (1991). Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiological Reviews 55*(2), 259–287. 113, 117, 125

Lovley, D. R., J. D. Coates, E. L. Blunt-Harris, E. J. P. Phillips, and J. C. Woodward (1996). Humic substances as electron acceptor for microbial respiration. *Nature 382*(1), 445–448. 117

Lovley, D. R. and E. J. P. Philips (1986). Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Applied and Environmental Biology 51*(4), 683–689. 99, 101, 119

Lürling, M., F. Eshetu, E. J. Faassen, S. Kosten, and V. L. M. Huszar (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology 58*(3), 552–559. 37, 60

Malecki, L. M., J. R. White, and K. R. Reddy (2004). Nitrogen and phosphorus flux rates from sediment in the lower St. Johns River estuary. *Journal of Environmental Quality 33*(4), 1545–1555. 23, 25, 99, 128, 138

Markensten, H., K. Moore, and I. Persson (2010). Simulated lake phytoplankton composition shifts toward cyanobacteria dominance in a future warmer climate. *Ecological Applications 20*(3), 752–767. 1, 5

McCarthy, M. J., R. T. James, Y. Chen, T. L. East, and W. S. Gardner (2009). Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical Lakes Okeechobee (Florida, USA) and Taihu (China). *Limnology 10*, 215–227. 17, 18

McCausland, M. A., P. A. Thompson, and S. I. Blackburn (2002). The effect of changes in light availability caused by mixing on the growth of *Anabaena circinalis* (Nostocales, Cyanobacteria) and *Aulacoseira* (Centrales, Bacillariophyceae). *Phycologia 40*(6), 530–541. 78

McCausland, M. A., P. A. Thompson, and S. I. Blackburn (2005). Ecophysiological influence of light and mixing on *Anabaena circinalis* (nostocales, cyanobacteria). *European Journal of Phycology 40*, 9–20. 30, 95, 96

Metcalf, J. S. and G. A. Codd (2009). Cyanobacteria, neurotoxins and water resources: Are there implications for human neurodegenerative disease? *Amyotrophic Lateral Sclerosis 10*, 74–78. 7

Meyers, P. A. and R. Ishiwatari (1993). Lacustrine organic geochemistry – an overview of indicators of organic matter sources and diagenesis in lake sediments. *Organic Geochemistry 20*(7), 867–900. 127

Meynendonckx, J., G. Heuvelmans, B. Muys, and J. Feyen (2006). Effects of watershed and riparian zone characteristics on nutrient concentrations in the River Scheldt Basin. *Hydrology and Earth System Sciences 10*, 913–922. 10

Mills, M. M., C. Ridame, M. Davey, J. L. Roche, and R. J. Geider (2004). Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature 429*, 292–294. 64

Mitchell, A. (2002). *Anaerobic nutrient cycles in freshwater sediments*. Ph. D. thesis, School of Science and Technology, Charles Sturt University. 117, 126, 128, 129, 130, 138

Mitchell, A. and D. S. Baldwin (1998). Effects of desiccation/oxidation on the potential for bacterially mediated P release from sediments. *Limnology and Oceanography 43*(3), 481–487. 23, 113

Mitrovic, S. M., L. C. Bowling, and R. T. Buckney (2001a). Responses of phytoplankton to in-situ nutrient enrichment; potential influences on species dominance in a river. *International Review of Hydrobiology 86*(3), 285–298. 14, 15

Mitrovic, S. M., L. C. Bowling, and R. T. Buckney (2001b). Vertical disentrainment of *Anabaena circinalis* in the turbid, freshwater Darling River, Australia: Quantifying potential benefits from buoyancy. *Journal of Plankton Research 23*(1), 47–55. 81

Mitrovic, S. M., L. Hardwick, and F. Dorani (2011). Use of flow management to mitigate cyanobacterial blooms in the Lower Darling River, Australia. *Journal of Plankton Research 33*(2), 229–241. 81

Mitrovic, S. M., R. L. Oliver, C. Rees, L. C. Bowling, and R. T. Buckney (2003). Critical flow velocities for the growth and dominance of *Anabaena circinalis* in some turbid freshwater rivers. *Freshwater Biology 48*, 164–174. 5, 30, 63, 81, 96, 135

Mohamed, Z. A. and A. M. Al Shehrib (2009). Microcystins in groundwater wells and their accumulation in vegetable plants irrigated with contaminated waters in Saudi Arabia. *Journal of Hazardous Materials 172*, 310–315. 7

Moon, J. B. and H. J. Carrick (2007). Seasonal variation of phytoplankton nutrient limitation in Lake Erie. *Aquatic Microbial Ecology 48*, 61–71. 35, 59

Moore, P. A., K. R. Reddy, and M. M. Fisher (1998). Phosphorus flux between sediment and overlying water in Lake Okechobee, Florida: Spatial and temporal variations. *Journal of Environmental Quality 27*(6), 1428–1439. 98

Mortimer, C. H. (1941). The exchange of dissolved substances between mud and water in lakes. *Journal of Ecology 29*(2), 208–329. 138

Mortimer, C. H. (1971). Chemical exchanges between sediment and water in the Great Lakes - speculations on probable regulatory mechanisms. *Limnology and Oceanography 16*(2), 387–404. 22, 98

Morton, S. D. and T. H. Lee (1974). Algal blooms – possible effects of iron. *Environmental Science & Technology 8*(8), 673–674. 63

Mugidde, R., R. E. Hecky, L. L. Hendzel, and W. D. Taylor (2003). Pelagic nitrogen fixation in Lake Victoria (East Africa). *Journal of Great Lakes Research 29*(29), 76–88. 81

Nealson, K. H. and D. Saffarini (1994). Iron and manganese in anaerobic respiration: Environmental significance, physiology, and regulation. *Annual Review of Microbiology 48*, 311. 113

Nemeth, Z., L. Gancs, G. Gemes, and A. Kolics (1998). pH dependence of phosphate sorption to aluminium. *Corrosion Science 40*(12), 2023–2027. 21, 22

NHMRC and NRMMC (2011). *Australian Drinking Water Guidelines Paper 6 National Water Quality Management Strategy*. National Health and Medical Research Council, National Resource Management Ministerial Council, Commonwealth of Australia, Canberra. 134, 135

Nõges, T., R. Laugaste, P. Nõges, and I. Tõnno (2008). Critical N:P ratio for cyanobacteria and N_2-fixing species in the large shallow temperate lakes Peipsi and Võrtsjärv, North-East Europe. *Hydrobiologia 599*, 77–86. 80

North, R. L., S. J. Guildford, R. E. H. Smith, S. M. Havens, and M. R. Twiss (2007). Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnology and Oceanography 52*(1), 315–328. 63, 78

Nowlin, W. H., J. L. Evarts, and M. J. Vanni (2005). Release rates and potential fates of nitrogen and phosphorus from sediments in a eutrophic reservoir. *Freshwater Biology 50*, 301–322. 20, 25, 99, 112

NRMMC (2006). *National protocol for the monitoring of cyanobacteria and their toxins in surface fresh waters*. In review. 42

OECD (2006). *OECD Guidelines for the testing of chemicals. Lemna sp. Growth Inhibition Test*. OECD/OCDE. xv, xvii, 65, 66, 82, 183

Ogbebo, F. E., M. S. Evans, M. J. Waiser, V. P. Tumber, and J. J. Keating (2009). Nutrient limitation of phytoplankton growth in Arctic lakes of the lower Mackenzie River Basin, northern Canada. *Canadian Journal of Fisheries and Aquatic Sciences 66*(2), 247–260. 55, 56

Olenina, I., S. Hajdu, L. Edler, A. Andersson, N. Wasmund, S. Busch, J. Göbel, S. Gromisz, S. Huseby, M. Huttunen, A. Jaanus, P. Kokkonen, I. Ledaine, and E. Niemkiewicz (2005). Biovolumes and size-classes of phytoplankton in the Baltic Sea. *HELCOM Baltic Sea Environment Proceedings 106*, 1–144. 42

Osborne, L. L. and D. A. Kovacic (1993). Riparian vegetated buffer strips in water-quality restoration and stream management. *Freshwater Biology 29*(2), 243–258. 26

Osborne, N. J. T., G. R. Shaw, and P. M. Webb (2007). Health effects of recreational exposure to Moreton Bay, Australia waters during a *Lyngbya majuscula* bloom. *Environment International 33*, 309–314. 7

Osborne, N. J. T., P. M. Webb, and G. R. Shaw (2001). The toxins of *Lyngbya majuscula* and their human and ecological health effects. *Environment International 27*(5), 381–392. 7

Padisák, J. (1997). *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding highly adaptive cyanobacterium: Worldwide distribution and review of its ecology. *Archiv für Hydrobiologie 4*, 563–593. 7

Paerl, H. W. (1988). Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnology and Oceanography 33*(4), 823–847. 2

Paerl, H. W. (2009). Controlling eutrophication along the freshwater-marine continuum: Dual nutrient (N and P) reductions are essential. *Esutraries and Coast 32*, 593–601. 12

Paerl, H. W., N. S. Hall, and E. S. Calandrino (2011). Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of the Total Environment 409*, 1739–1745. 1, 12

Paerl, H. W. and J. Huisman (2008). Blooms like it hot. *Science 320*(5872), 57–58. 1, 5

Paerl, H. W. and T. G. Otten (2013). Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microbial Ecology 65*, 995–1010. 1, 12

Paerl, H. W. and J. T. Scott (2010). Throwing fuel on the fire: Synergistic effects of excessive nitrogen inputs and global warming on harmful algal blooms. *Environmental Science & Technology 44*(20), 7756–7758. 12

Paul, L. (2003). Nutrient elimination in pre-dams: Results of long term studies. *Hydrobiologia 504*, 289–295. 29

Penn, M. R., M. T. Auer, S. M. Doerr, C. T. Driscoll, C. M. Brooks, and S. W. Effler (2000). Seasonality in phosphorus release rates from the sediments of a hypereutrophic lake under a matrix of pH and redox conditions. *Canadian Journal of Fisheries and Aquatic Sciences 57*(5), 1033–1041. 99, 112

Perkins, R. G. and G. J. C. Underwood (2000). Gradients of chlorophyll a and water chemistry along an eutrophic reservoir with determination of the limiting nutrient by *in situ* nutrient addition. *Water Research 34*(3), 713–724. 14, 15

Perrone, U., A. Facchinelli, and E. Sacchi (2008). Phosphorus dynamics in a small eutrophic Italian lake. *Water Air Soil Pollution 189*, 335–351. 21, 99

Peterjohn, W. T. and D. L. Correll (1984). Nutrient dynamics in an agricultural watershed: Observations on the role of a riparian forest. *Ecology 65*(5), 1466–1475. 26

Petterson, K. (1998). Mechanisms for internal loading of phosporus in lakes. *Hydrobiologia 373*, 21–25. 20

Pick, F. R. and D. R. S. Lean (1987). The role of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. *New Zealand Journal of Marine and Freshwater Research 21*, 425–434. 16, 17

Piehler, M. F., J. Dyble, P. H. Moisander, A. D. Chapman, J. Hendrickson, and H. W. Paerl (2009). Interactions between nitrogen dynamics and the phytoplankton community in Lake George, Florida, USA. *Lake and Reservoir Management 25*, 1–14. 59, 81

Pilotto, L., P. Hobson, M. D. Burch, G. Ranmuthugala, R. Attewell, and W. Weightman (2007). Acute skin irritant effects of cyanobacteria (blue-green algae) in healthy volunteers. *Australian and New Zealand Journal of Public Health 28*(3), 220–224. 7

Pollman, C. D. and R. T. James (2011). A simple model of internal loading of phosphorus in Lake Okeechobee. *Lake and Reservoir Management 27*, 15–27. 98

Potgieter, J. H. (1991). Adsorption of methylene blue on activated carbon. *Journal of Chemical Education 68*(4), 349–350. 131

Prescott, G. W. (1978). *How to Know the Freshwater Algae* (3 ed.). Wm.C. Brown Company Publishers. Dubuque, Iowa. 42

Price, N. M., L. F. Andersen, and F. M. Morel (1991). Iron and nitrogen nutrition of equatorial Pacific plankton. *Deep Sea Research 38*(11), 1361–1378. 78

Qin, B., G. Zhu, G. Gao, Y. Zhang, W. Li, H. W. Paerl, and W. W. Carmichael (2010). A drinking water crisis in Lake Taihu, China: Linkage to climatic variability and lake management. *Environmental Management 45*, 105–112. 8, 9, 35

Quiblier, C., C. Leboulanger, S. Sané, and P. Dufour (2008). Phytoplankton growth control and risk of cyanobacterial blooms in the lower Senegal River delta region. *Water Research 42*, 1023–1034. 56

Quin, B., G. Zhu, L. Zhang, L. Luo, G. Gao, and B. Gu (2006). Estimation of internal nutrient release in large shallow Lake Taihu, China. *Science in China: Series D Earth Sciences 49*, 38–50. 24

R Development Core Team (2011). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. ISBN 3-900051-07-0. 43, 66, 87, 102, 119

Raven, J. A., M. C. W. Evans, and R. E. Korb (1999). The role of trace metals in photosynthetic electron transport in O_2-evolving organisms. *Photosynthesis Research 60*, 111–149. 63

Reddy, K. R., M. M. Fisher, and D. Ivanoff (1996). Resuspension and diffusive flux of nitrogen and phosphorus in a hypereutrophic lake. *Journal of Environmental Quality 25*(2), 363–371. 114

Reynolds, C. S., R. L. Oliver, and A. E. Walsby (1987). Cyanobacterial dominance: The role of buoyancy regulation in dynamic lake environments. *New Zealand Journal of Marine and Freshwater Research 21*, 379–390. 2, 5

Robarts, R. D. (1985). Hypertrophy, a consequence of development. *International Journal of Environmental Studies 25*, 167–175. 8

Robarts, R. D. and T. Zohary (1987). Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research 21*, 391–399. 2

Roden, E. E. and J. W. Edmonds (1997). Phosphate mobilization in iron-rich anaerobic sediments: Microbial Fe(III) oxide reduction versus iron-sulfide formation. *Archiv für Hydrobiologie 139*(3), 347–378. 23

Ryan, N. J., S. M. Mitrovic, and L. C. Bowling (2008). Temporal and spatial variability in the phytoplankton community of Myall Lakes, Australia, and influences of salinity. *Hydrobiologia 608*, 69–86. 18

Rydin, E. (2000). Amount of phosphorus inactivated by alum treatments in Washington lakes. *Limnology and Oceanography 45*(1), 226–230. 128

Saito, M. A., T. J. Goepfert, and J. T. Ritt (2008). Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. *Limnology and Oceanography 53*(1), 276–290. 56

Salvia-Castellvi, M., A. Dohet, P. V. Borght, and L. Hoffmann (2001). Control of the eutrophication of the reservoir of Esch-sur-Sûre (Luxembourg): Evaluation of the phosphorus removal by predams. *Hydrobiologia 459*, 61–71. 29

Saunders, D. L. and J. Kalff (2001). Nitrogen retention in wetlands, lakes and rivers. *Hydrobiologia 443*, 205–211. 20, 25, 98

SCA (2010). *Sydney Catchment Authority Cyanobacteria Risk Profile*. Sydney Catchment Authority. 2, 19

Schabhüttl, S., P. Hingsamer, G. Weigelhofer, T. Hein, A. Weigert, and M. Striebel (2013). Temperature and species richness effects in phytoplankton communities. *Oecologia 171*, 527–536. 37

Schade, J. D., J. F. Espeleta, C. A. Klausmeier, M. E. McGroddy, S. A. Thomas, and L. Zhang (2005). A conceptual framework for ecosystem stoichiometry: Balancing resource supply and demand. *Oikos 109*, 40–51. 137, 139

Schallenberg, M. and J. Kalff (1993). The ecology of sediment bacteria in lakes and comparisons with other aquatic ecosystems. *Ecology 74*(3), 919–934. 117

Scheffer, M. (1998). *Ecology of Shallow Lakes*. Chapman and Hall. 25

Scheffer, M., S. Rinaldi, A. Gragnani, L. R. Mur, and E. H. van Nes (1997). The dominance of filamentous cyanobacteria in shallow, turbid lakes. *Ecology 78*(1), 272–282. 2, 5

Schindler, D. W. (1977). Evolution of phosphorus limitation in lakes. *Science 195*(4275), 260–262. 11, 12, 13, 15, 80, 96

Schindler, D. W. (1978). Factors regulating phytoplankton production and standing crop in the world's freshwaters. *Limnology and Oceanography 23*(3), 478–486. 11, 12

Schindler, D. W. (1998). Replication versus realism: Need for ecosystem-scale experiments. *Ecosystems 1*, 323–334. 36

Schindler, D. W. (2006). Recent advances in the understanding and management of eutrophication. *Limnology and Oceanography 51*(1), 356–363. 9, 10

Schindler, D. W. and R. E. Hecky (2008). Reply to Howarth and Paerl: Is control of both nitrogen and phosphorus necessary? *Proceedings of the National Academy of Sciences 105*(49). 12

Schindler, D. W. and R. E. Hecky (2009). Reply to Bryhn and Håkanson: Models for the baltic agree with our experiments and observations in lakes. *PNAS 106*(1). 12

Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng, and S. E. M. Kasian (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences 105*(32), 11254–11258. 9, 10, 12, 13, 36, 62

Schubert, C. J., E. Durisch-Kaiser, B. Wehrli, B. Thamdrup, P. Lam, and M. M. M. Kuypers (2006). Anaerobic ammonium oxidation in a tropical freshwater system (Lake Tanganyika). *Environmental Microbiology 8*(10), 1857–1863. 111

Scott, J. T. and M. J. McCarthy (2010). Nitrogen fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. *Limnology and Oceanography 55*(3), 1265–1270. 13

Seitzinger, S. P. (1988). Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnology and Oceanography 33*(4), 702–724. 112

Shanableh, A. M. and M. M. Elsergany (2013). Removal of phosphate from water using six Al-, Fe-, and Al-Fe-modified bentonite adsorbents. *Journal of Environmental Science and Health, Part A 48*, 223–231. 131

Shapiro, J. (1984). Blue-green dominance in lakes: The role and management significance of pH and CO_2. *Internationale Revue der gesamten Hydrobiologie und Hydrographie 69*, 765–780. 2, 5

Shapiro, J. (1997). The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. *Freshwater Biology 37*, 307–323. 2, 5

Sherman, B., B. Whittington, and R. Oliver (2000). The impact of artificial destratification on water quality in Chaffey Reservoir. *Archiv für Hydrobiologie 55*, 15–29. 31

Sherman, B. S., I. T. Webster, G. J. Jones, and R. L. Oliver (1998). Transitions between *Aulacoseira* and *Anabaena* dominance in a turbid river weir pool. *Limnology and Oceanography 43*(8), 1902–1915. xii, 30, 63, 78, 103

Sigee, D. C. (2005). *Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment*. John Wiley & Sons, Ldt. 4, 5, 12, 15, 24, 116

Sivonen, K. and G. Jones (1999). *Toxic Cyanobacteria in Water*, Chapter 3, pp. 41–111. E & FN Spon, an imprint of Routledge, London. 5

Smith, S. V. (1984). Phosphorus versus nitrogen limitation in the marine environment. *Limnology and Oceanography 29*(6), 1149–1160. 11

Smith, V. H. (1983). Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science 221*(4611), 669–671. 15, 80

Smith, V. H. (1985). Predictive models for the biomass of blue-green algae in lakes. *Water Resources Bulletin 21*(3), 433–439. 81

Smith, V. H. (2003). Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environmental Science and Pollution Research 10*(2), 126–139. 8

Smolders, A. J. P., L. P. M. Lamers, E. C. H. E. T. Lucassen, G. Van der Welde, and J. G. M. Roelofs (2006). Internal eutrophication: How it works and what to do about it - a review. *Chemistry and Ecology 22*(2), 93–111. 24, 25, 26, 28

Soares, R. M., M. Yuan, J. C. Servaites, A. Delgado, V. F. Magalhães, E. D. Hilborn, W. W. Carmichael, and S. M. F. O. Azevedo (2006). Sublethal exposure from microcystins to renal insufficiency patients in Rio de Janeiro, Brazil. *Environmental Toxicology 21*(2), 95–103. 6

Søndergaard, M., J. P. Jensen, and E. Jeppesen (1999). Internal phosphorus loading in shallow Danish lakes. *Hydrobiologia 408*, 145–152. 20

Søndergaard, M., J. P. Jensen, and E. Jeppesen (2001). Retention and internal loading of phosphorus in shallow eutrophic lakes. *Scientific World Journal 1*, 427–42. 10, 20, 25, 98

Søndergaard, M., J. P. Jensen, and E. Jeppesen (2003). Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia 506*, 135–145. 10, 20, 24, 25, 98

Song, C., X. Cao, J. Li, Q. Li, G. Chen, and Y. Zhou (2006). Contributions of phosphatase and microbial activity to internal phosphorus loading and their relation to lake eutrophication. *Science in China: Series D Earth Sciences 49*, 102–113. 22

Soranno, P. A., S. L. Huber, S. R. Carpenter, and R. C. Lathrop (1996). Phosphorus loads to surface waters: A simple model to account for spatial pattern of land use. *Ecological Applications 6*(3), 865–878. 10

Spears, B. M., L. Carvalho, R. Perkins, and D. M. Paterson (2008). Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. *Water Research 42*, 977–986. 20

Spivak, A. C., M. J. Vanni, and E. M. Mette (2011). Moving on up: Can results from simple aquatic mesocosm experiments be applied across broad spatial scales? *Freshwater Biology 56*, 279–291. 36, 37

Steenbergh, A. K., P. L. E. Bodelier, H. L. Hoogveld, C. P. Slomp, and H. J. Laanbroek (2011). Phosphatases relieve carbon limitation of microbial activity in Baltic Sea sediments along a redox-gradien. *Limnology and Ocaenography 56*(6), 2018–2026. 127

Sterner, R. W. (2008). On the phosphorus limitation paradigm for lakes. *International Review of Hydrobiology 93*(4-5), 433–445. 11, 35, 137

Sterner, R. W., T. M. Smutka, R. Michael, L. McKay, Q. Xiaoming, E. T. Brown, and R. M. Sherrell (2004). Phosphorus and trace metal limitation of algae and bacteria in Lake Superior. *Limnology and Oceanography 49*(2), 495–507. 63

Stewart, I., A. A. Seawright, and G. R. Shaw (2008). Chapter 28: Cyanobacterial poisoning in livestock, wild mammals and birds - an overview. In *International Symposium on Cyanobacterial Algal Blooms - State of the Science and Research Needs*, http://www.epa.gov/cyanohabssymposium/monograph.html, accessed 7/7/2010. 1, 6

Stockner, J. G. and K. S. Shortreed (1988). Response of *Anabaena* and *Synechococcus* to manipulation of nitrogen:phosphorus ratios in a lake fertilization experiment. *Limnology and Oceanography 33*(6), 1348–1361. 15, 17

Stookey, L. L. (1970). Ferrozine-a new spectrophotometric reagent for iron. *Analytical Chemistry 42*(7), 779–781. 101, 119

Straškraba, M. and J. G. Tundisi (1999). *Guidelines of Lake Management - Reservoir Water Quality Management*, Volume 9. International Lake Environment Committee Foundation. 28, 29, 30

Streble, H. and D. Krauter (2008). *Das Leben im Wassertropfen* (10 ed.). Franckh-Kosmos Verlags-GmbH& Co. KG, Stuttgart. 42

Suttle, C. A. and P. J. Harrison (1988). Ammonium and phosphate uptake rates, N:P supply ratios, and evidence for N and P limitation in some oligotrophic lakes. *Limnology and Oceanography 33*(2), 186–202. 15, 17

Texeira, M. G. L. C., M. C. N. Costa, V. L. P. de Carvalho, M. D. S. Pereira, and E. Hage (1993). Gastroenteritis epidemic in the area of the Itaparica Dam, Bahia. *Bulletin of the Pan American Health Organization 114*, 502–512. 6

Thamdrup, B. and T. Dalsgaard (2002). Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied and Environmental Microbiology 68*(3), 1312–1318. 111

Thiel, T. and M. Leone (1986). Effect of glutamine on growth and heterocyst differentiation in the cyanobacterium *Anabaena variabilis*. *Journal of Bacteriology 168*(2), 769–774. 94

Tilman, D., S. S. Kilham, and P. Kilham (1982). Phytoplankton community ecology: The role of limiting nutrients. *Annual Review of Ecology and Systematics 13*, 349–372. 11, 15

Tilzer, M. M. (1987). Light-dependence of photosynthesis and growth in cyanobacteria: Implications for their dominance in eutrophic lakes. *New Zealand Journal of Marine and Freshwater Research 21*, 401–412. 17, 63

Tomioka, N., A. Imai, and K. Komatsu (2011). Effect of light availability on *Microcystis aeruginosa* blooms in shallow hypereutrophic Lake Kasumigaura. *Journal of Plankton Research 33*(8), 1263–1273. 63

Törnblom, E. and E. Rydin (1998). Bacterial and phosphorus dynamics in profundal Lake Erken sediments following the deposition of diatoms: A laboratory study. *Hydrobiologia 364*, 55–63. 117

Torres, I. C., K. S. Inglett, and K. R. Reddy (2011). Heterotrophic microbial activity in lake sediments: Effects of organic electron donors. *Biogeochemistry 104*, 165–181. 117

Triplett, L. D., D. R. Engstrom, and M. B. Edlund (2009). A whole-basin stratigraphic record of sediment and phosphorus loading to the St. Croix River, USA. *Journal of Paleolimnology 41*, 659–677. 9

Tsukada, H., S. Tsujimura, and H. Nakahara (2006). Seasonal succession of phytoplankton in Lake Yogo over 2 years: Effect of artificial manipulation. *Limnology 7*, 3–14. 31

Twiss, M. R., J.-C. Auclair, and M. N. Charlton (2000). An investigation into iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques. *Canadian Journal of Fisheries and Aquatic Sciences 57*(1), 86–95. 63

Ulrich, K.-U. and R. Pöthig (2000). Precipitation of aluminium and phosphate affected by acidification. *Acta hydrochimica et hydrobiologica 28*, 313–322. 128, 129

Vanni, M. J., J. S. Andrews, W. H. Renwick, M. J. Gonzalez, and S. J. Noble (2006). Nutrient and light limitation of reservoir phytoplankton in relation to storm-mediated pulses in stream discharge. *Archiv für Hydrobiologie 167*(1), 421–445. 19

Velzeboer, R. M. A., P. D. Baker, and J. Rositano (2001). Saxitoxins associated with the growth of the cyanobacterium *Anabaena circinalis* (nostocales, cyanophyta) under varying sources and concentrations of nitrogen. *Phycologia 40*(3), 305–312. 81, 95

Vis, C., A. Cattaneo, and C. Hudon (2008). Shift from chlorophytes to cyanobacteria in benthic macroalgae along a gradient of nitrate depletion. *Journal of Phycology 44*, 38–44. 16

Vrede, T., A. Ballantyne, C. Mille-Lindblom, G. Algesten, C. Gudasz, S. Lindahl, and A. K. Brunberg (2009). Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. *Freshwater Biology 54*, 331–344. 14, 16, 59, 62, 63, 81, 96

Vrede, T. and L. J. Tranvik (2006). Iron constraints on planktonic primary production in oligotrophic lakes. *Ecosystems 9*, 1094–1105. 63

Wacklin, P., L. Hoffmann, and J. Komárek (2009). Nomenclatural validation of the genetically revised cyanobacterial genus *Dolichospermum* (Ralfs ex Bornet et Flahault) comb. nova. *Fottea 9*(1), 59–64. 6

Wagner, C. and R. Adrian (2009). Cyanobacteria dominance: Quantifying the effects of climate change. *Limnology and Oceanography 54*(6), 2460–2468. 1, 5

Walsby, A. E., P. K. Hayes, R. Boje, and L. J. Stal (1997). The selective advantage of buoyancy provided by gas vesicles for planktonic cyanobacteria in the Baltic Sea. *New Phytologist 136*, 407–417. 30

Wang, D., Q. Huang, C. Wang, M. Ma, and Z. Wang (2007). The effects of different electron donors on anaerobic nitrogen transformations and denitrification processes in Lake Taihu sediments. *Hydrobiologia 581*, 71–77. 111, 117

Wang, S.-H., A. R. Dzialowski, J. O. Meyer, F. deNoyelles, Jr., N.-C. Lim, W. Spotts, and D. G. Huggins (2005). Relationships between cyanobacterial production and the physical and chemical properties of a Midwestern Reservoir, USA. *Hydrobiologia 541*, 29–43. 16

Waterbury, J. B. (2006). *The Prokaryotes - Volume 4: Bacteria: Firmicutes, Cyanobacteria*, Chapter 21. Springer New York. 4

Watts, C. J. (2000). The effect of organic matter on sedimentary phosphorus release in an Australian reservoir. *Hydrobiologia 431*, 13–25. 113, 117

Weber, K. A., L. A. Achenbach, and J. D. Coates (2006). Microorganisms pumping iron: Anaerobic microbial iron oxidation and reduction. *Nature Reviews 4*, 752–764. 99

Welch, E. B. and G. D. Cooke (1999). Effectiveness and longevity of phosphorus inactivation with alum. *Lake and Reservoir Management 15*(1), 5–27. 27

Westhorpe, D. P. and S. M. Mitrovic (2012). Dissolved organic carbon mobilisation in relation to variable discharges and environmental flows in a highly regulated lowland river. *Marine and Freshwater Research 63*, 1218–1230. 136

Wilhelm, S. and R. Adrian (2008). Impact of summer warming on the thermal characteristics of a polymictic lake and consequences for oxygen, nutrients and phytoplankton. *Freshwater Biology 53*, 226–237. 99, 112

Willén, E. (2001). Phytoplankton and water quality characterization: Experiences from the Swedish large lakes Mälaren, Hjälmaren, Vättern and Vänern. *Ambio 30*, 529–538. 8, 9, 11, 35

Wood, S. A., M. J. Prentice, K. Smith, and D. P. Hamilton (2010). Low dissolved inorganic nitrogen and increased heterocyte frequency: Precursors to *Anabaena planktonica* blooms in a temperate, eutrophic reservoir. *Journal of Plankton Research 32*(9), 1315–1325. 16

Xu, H., G. Zhu, B. Qin, and H. W. Paerl (2013). Growth response of *Microcystis* spp. to iron enrichment in different regions of Lake Taihu, China. *Hydrobiologia 700*, 187–202. 36, 59, 63, 77

Yan, L.-G., Y.-Y. Xu, H.-Q. Yu, X.-D. Xin, Q. Wei, and B. Du (2010). Adsorption of phosphate from aqueous solution by hydroxy-aluminum, hydroxy-iron and hydroxy-iron-aluminum pillared bentonites. *Journal of Hazardous Materials 179*, 244–250. 131

Yao, W. and F. J. Millero (1996). Adsorption of phosphate on manganese dioxide in seawater. *Environmental Science and Technology 30*, 536–541. 21

Zapomělová, E., K. Řeháková, J. Jezberová, and J. Komárková (2010). Polyphasic characterization of eight planktonic *Anabaena* strains (Cyanobacteria) with reference to the variability of 61 *Anabaena* populations observed in the field. *Hydrobiologia 639*, 99–113. 81, 95

Zerkle, A. L., C. H. House, R. P. Cox, and D. E. Canfield (2006). Metal limitation of cyanobacterial N_2 fixation and implications for the Precambrian nitrogen cycle. *Geobiology 4*, 285–297. 94

Zhang, C.-C., S. Laurent, S. Sakr, L. Peng, , and S. Bédu (2006). Heterocyst differentiation and pattern formation in cyanobacteria: A chorus of signals. *Molecular Microbiology 59*(2), 367–375. 94

Zhao, Y., S.-M. Bian, H.-N. Zhou, and J.-F. Huang (2006). Diversity of nitrogenase systems in diazotrophs. *Journal of Integrative Plant Biology 48*(7), 745–755. 64, 78

Zhou, L., H. Yu, and K. Chen (2002). Relationship between microcystin in drinking water and colorectal cancer. *Biomedical and Environmental Sciences 15*, 166–171. 7

Appendix A

Nutrient limitation in Grahamstown Dam

Table A.1: Mean nutrient concentrations in μg/L $\pm$ standard errors (n=3) in all nurient enrichment assays measured on day 0 and day 4. Initial concentration (on day 0) were measured in samples taken from the filtered bulk water the experimental bottles were filled with. Amended nutrients (treatment PN on day 0) were measured in surrogate experimental bottles.

	Day 0				Day 4							
Treatment	initial	initial	PN	PN	C	C	N	N	P	P	PN	PN
Nutrient	N	P	N	P	N	P	N	P	N	P	N	P
April 2009 – Site 1	5.2±0.0	0.0±0.0	411±16	185±0	2.5±1.0	0.0±0.0	371±2	0.3±0.3	0.6±0.2	156±4	358±11	153±1
Site 2	8.6±0.7	1.0±1.0	390±1	186±3	0.3±0.2	0.0±0.0	368±3	4.5±2.4	0.6±0.6	147±13	339±8	154±2
Site 3	5.2±0.1	0.3±0.3	742±153	163±26	1.2±0.3	0.0±0.0	355±1	0.1±0.1	1.4±0.6	155±8	317±8	138±2
August 2009 – Site 1	0.0±0.0	0.0±0.0	474±2	186±1	0.0±0.0	1.8±1.0	458±3	0.0±0.0	0.0±0.0	151±7.2	454±2	158±4
Site 2	2.5±0.3	1.1±0.7	486±5	200±4	1.8±1.0	0.0±0.0	469±4	1.1±1.1	0.0±0.0	167±3	464±19	161±5
Site 3	2.0±0.1	2.4±2.4	470±1	178±5	1.3±0.7	0.0±0.0	460±3	0.0±0.0	0.2±0.2	150±5	445±0.6	151±9
Feburary 2010 – Site 1	3.2±0.1	1.2±0.1	379±0.9	175±0	3.9±2.6	1.1±0.3	360±2	1.8±0.5	1.5±0.2	152±3	247±41	110±9
Site 2	3.3±1.0	0.9±0.1	308±171	177±0.7	1.3±0.2	0.9±0.1	295±6	1.2±0.3	5.6±3.5	154±3.7	234±4	133±1
Site 3	3.0±1.0	1.2±0.2	327±2	181±2	1.2±0.1	1.1±0.1	287±5	1.3±0.3	1.2±0.1	155±1	221±3	130±2
May 2010 – Site 1	9.2±2.9	2.5±0.8	667±38	240±4	5.0±1.2	2.0±0.4	616±79	2.1±0.6	1.8±0.4	238±2.5	652±20	230±3
Site 2	2.5±0.7	1.7±0.4	615±120	248±14	1.8±0.3	2.1±0.4	440±4.3	3.3±1.7	1.1±0.2	243±0.3	422±11	156±77
Site 3	3.1±1.3	1.8±0.3	450±6	142±1.5	1.6±0.2	2.5±0.5	393±30	2.5±0.1	1.5±0.2	134±2.5	372±2.1	129±2
December 2010 – Site 1	6.1±1.7	5.0±0.9	413±12	151±5	1.4±0.1	3.6±0.3	415±6	3.6±0.1	2.2±1.3	129±2.4	316±7	105±1
Site 2	4.2±0.6	3.4±0.3	436±1.5	167±2.5	1.3±0.3	3.1±0.3	422±6	2.6±0.0	2.0±0.5	132±1	334±1.3	113±1
Site 3	1.4±0.1	3.0±0.1	427±1	162±2	1.2±0.3	4.4±0.7	396±4	3.9±0.2	1.4±0.1	134±1.3	239±27	118±2
May 2011 – Site 1	6.3±0.6	6.5±3.4	436±4	191±2	7.6±2.0	0.1±0.1	362±5.2	0.0±0.0	10.3±3.0	152±10	103±8	137±1
Site 2	8.4±0.6	0.3±0.3	416±22	184±1	0.8±0.4	0.0±0.0	344±2	0.0±0.0	1.3±0.2	143±1	308±8	140±2
Site 3	14.9±0.3	6.2±0.4	384±9	198±1	5.1±3.7	1.3±0.3	334±12	1.5±0.7	6.9±3.7	171±2.7	314±11	169±3
August 2011 – Site 1	20.8±3.1	0.8±0.6	494±6	183±2	11.3±0.6	0.5±0.3	457±11	0.1±0.1	0.8±0.6	141±2.6	444±4	141±1
Site 2	16.5±3.8	1.0±0.6	482±7	181±2	1.0±0.2	0.3±0.2	433±16	0.0±0.0	0.0±0.0	145±2	418±4	144±1
Site 3	19.9±0.4	1.2±0.3	487±9	186±2	129±118	53±51	465±3	0.6±0.2	0.3±0.2	157±1	272±131	104±52

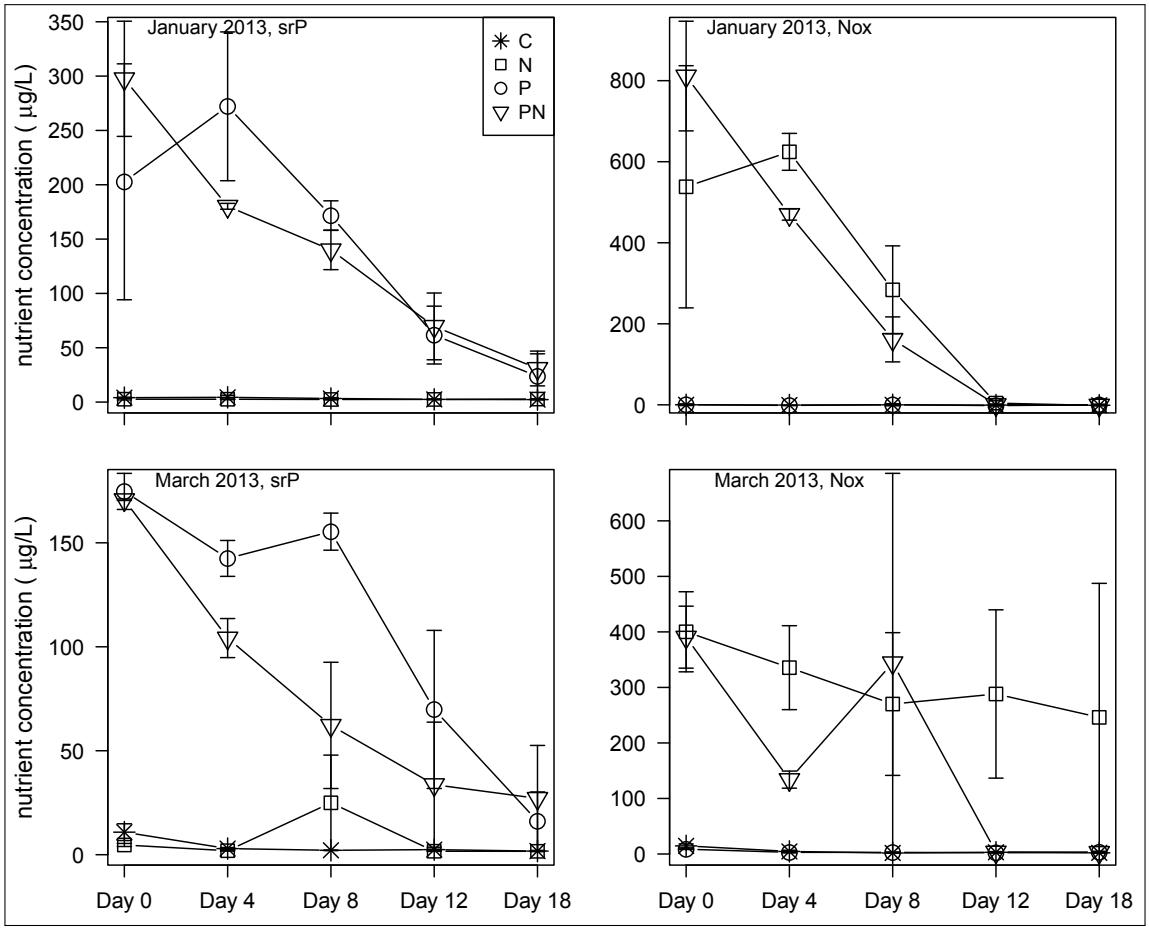

Figure A.1: Nurient concentrations in μg/L in the mesocosm assays. Error bars are one standard error from the mean, n=3.

Appendix B

Effects of light and trace metals on cyanobacterial growth in Grahamstown Dam

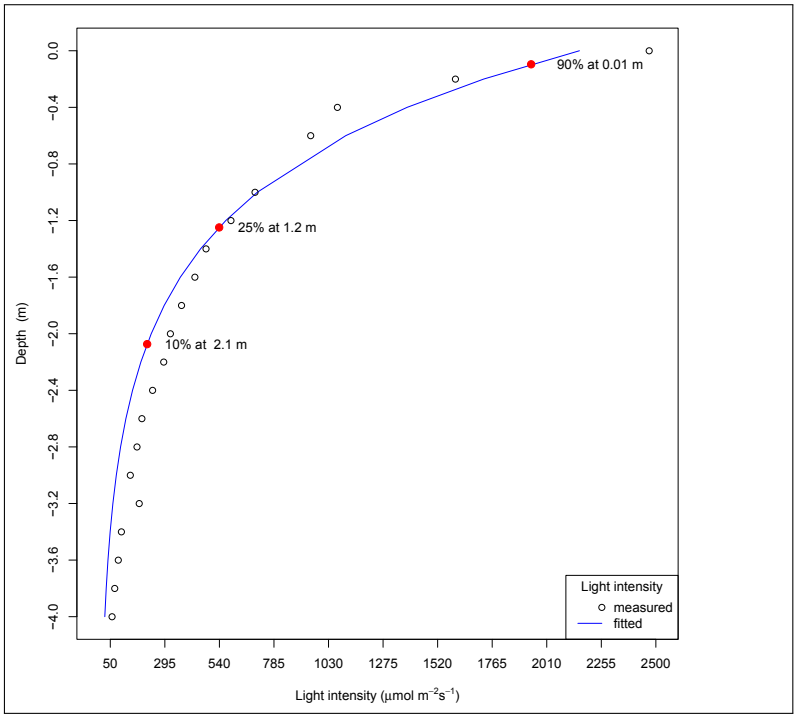

Figure B.1: Profile of the light intensity measured at the beginning of the light level assay. The line represents the result of a nonlinear regression (performed with the nls function of the statistics software R) of the measured values (R^2=0.96). Depth corresponding to 10, 25 and 90 % light intensity were calculated with the model fitted in the nonlinear regression.

Appendix C

Assessing the importance of the nutrients N and P for the growth of *Anabaena circinalis*

Table C.1: Swedish Standard (SIS) *Lemna* growth medium (OECD, 2006).

Stock solution			Prepared medium		
No.	Substance	g/L	mg/L	Element	mg/L
Ia	$NaNO_3$	17.0	85	Na; N	32; 14
Ib	KH_2PO_4	2.68	13.4	K; P	6.0; 2.4
II	$MgSO_4 \cdot 7H_2O$	15	75	Mg; S	7.4; 9.8
III	$CaCl_2 \cdot 2Hl_2O$	7.2	36	Ca; Cl	9.8; 17.5
IV	Na_2CO_3	4.0	20	C	2.3
V	H_3BO_3	1.0	1.0	B	0.17
	$MnCl_2 \cdot 4H_2O$	0.20	0.20	Mn	0.056
	$Na_2MoO_4 \cdot H_2O$	0.010	0.010	Mo	0.0040
	$ZnSO_4 \cdot 7H_2O$	0.050	0.050	Zn	0.011
	$CuSo_4 \cdot 5H_2O$	0.0050	0.0050	Cu	0.0013
	$CoCl_2 \cdot H_2O$	0.010	0.010	Co	0.0020
VI	$FeCl_3 \cdot 6H_2O$	0.17	0.84	Fe	0.17
	$Na_2EDTA \cdot 6H_2O$	0.28	1.4	-	-
VII	MOPS (buffer)	490	490	-	-

Stock solutions were stored at 4°C until use. To prepare one litre of SIS medium, the following are added to 900 ml of deionised water:

- 5 ml of stock solution Ia
- 5 ml of stock solution Ib
- 5 ml of stock solution II
- 5 ml of stock solution III
- 5 ml of stock solution IV
- 1 ml of stock solution V
- 5 ml of stock solution VI
- 1 ml of stock solution VII

The pH is adjusted to 6.5 $\pm$0.2 with either 0.1 or 1 Molar HCl or NaOH, and the volume is adjusted to one litre with ultrapure water.

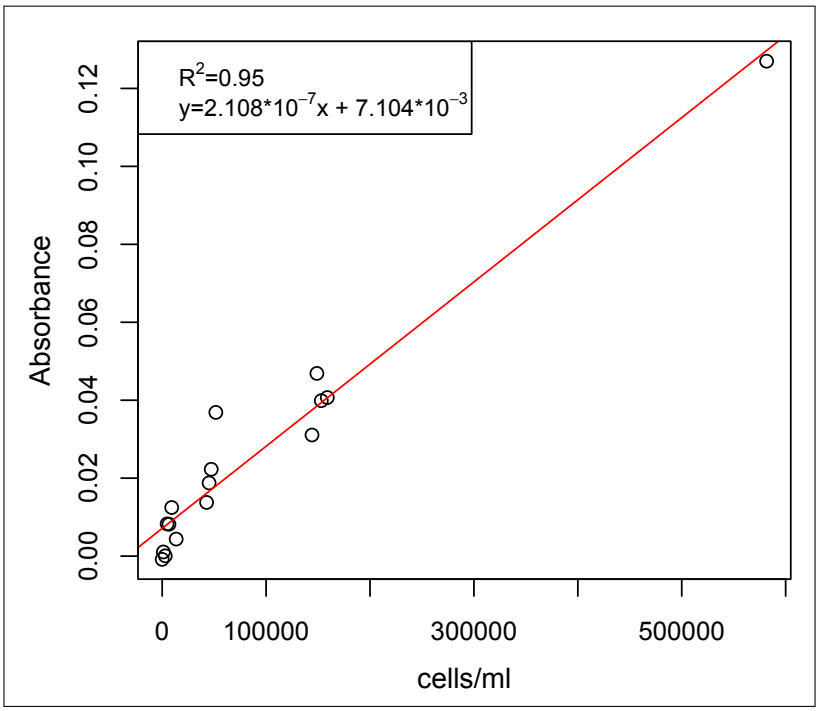

Figure C.1: Calibration curve of optical density (OD) and cell counts. OD was measured at 560 nm.

Table C.2: Base mixture for preparation of *Lemna* base medium (LM-N&P) used for batch experiments.

Solution	Component	Volume (ml)
II	$MgSO_4$	100
III	$CaCl_2$	100
V	Trace elements	20
VI	Fe/EDTA	100
VII	MOPS buffer	20
Total volume		340

Appendix D

Nutrient release from the sediments in Grahamstown Dam

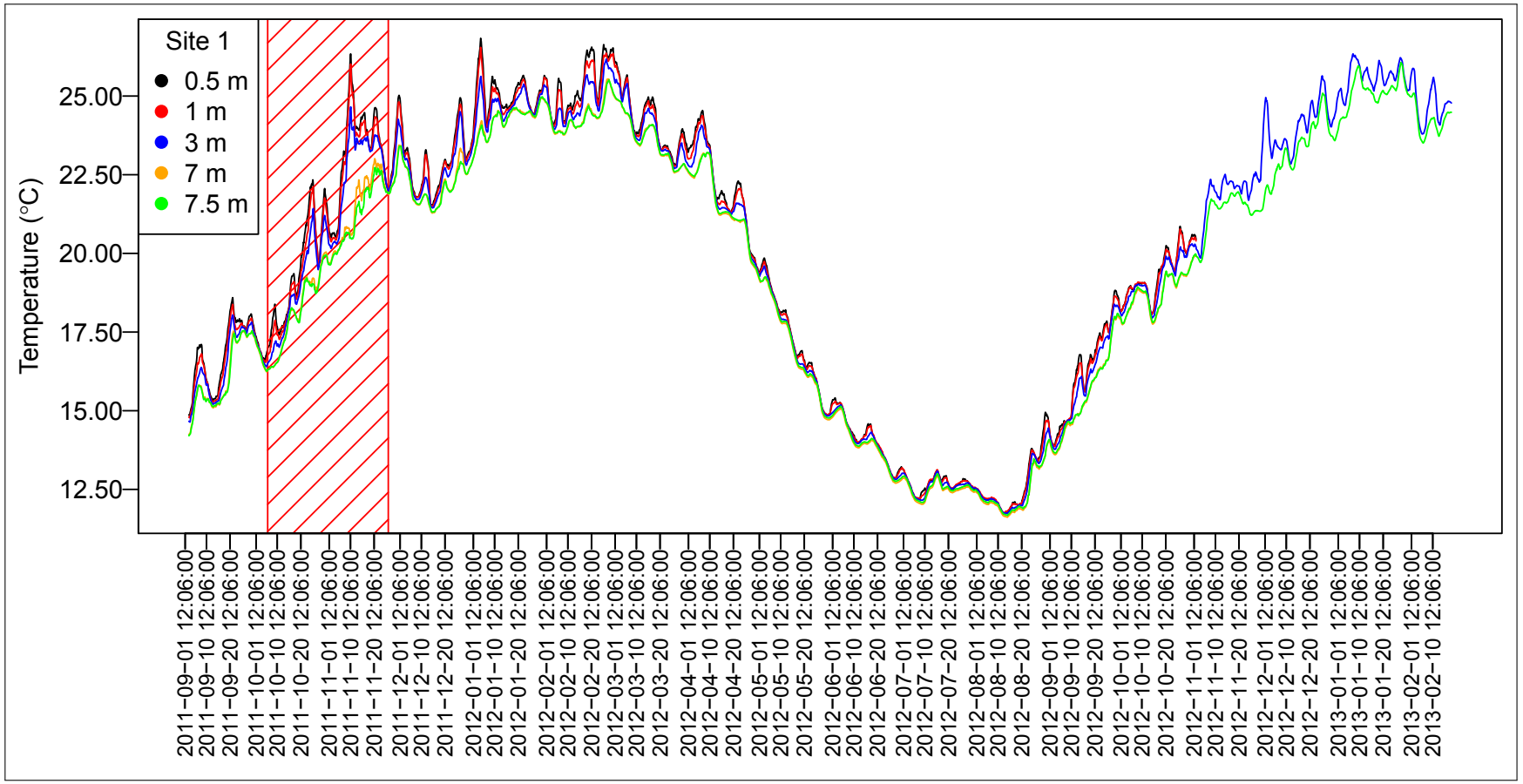

Figure D.1: Temperature profile of the water column at Site 1 in Grahamstown Dam. Data was smoothed with a rolling mean with a window of 100 observations. Temperature loggers at 0.5, 1 and 7 m stopped recording on the 2/11/2012. The shaded area represents a period with pronounced thermal stratification. Data from that periods is shown in more detail in Figure D.4.

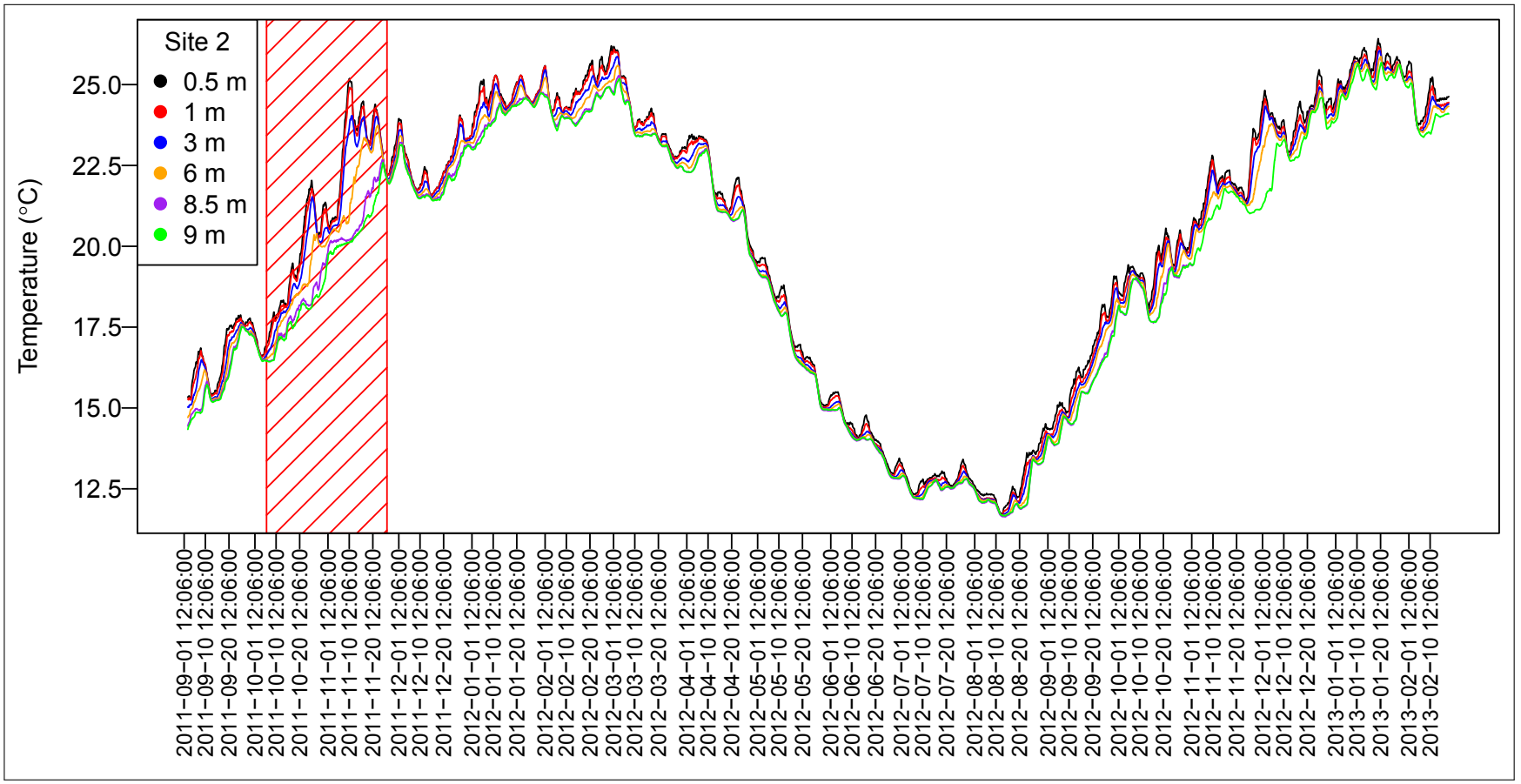

Figure D.2: Temperature profile of the water column at Site 2 in Grahamstown Dam. Data was smoothed with a rolling mean with a window of 100 observations. The temperature logger at 8.5 m was accidentally detached when downloading data on the 1/11/2012 and reattached on the 27/11/2012. Data recorded between those dates is not shown. The shaded area represents a period with pronounced thermal stratification. Data from that periods is shown in more detail in Figure 5.2.

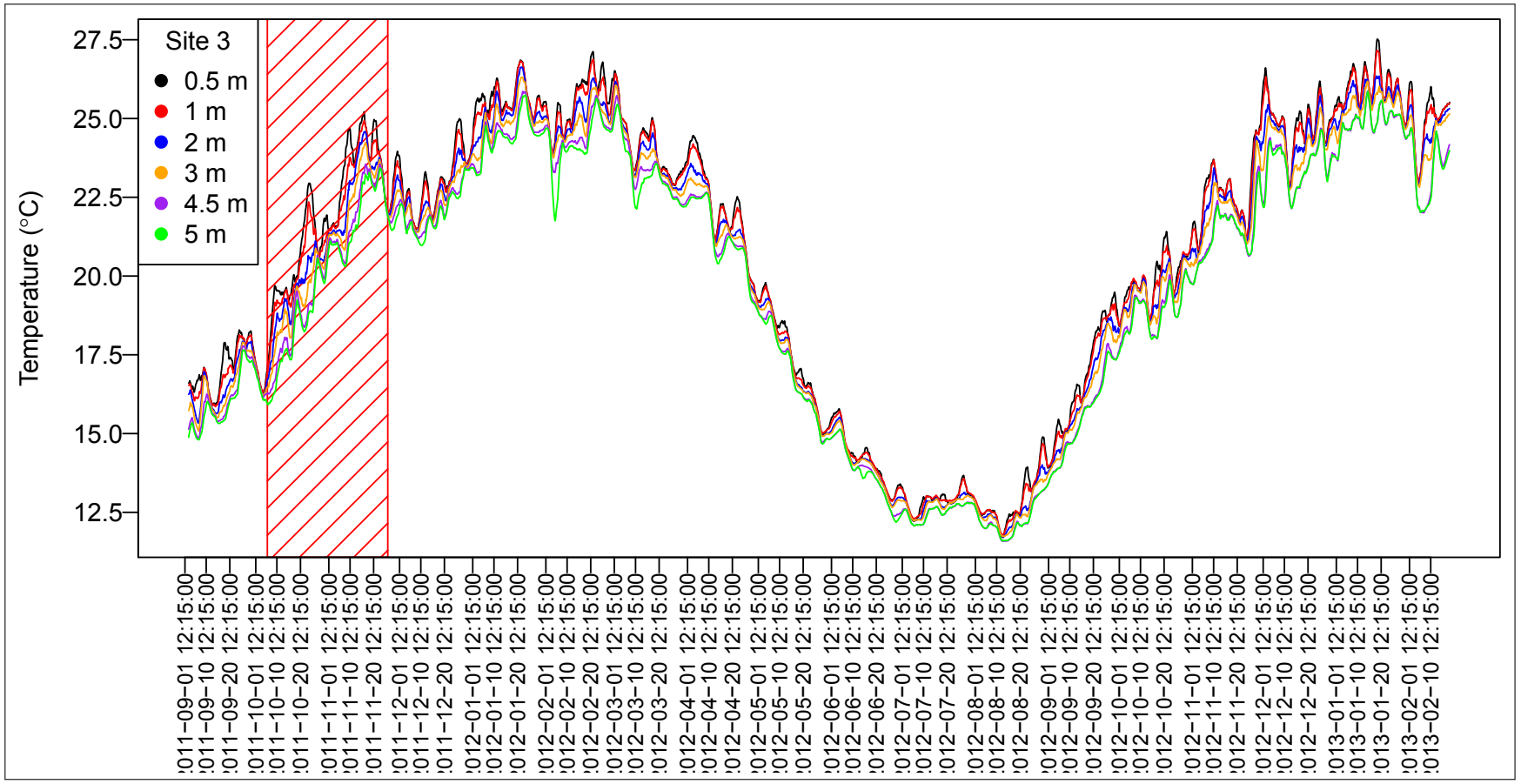

Figure D.3: Temperature profile of the water column at Site 3 in Grahamstown Dam. Data was smoothed with a rolling mean with a window of 100 observations. The shaded area represents a period with pronounced thermal stratification. Data from that periods is shown in more detail in Figure D.5.

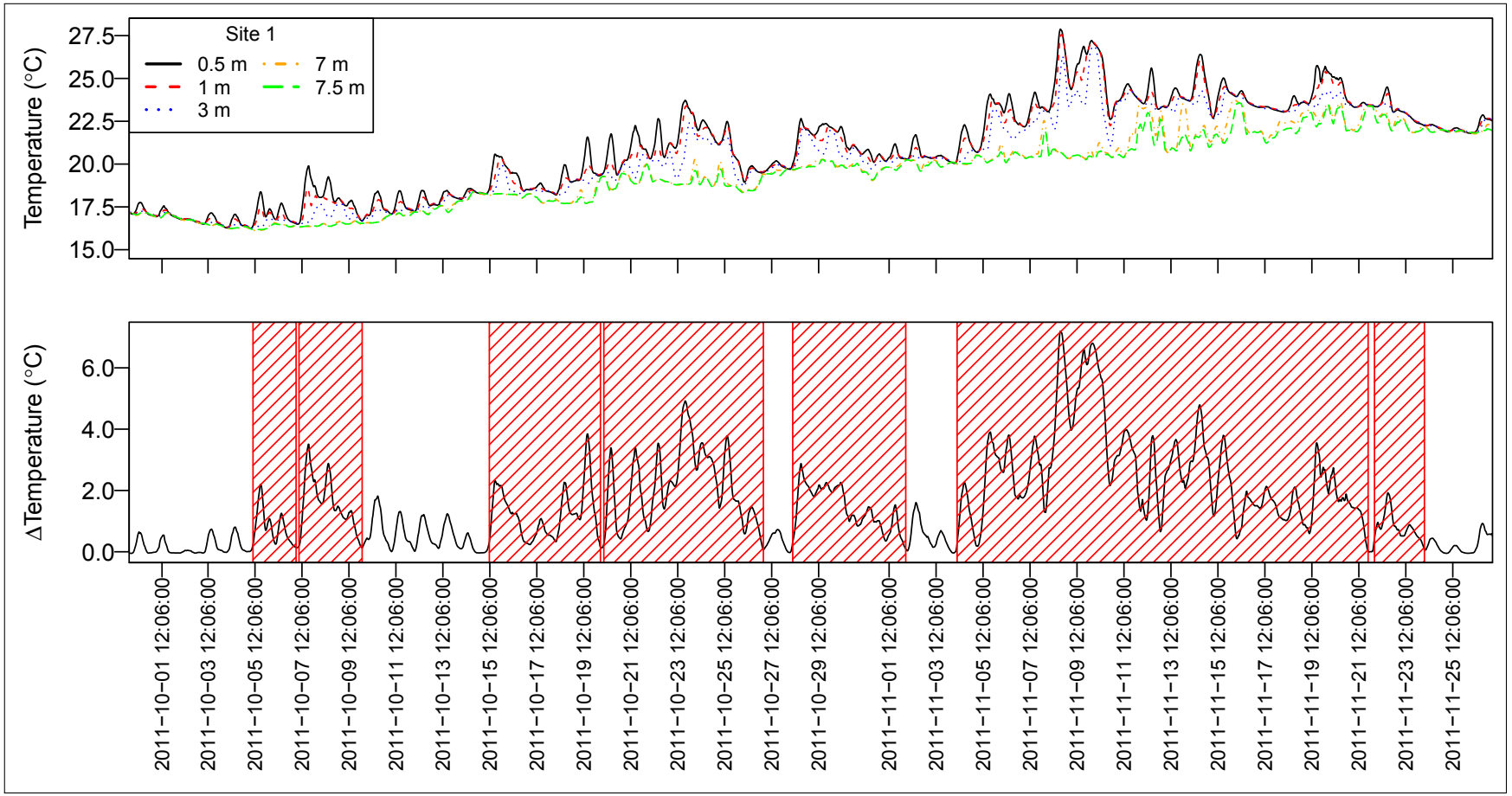

Figure D.4: Temperature profile of the water column and temperature differences between different water depths at Site 1 in Grahamstown Dam during the period of the most pronounced stratification between September 2011 and February 2013. Data was smoothed with a rolling mean with a window of 48 observations. Shaded areas highlight temperature differences larger than 0.15°C and lasting for more than 24 hours. Data was smoothed with a rolling mean with a window of 10 observations.

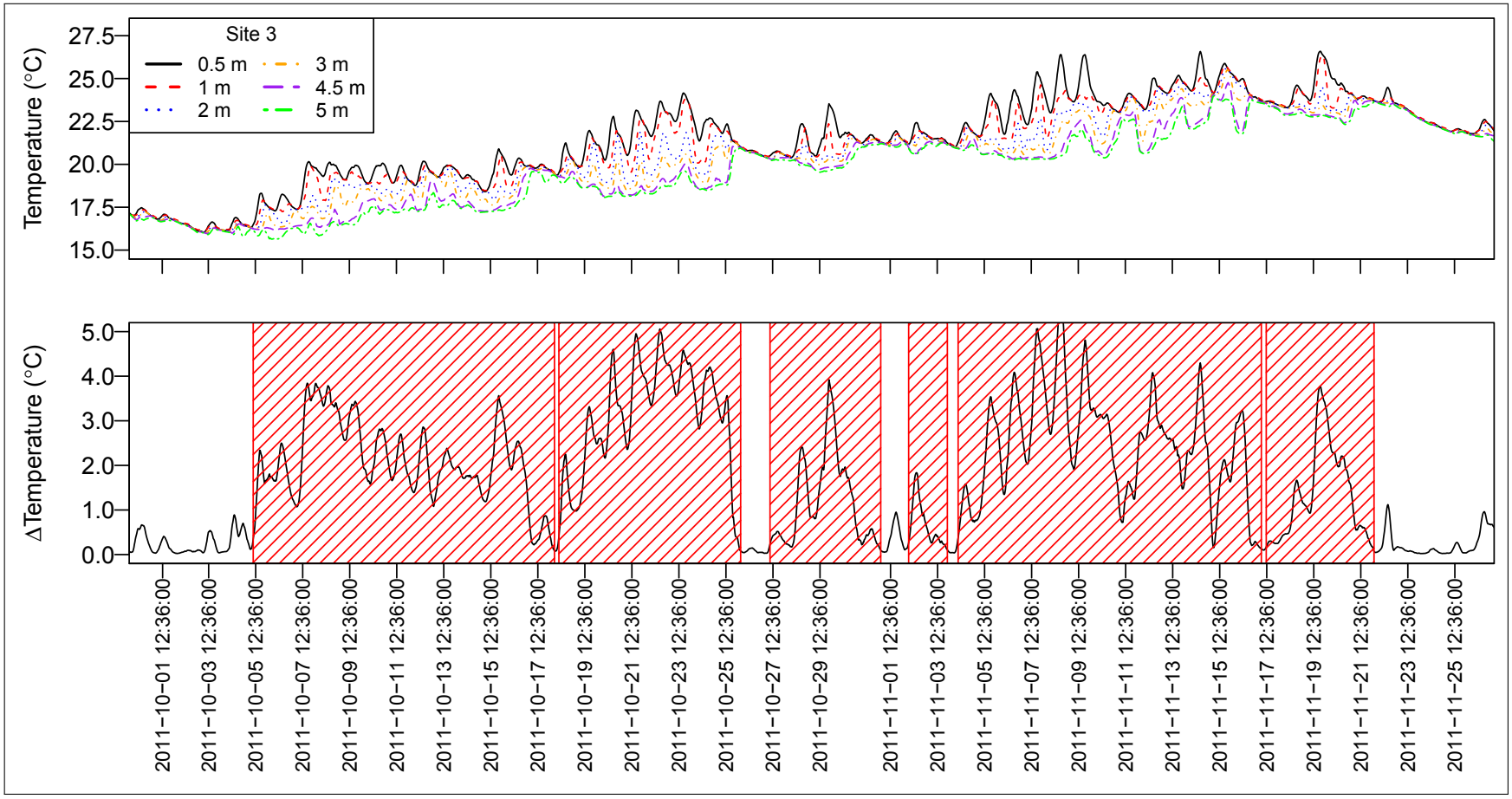

Figure D.5: Temperature profile of the water column and temperature differences between different water depths at Site 3 in Grahamstown Dam during the period of the most pronounced stratification between September 2011 and February 2013. Data was smoothed with a rolling mean with a window of 48 observations. Shaded areas highlight temperature differences larger than 0.15°C and lasting for more than 24 hours. Data was smoothed with a rolling mean with a window of 10 observations.