Nutrients and their role in promoting cyanobacterial growth along the Murray River, Australia

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Terence A. Rogers

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Freshwater and Estuarine Research Group
School of Life Sciences
University of Technology Sydney

Certificate of original authorship

I, Terence Rogers, declare that this thesis is submitted in fulfilment of the requirements for the award of Masters of Science, in the Faculty of Science at the University of Technology Sydney. This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by an Australian Government Research Training Program.

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Preface

This thesis consists of five chapters. Chapters 2 to 4 have been written as separate articles that are in review, or are in preparation for submission to peer reviewed scientific journals. These papers are included as their submitted form, and as such, some repetition occurs between chapters. To prevent unnecessary duplication, a single reference list has been provided at the end of this thesis.

This thesis is a compilation of my own work, carried out with guidance from my supervisors and others. I conceptualized this research, conducted all data collection and analysis and wrote the manuscript. Publication details and contributions of co-authors are detailed below.

Chapter 2: Rogers, T.A., Walton, J.E., Facey, J.A., Kobayashi, T., Williamson, N., Mitrovic S.M. Co-limitation by nitrogen and phosphorus for phytoplankton growth while phosphorus limits diazotrophic cyanobacteria in the Murray River, Australia.

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Abstract

Nutrients are important in structuring the phytoplankton community and regulating cyanobacterial blooms. In riverine environments, the prevalence of cyanobacterial blooms has increased, therefore the need to understand how nutrients are interacting with phytoplankton is significant. The macronutrients nitrogen (N) and phosphorus (P) are known drivers of cyanobacterial growth, and micronutrients such as sulfur and the trace metals iron, cobalt and manganese can also influence bloom formation. This thesis examined what macronutrients limit cyanobacterial and phytoplankton growth, spatial and temporal nutrient dynamics and sediment nutrient release within the Murray River, Australia. A combination of lab-based incubation experiments, *in-situ* microcosm experiments and long-term seasonal monitoring was conducted. Microcosm experiments were run *in-situ* over 2020–2021 to examine whether N or P or a combination of both, limited phytoplankton and cyanobacterial growth during warmer temperature bloom periods. These experiments indicated that the overall phytoplankton community may be limited by P or co-limited by N and P, whereas most N-fixing cyanobacteria were limited by P only. The control of P in the river may be a useful way to reduce nitrogen fixing cyanobacterial bloom concentrations. Monitoring of nutrient concentration including differences between surface and bottom water column concentration across the five sites was performed to see how they changed spatially and temporally. This was conducted at four weir pool and one riverine site across 24 months along the Murray River. There were only minor differences between top and bottom waters suggesting bottom waters and hence sediments are not a major source. Filtered (0.45 um) available nutrient concentrations were very low generally, suggesting nutrients are possibly locked up in phytoplankton biomass. However, NO_x concentrations decreased downstream. Total nutrient concentrations were low for TP, however TN increased downstream. I also investigated experimentally the release of nutrients from riverine sediments from Kulnine and Mildura weir pools under oxic and anoxic conditions. Under anoxic treatments there were clear releases of ammonium, iron, cobalt, manganese, and sulfur. Similarly, ammonium, cobalt, manganese and sulfur were released under oxic conditions, while NO_x was also released just at Kulnine. We found no release of P from sediments. These studies provide novel information for the understanding of nutrients and their role in phytoplankton and cyanobacterial growth in the Murray River. Results from this study may be useful in the management of cyanobacterial blooms as they provide a better understanding of what might be influencing these blooms.

Chapter 1: Introduction

1.1 Background

Once thought to be a rare event, algal blooms in Australian rivers are increasing in occurrence and severity. In 2016 a toxic cyanobacterial bloom affected 1,600 km of the Murray River and this was the fourth large bloom in the river in 10 years (Bowling et al. 2018). This is important as the Murray River is the longest river of the second largest drainage in Australia (the Murray Darling Basin), spanning 2,530 km and discharging ~12,000 GL of water annually (MDBA, 2022). The summer of 2018–2019 also saw mass fish kills in the Lower Darling River near Menindee influenced by extreme weather, algal blooms, low river flows and water column mixing events (MDBA, 2022). With growing pressure on water resources and future climate scenarios predicting higher temperatures and substantial reductions in rainfall, the warm low-flow conditions that often drive algal bloom formation are likely to become more common (Paerl and Otten, 2013). Exposure to toxic cyanobacteria (blue-green algae) is the main concern associated with algal blooms as these are a serious public health risk. Toxic cyanobacteria can affect water supplies, stock watering and recreation though production of hepatotoxins (liver toxins which can also be carcinogens), cytotoxins, neurotoxins (some implicated in neurodegenerative diseases) and/or dermatoxins (skin irritants) (Carmichael, 1992). The scale and time-critical nature of managing blooms necessitates professional and informed management. A key requirement for this a good understanding of the conditions leading to the development of toxic cyanobacterial blooms in the Murray River.

Cyanobacteria, commonly referred to as blue-green algae, are photoautotrophic prokaryotes that can exist as independent cells, or as colonies / filaments (Whitton and Potts, 2007). Sometimes these colonies / filaments grow so large that they form visible mats at the water's surface (scums). Although cyanobacteria are an integral part of the River Murray's ecology, and blooms are not abnormal (Shiel et al. 1982), the recent years have seen an increase in the frequency and magnitude of these blooms (Bowling et al. 2018). They are an economic (Falkenmark et al. 2007), social (Falconer, 2001), and environmental problem (Gehrke and Harris, 1994). Part of this burden is due to toxic substances (cyanotoxins) produced by a few species of cyanobacteria (Carmichael, 1992; Catherine et al. 2013), leading to the development of Harmful Algal Blooms (HABs). There are three main perpetrators in the

Murray River. These are *Dolichospermum circinale* – capable of producing saxitoxins (Daneshian et al. 2013); *Microcystis aeruginosa* – responsible for the release of microcystin-LR (Falconer, 2001); and *Chrysosporum ovalisporum* – capable of producing the hepatotoxic and cytotoxic cylindrospermopsin (Shaw et al. 1999).

The occurrences of cyanobacterial blooms are influenced by an array of environmental factors. These include light attenuation (Huisman et al. 1999), pH (Paerl et al. 2011), water temperature (Davis et al. 2009), nutrient levels (Vézie et al. 2002), zooplankton and protozoan grazing (Canter and Lund, 1968), salinity (Tonk et al. 2007), river flow (Mitrovic et al. 2003), turbulence (O'Brien et al. 2004) and buoyancy regulation (Reynolds et al. 1987). In this study I focus on the role of nutrients in the Murray River.

1.2 Nutrients and cyanobacteria

Phytoplankton growth depends upon the availability of macronutrients within their environment. Historically, rivers such as the low-lying Murray River would derive much of their nutrients from natural inflows as a result of floods or heavy rain bringing in catchment-based nutrients. As a result of catchment modification, such events bring more anthropogenically derived nutrients from farming and urban areas. As lowland river systems such as the Murray have seen considerable development in the form of flow regulation (Walker and Thoms, 1993) structures such as weirs and dams have been built along the river. These can act as sediment traps (Olive and Olley, 1997; McCarthy et al. 2004) allowing finer sediments suspended in the column to settle, leading to sedimentation of nutrient rich organic matter, settling of adsorbed sediments and possible water column resuspension of nutrients (Webster et al. 2001; McCarthy et al. 2004; Boström et al. 1988). Also, sediments can sometimes act as nutrient sources and as sinks, depending on flow rate and seasonal thermal stratification patterns (Baldwin and Williams, 2007; Müller and Mitrovic 2015). Further, during these times of lower or reduced flow (such as in droughts) water impoundments such as weirs may lead to extended periods of thermal stratification, a state that particularly favours the potentially toxic cyanobacteria to bloom (Bormans et al., 2005; Webster et al. 2000; Mitrovic et al. 2003; Mitrovic et al., 2011). It can also lead to low bottom water (hypolimnial) dissolved oxygen concentrations due to biological processes (Turner and Erskine 2005; Rowe 2001; Sigee 2005; Becker et al. 2010). Therefore, thermal stratification events may result in changes to the sediment chemistry and redox potential which may result in nutrient release from sediments (Mortimer, 1971; Mueller et al. 2016). Typically, both nitrogen (N) and phosphorus (P) released from sediments are readily available for cyanobacterial use, where their contribution can lead to blooms or increased phytoplankton abundances (Jones and Poplawski, 1998; Xie et al. 2003; Bormans et al. 2004; Baldwin and Williams, 2007; Song et al. 2006).

When looking at cyanobacterial blooms, and how nutrients contribute to their formation, it is important to look at how available nutrients are in the water column, their sources and how they influence cyanobacteria and other phytoplankton. The role of nutrients in cyanobacterial blooms around the world is variable. However, in rivers of the Murray Darling Basin for example, other factors are more readily known to influence cyanobacterial blooms such as warmer water temperatures (Bowling and Baker, 1996; Whiterod and Sherman, 2012; Bowling et al., 2013), increased transparency & lower turbidity (Bowling and Baker, 1996; Bormans and Webster, 1997), low flows (Bormans and Webster, 1997) and low surface winds (Webster et al. 2000) as well as thermal stratification (Bormans and Webster, 1997; Mitrovic et al. 2003). Factors such as these can control or dictate cyanobacterial bloom formation, but nutrient availability may also play a role in determining which species may bloom and the overall biomass of blooms. Therefore, understanding how blooms are influenced by nitrogen and/or phosphorus is important. Phosphorus is often considered the main limiting nutrient to phytoplankton growth (Schindler et al. 2008) and many studies have found phosphorus plays an important role in bloom formation (Bowling and Baker, 1996; Ha et al. 1999; Davis and Koop, 2006; Webster et al. 2000; Bowling et al. 2013). However, nitrogen is sometimes also limiting and the formation of blooms can be driven by nitrogen (De Leon and Yunes, 2001; El-Manawy and Amin, 2004; Krogmann et al. 1986; McCarthy et al. 2009). In some waters, co-limitation occurs where both P and N are limiting, and increased growth only occurs when both are added together (Müller and Mitrovic 2015; Elser et al. 2007). Understanding how nutrients interact with flow and how they generally influence phytoplankton and cyanobacterial growth is important in river management.

1.3 Scope and need for this study

This thesis is part of larger study funded by the Murray Darling Basin Authority “Flow and stratification based algal bloom prediction model for the Murray River” examining how flow, thermal stratification, weather and nutrient patterns influence cyanobacterial growth. This was a multi-person project involving UTS, University of Sydney, Department of Environment and the Murray Darling Basin Authority. In this thesis I present results I have collected during the study on nutrients and their potential influence on cyanobacteria. I at times refer to other data such as thermal stratification data collected in the broader study.

1.4 Aims of thesis

The overall aim of my study is to understand more about how nutrients may influence cyanobacterial growth in the Murray River. This was done by a series of field and laboratory experimental studies and a field monitoring campaign. The aims and need for each chapter are given below.

Chapter 2 - I examined which nutrients are limiting cyanobacterial growth and other phytoplankton across different sites along the Murray River. This will allow a better understanding of how nutrients may be affecting phytoplankton and cyanobacterial growth in the Murray River.

Specific aim was:

- To understand whether N and P or a combination of them, limit phytoplankton and cyanobacterial growth during warmer temperature periods, across multiple weir pool and riverine sites in the Murray River

Chapter 3 - I examined top and bottom differences in nutrient concentration within the water column, and if they changed spatially or temporally. I also assessed nutrient concentrations seasonally, across different sites along the Murray River.

Specific aims were:

- To monitor nutrient concentrations, including longitudinal and seasonal trends in the Murray River over a 24-month period, to understand their possible role in cyanobacterial blooms.
- To compare nutrient concentrations in surface and bottom waters to understand the role of sediment derived nutrients.

Chapter 4 - I examined the possible role of sediment nutrient release from two weir pool sties on the Murray River, Australia. I conducted two incubation experiments with sediments under oxic and anoxic conditions to see what was released.

Specific aim was:

- To determine which nutrients are released from sediments under oxic and anoxic conditions and their respective concentrations in the overlying water in experimental chambers.

Chapter 5 - In this chapter, results of chapters 2, 3 and 4 are discussed and overall conclusions are drawn.

Chapter 2: Co-limitation by nitrogen and phosphorus for phytoplankton growth while phosphorus limits diazotrophic cyanobacteria in the Murray River, Australia.

2.1 Abstract

The macronutrients such as nitrogen (N) and phosphorus (P) play essential roles in the growth, development and structure of phytoplankton communities and formation of blooms. Importantly, these nutrients can limit the growth of bloom species, and ultimately the structure of the phytoplankton communities present. This is particularly important as nutrients could then influence the selection of diazotrophic or non-diazotrophic cyanobacterial blooms in riverine environments. To assess the influence of N and P on the cyanobacterial and wider phytoplankton community, we conducted six in-situ nutrient amendment experiments across five riverine sites along the Murray River, Australia. Cyanobacterial blooms were present at Torrumbarry, Euston, Mildura and Kulnine sites at the onset of experiments. Dominating cyanobacterial species during the study included the diazotrophic *Dolichospermum crassum*, *Dolichospermum planctonicum* and *Chrysosporum ovalisporum*. Using chlorophyll-a as a surrogate measure of the phytoplankton community biomass, we found evidence of co-limitation by P and N at Corowa, Euston and Kulnine, while P was found to be limiting at Torrumbarry and Mildura. Cyanobacterial species responses to nutrient additions indicated P was limiting their growth in the Torrumbarry, Euston, and Mildura experiments. Ambient concentrations of available nutrients were low at these sites which may have influenced the diazotrophic species response to P amendment. Ambient concentrations were higher at Kulnine at the onset of experimentation, and diazotrophic cyanobacteria were not limited by nutrients. We conclude that P is limiting diazotrophic cyanobacterial growth, and P reductions will likely be most effective in reducing cyanobacterial biomass in the Murray River.

2.2 Introduction

Riverine systems are becoming increasingly threatened by anthropogenic alteration and degradation. Catchment modification, urbanisation, agricultural development and in-stream alterations have changed many natural limnological processes (Cassardo and Jones, 2011; Dudgeon, 2019). These processes can influence the availability of macronutrients such as nitrogen (N) and phosphorus (P), increasing the risk of nutrient over-enrichment and consequent excessive algal and macrophyte growth, known as eutrophication. This can also drive the growth of phytoplankton including planktonic cyanobacteria (blue-green algae) to bloom proportions (Davis and Koop, 2006; Paerl et al. 2019). This is concerning as some cyanobacteria produce an array of toxic compounds, collectively known as cyanotoxins, implicated in many instances of human and animal illness and death (de la Cruz et al. 2012; Carmichael, 1992; Mowe et al. 2015). Bloom events have the capacity to cause severe environmental, economic and human health problems (Carmichael, 2001; Paerl and Otten, 2013; Pilotto et al. 1997; Testai et al. 2016).

In large lowland riverine environments, N and P can be derived from outside the water column from the catchment (allochthonous) as well as internally within the water column (sediments and recycling) (Davis and Koop, 2006; Vink et al. 2005). Increases in anthropogenic point and non-point source pollution from sewage outfalls, cleared land and agricultural development can elevate N and P levels in rivers and drive increased phytoplankton growth (Davis and Koop, 2006; Mosley and Fleming, 2010, Mainstone 2002). Furthermore, impoundments made by dams and weirs on rivers increase water residence times, stimulating thermal and oxygen stratification (Bormans et al. 2004; Mitrovic et al. 2011). This may aid the release of sediment-bound nutrients to the water column during anoxic bottom water periods, which can be redistributed by a mixing event, or exported downstream, particularly in the case of bottom release impoundments (Jung et al. 2014; Paerl, 2008).

Growth of phytoplankton is heavily dependent on the availability of N and P, meaning they can become the limiting factor for growth (Elser et al. 2007). Based on Liebig's law of the minimum, nutrient limitation is the limitation of the potential rate of net primary production by resources (Harpole et al. 2011; Howarth, 1988). It implies that only single nutrients can limit primary productivity. However, it has been found that N and P can co-limit productivity (Elser et al. 2007; Harpole et al. 2011; Müller and Mitrovic, 2015; Paerl et al. 2011). Harpole et al. (2011) defines co-limitation as either simultaneous (where if two nutrients are added together they elicit a response, but individual additions show no response) or independent co-limitation (where there is a greater response from dual nutrient additions, contrasted to the single nutrient additions). Based on the work of Schindler et al. (2008), the current paradigm in freshwater systems is that in most situations P is limiting. However, this is challenged by many studies finding either N limitation (Krogmann et al. 1986; McCarthy et al. 2009) or co-limitation by N and P (Müller and Mitrovic, 2015; Paerl et al. 2011). Understanding what nutrients limit phytoplankton growth can help in the development of effective targeted management actions to reduce potentially toxic phytoplankton blooms (Wurtsbaugh et al. 2019).

One of Australia's major rivers is the Murray River, spanning over 2000 km in length and passing through 3 states. The prevalence of cyanobacterial blooms has increased in the Murray River (Bowling et al. 2018; Walker and Hillman, 1982), with nearly all blooms originating in weir pools (Al-Tebrineh et al. 2012; Scholz et al. 2003) or reservoirs (Bowling et al. 2018, 2013). These blooms are typically monospecific, and several reoccurring species dominate such as *Microcystis flos-aquae*, *Dolichospermum circinale* and *Cylindrospermopsis raciborskii*. In recent years, new species such as *Chrysosporum ovalisporum* have bloomed along most of the Murray River, with its origins thought be from the large upstream Hume Dam (Bowling et al. 2018). *Dolichospermum, Cylindrospermopsis* and *Chrysosporum* are all able to fix atmospheric N and some species in these genera have the ability to produce different cyanotoxins (Carmichael, 2001). *Dolichospermum* spp. have been known to produce saxitoxins, with *Cylindrospermopsis* and *Chrysosporum* known to produce cylindrospermopsin (Carmichael, 2001; Falconer, 2001).

Little information on the limiting nutrients for phytoplankton growth in the Murray River are available. Harris (2001) suggested that the Murray River is likely N-limited due to denitrification processes within the river. In-situ nutrient enrichment experiments in Lake Mulwala (a large lake formed by a weir impoundment along the river) found strong evidence of P limitation as well as co-limitation of N and P to phytoplankton growth (Walker and Hillman, 1982). However, these experiments were carried out from June to August (austral winter) and no cyanobacterial bloom was present at the time (diatoms and green algae dominated in their samples). During the cyanobacterial blooms, there have been no direct tests of limiting nutrients and the available literature has only commented on the possible influence of nutrients as drivers (Bowling et al. 2018; Scholz et al. 2003). Knowing what nutrients to target for nutrient reduction strategies and risk management frameworks is a major gap for management of blooms in the river.

Our research strives to fill these gaps, particularly for the dominating cyanobacterial species that are increasingly prevalent across weir pools in the Murray River. This study aims to understand whether N and P or a combination of them, limit phytoplankton and cyanobacterial growth during warmer temperature periods, across multiple weir pool and riverine sites in the Murray River spanning approximately 1300 km. We hypothesised that phytoplankton would be P-limited during summer periods and dominant cyanobacteria would also be limited by P across all sites.

2.3 Methods

2.3.1 Study sites

The Murray Darling Basin is Australia's second largest river catchment system, which sits in the South-East of the country and spans across five states and territories. Within the basin is the country's largest river, the Murray, which runs for ~2530 km and is the terminus for the Basin. Post-colonial regulation of the river in the form of weirs, locks and channels has allowed for the development of towns and agriculture along the river. The development of The Snowy Mountains Scheme, a hydroelectric and irrigation project, has also influenced the Murray. The project meant water is diverted into the watershed of the Murray River from other headwaters where it is then stored and controlled in dams. This water is then released into the Murray and re-regulated in Hume Dam for irrigation and anthropogenic purposes (Snowy Hydro Limited, 2021). Consequently, these riverine alterations such as dams have created ideal conditions for cyanobacteria to bloom (Scholz et al. 2003).

Weir pools within the Murray River are an area of interest as they are the major contributor to flow reduction in-stream and consequentially are the sites of recurring cyanobacterial blooms (Bowling et al. 2018; Scholz et al. 2003; Walker and Hillman 1982). These weirs are also often situated alongside major townships on the river so are used for drinking water, recreation and irrigation. Four weir pools and one riverine site were selected along the Murray River, which forms a large part of the border between New South Wales and Victoria, Australia (Figure 1). The weir pool sites chosen for this study are along approximately 1300 km of river and are often influenced by irrigation canals, tributaries and anabranches. Sites were monitored during the summer months of 2019–2020 and 2020–2021 in conjunction with obtaining algal bloom reports from the New South Wales State Government's Murray and Sunraysia Regional Algae Coordinating Committees (RACCs) so that experiments would coincide with potentially toxic cyanobacterial blooms if possible.

Microcosm nutrient-enrichment experiments were conducted at Mildura and Euston in January 2020, Torrumbarry in February 2020, as well as Kulnine, Corowa and Mildura again in April 2021. Lock 9 (Kulnine Weir) sits 765 km from the mouth of the Murray River (Figure 2.1). The weir pool extends for ~63 km, while the river is mostly ~130 m wide and ~5 m deep along its length. The weir is a source of water for both Lake Victoria and Lake Cullulleraine, and it lies downstream of the Murray-Darling confluence, making it the only site to be potentially affected by the Darling River. Wallpollo Creek and the Great Darling Anabranch both meet the Murray in the Kulnine weir, however they very rarely flow and usually act as large backwaters. The riparian vegetation is quite extensive for most of the weir pool, with much of the riverbank untouched and heavily vegetated. The area selected for experimental setup was within 2 m of the bank (141.600282 °E, -34.192232 °S) and had a northerly aspect for even sun exposure.

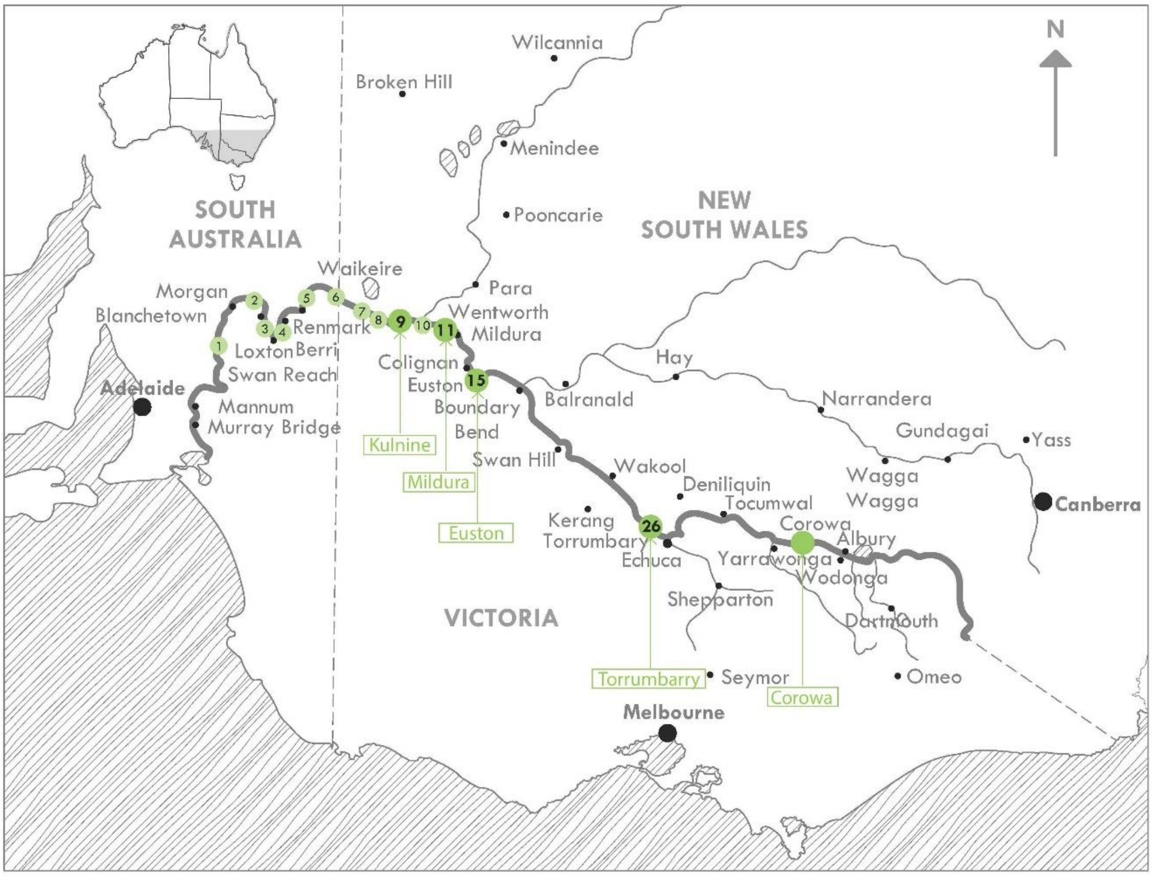


Figure 2.1. Map of the Murray River, Australia, with the five sites chosen for experimental setup.

Mildura Weir, lock 11 is situated 113 km upstream of the Kulnine weir (Figure 2.1). The weir has a lock which is separated by a small island that at capacity creates a weir pool ~60 km long. Upstream of the weir, the river is ~176 m wide and ~5 m deep, and has very few tributaries. The weir pool riparian zone is subject to a large amount of urban land, such as the town of Mildura, which along with many farms and sparse riparian vegetation, are likely to be large contributors to allochthonous derived nutrients. The area selected for experimental setup was within 4 m of the riverbank (142.175219 °E, -34.182928 °S) and had a south westerly aspect, which allowed for prolonged sun exposure and part shade evenly for all treatment bottles.

Euston weir, lock 15 is the next upstream weir, 232 km upstream of Mildura (Figure 2.1). The weir pool is typically ~120 m wide and ~5 m deep, creating a variable weir pool length of 60–100 km. The majority of the riparian zone for this weir pool is native vegetation, however both Euston and Robinvale share its banks close to the weir. The Murrumbidgee River and Edward/Wakool Anabranch are two major confluences within the Euston weir pool and are potentially important nutrient sources. The nutrient enrichment setup was within 4 m of the riverbank (142.744813 °E, -34.581027 °S) and had an easterly aspect, with some shade in the late afternoons.

Torrumbarry weir, 528 km upstream of Euston was the most upstream site, ~1638 km from the Murray River's mouth (Figure 2.1). The weir pool close to the weir is ~70 m wide and ~6 m deep however the pool shallows to ~4 m a short distance upstream. The weir pools riparian zones are heavily dominated by vegetation, as it runs through Gunbower National Park, with the only major urban influence being Echuca ~40 km upstream. The Campaspe and Goulburn rivers are two tributaries of the weir pool that are fed by large dams, with some of this water exiting the system through an irrigation channel and into Gunbower Creek. The nutrient enrichment experimental setup had an easterly aspect and was setup within 5 m of the riverbank (144.466037 °E, -35.944616 °S). Treatment bottles were suspended from fixed floating buoys and had limited late afternoon shade.

Finally, Corowa is the only non-weir pool riverine site of the study. The study area is ~440 km upstream of Torrumbarry weir, and is typically ~70–80 m wide and ~3 m deep. The riverbanks are moderately vegetated with mostly agricultural land beyond the vegetation within the floodplain, while upstream is the tributary of the Kiewa River and the large Hume Dam. The nutrient enrichment setup was placed within 2 m of the riverbank (146.378395 °E, -36.017845 °S) and had a westerly aspect, where treatment bottles experienced some shade during late afternoon hours.

2.3.2 Microcosm enrichment assays

In-situ microcosm nutrient assays were undertaken at each site to determine the limiting nutrients of cyanobacterial growth and to learn how increased nutrient availability influenced phytoplankton community structure. Each microcosm experiment lasted for 8 days, and experimental setup was similar to Facey et al. (2021). Microcosm treatments consisted of a Control (C), Nitrogen (N), Phosphorus (P), and a Nitrogen/Phosphorus combination (NP), which were all tested in triplicate per site. Amended nutrient addition concentrations were 500 µg N/L in the form of KNO_3 (N treatment) and 200 µg P/L in the form of KH_2PO_4 (P treatment) for each microcosm treatment. A combination of both were used at the same concentrations for the NP treatment. The amended nutrient concentrations were selected so nutrient availability was in excess but within a natural range expected in inland Australian river systems (Furst et al. 2019).

The experimental setup consisted of 3-L polyethylene bottles suspended with rope within the river with floats. Bottles were positioned within the euphotic zone near the water surface, ensuring that underwater light was not a factor limiting phytoplankton growth during the experiments. Microcosm treatments bottles were pre-rinsed and then filled from a large plastic tub with approximately 120 L of surface river water, which was pre-filtered through a 75-µm plankton net to exclude zooplankton, preventing/reducing any phytoplankton change due to grazing. The fraction left in the net was analysed post experiment for each site to ensure that it did not exclude any phytoplankton that were absent from the filtrate. Bottles were left with a small air space and nutrient amendments were added using calibrated pipettes. Bottles were lightly mixed through inversion and set along the stringline.

Water samples were collected and physicochemical conditions (temperature, dissolved oxygen, pH and conductivity) were measured from the sample using a Hydrolab field hand-meter Surveyor and an MS5 minisonde probe. Physicochemical conditions, phytoplankton, chlorophyll-a and dissolved nutrients were collected in triplicate on day 0 and on day 8 using the methods outlined below. Single replicates of all parameters were taken from each microcosm bottle on day 8, and dissolved nutrients were collected from three surrogate bottles after the nutrient additions on day 0.

2.3.3 Nutrient Sampling and analysis

Water samples for the measurements of dissolved nutrients were filtered through a pre-rinsed 0.45 µm Sartorius cellulose acetate syringe filter into new, pre-rinsed with sample, 200 mL polyethylene bottles. Between 100–150 mL was collected from each sample and bottles were immediately frozen for transport to the laboratory. Analysis of the nutrient species included filterable reactive phosphorus (FRP) and oxidised nitrogen (nitrate and nitrite or NO_x), and were determined photometrically using a QuikChem 8500 Lachat nutrient analyser (Loveland USA) following APHA (1995) methods. Phosphorus as FRP was measured with the molybdite blue method using ascorbic acid as the reductant (Murphy and Riley, 1962). NO_x was analysed with the sulphanilamide method after reduction by a cadmium column (APHA, 1995; Hosomi & Sudo, 1986).

2.3.4 Phytoplankton collection and enumeration

Phytoplankton samples (in triplicate: 200 mL each) were collected from the tub on day 0 and from each microcosm bottle on day 8 of the experiments and were preserved in Lugol's iodine solution. Phytoplankton in the samples were identified using an Olympus BX41 compound light microscope and a Sedgewick-Rafter counting chamber. Enumeration followed methods described by Hötzel & Croome (1999). If required, a 5x concentration was made by settling 50 mL of the sample in measuring cylinders for 24 hours. The cylinder was checked for any floating phytoplankton and the upper 40 mL was removed. All taxa were identified to genus level except for potentially toxic cyanobacteria that were identified to species level.

2.3.5 Chlorophyll-a analysis

For day 0 and 8 of the experiments, chlorophyll-a concentrations were measured using the methods described in Müller & Mitrovic (2015), by filtering 200 mL of water sample through a 0.7 µm pore-sized Whatman glass fibre filter using a Mitivac vacuum hand pump. The filters were then wrapped in aluminium foil and kept frozen until analysed. Prior to analyses, 10 mL of 90% ethanol was added to the glass fibre filters in clean 10 mL centrifuge tubes and subsequently heated in a 75°C water bath for 10 minutes. Remnant particles were settled by centrifuging at 3000 rpm for 10 minutes. The supernatant was analysed spectrophotometrically using a Varian Cary 50 Bio UV Spectrophotometer.

2.3.6 Statistical analysis

All statistical analyses were carried out using the software SigmaPlot 12.5, Primer 6 and CANOCO. One-Way analysis of variance (ANOVA) with significance of $\alpha = 0.05$ was carried out on phytoplankton biovolume, cyanobacterial biovolume, individual species biovolumes and chlorophyll-a concentrations from each microcosm assay using SigmaPlot 12.5. Bonferroni post-hoc t-test was used for each experiment to determine differences within treatments. Homogeneity of variance was tested with the Levene statistic. Community analysis (SIMPER) was performed on Primer 6 software. Redundancy analysis (RDA) was performed using CANOCO version 4.5 at all sites. In the RDA analysis, NO$_x$ and FRP were used as explanatory environmental factors to assess their effects on the phytoplankton community density and structure, with automatic forward selection. While a Monte-Carlo permutation (999 permutations without restriction) was used to assess the significance of canonical axes and environmental variables on the phytoplankton community at each site. The top 10 biovolumes of species or genera were selected for analysis at each site.

2.4 Results

Cyanobacterial blooms were present at most sites across the course of this study. Upon the commencement of experiments in 2020, Torrumbarry, Euston and Mildura were experiencing blooms dominated by the diazotrophic cyanobacteria *Dolichospermum crassum* (RACC, 2022). Similarly, in 2021, experiments at Mildura and Kulnine had *Dolichospermum planctonicum* and *Chrysosporum ovalisporum* blooming. Being a riverine site, Corowa was not experiencing blooms at the onset of experimentation. Ambient dissolved NO$_x$ and FRP concentrations were low at Torrumbarry, Euston and Mildura sites on Day 0 of the experiments. Similarly, FRP was minimal at Corowa, although NO$_x$ was the highest of all sites (62 μg/L). At Kulnine, FRP concentration was the highest of all sites (23 μg/L), but NO$_x$ concentrations were not notably elevated (Table 2.1).

Table 2.1 Ambient and amended concentrations (μg/L) of nutrients at the onset of experiments. Values are mean± SE

	Corowa – April 2021	**Torrumbarry – February 2020**	**Euston – January 2020**	**Mildura – January 2020**	**Mildura – April 2021**	**Kulnine – April 2021**
NO$_x$ Day 0	62.00	1.86±2.37	9.70±0.10	4.45±0.66	10.00±1.00	20.50±11.50
FRP Day 0	7.00	3.28±0.09	1.82±0.14	1.59±0.30	5.00±1.00	23.00±18.00
NO$_x$ amended	452.50±127.50	654.58±22.72	531.48±11.02	484.13±47.73	625.00±5.00	387.50±17.50
FRP amended	185.00±25.00	237.00±6.05	191.35±4.77	163.93±10.55	227.50±2.50	225.00±5.00

2.4.1 Chlorophyll-a responses to nutrients

Chlorophyll-a concentrations were significantly different between controls and treatments at all sites (One-Way ANOVA: $p<0.05$). At Mildura, chlorophyll-a increased significantly in the P treatment in 2020 ($p<0.001$) and 2021 ($p<0.001$) experiments when compared to the controls (Figure 2.2). Bonferroni's pairwise comparison showed the treatment effect of P was significantly greater than the NP treatment ($p = 0.001$) in 2020. In 2021, however, the NP treatment was significantly greater than the P treatment ($p<0.001$) indicating some co-limitation. This co-limitation was also seen at Torrumbarry, where P and NP treatments were significantly greater than the control ($p = 0.017$ and 0.004 respectively) and the NP treatment was significantly greater than P treatment ($p = 0.017$). Co-limitation also occurred at both Kulnine and Corowa, with NP the only treatment significantly greater than the control ($p<0.001$). At Euston the NP treatment showed significantly greater chlorophyll-a concentration than the control (One-Way ANOVA: $p = 0.024$) while the response from the P treatment showed no statistical difference to the control or NP treatment (Figure 2.2). This response was similar to that observed at Mildura in 2021; however, there was a significant difference at Mildura between the P and NP treatments.

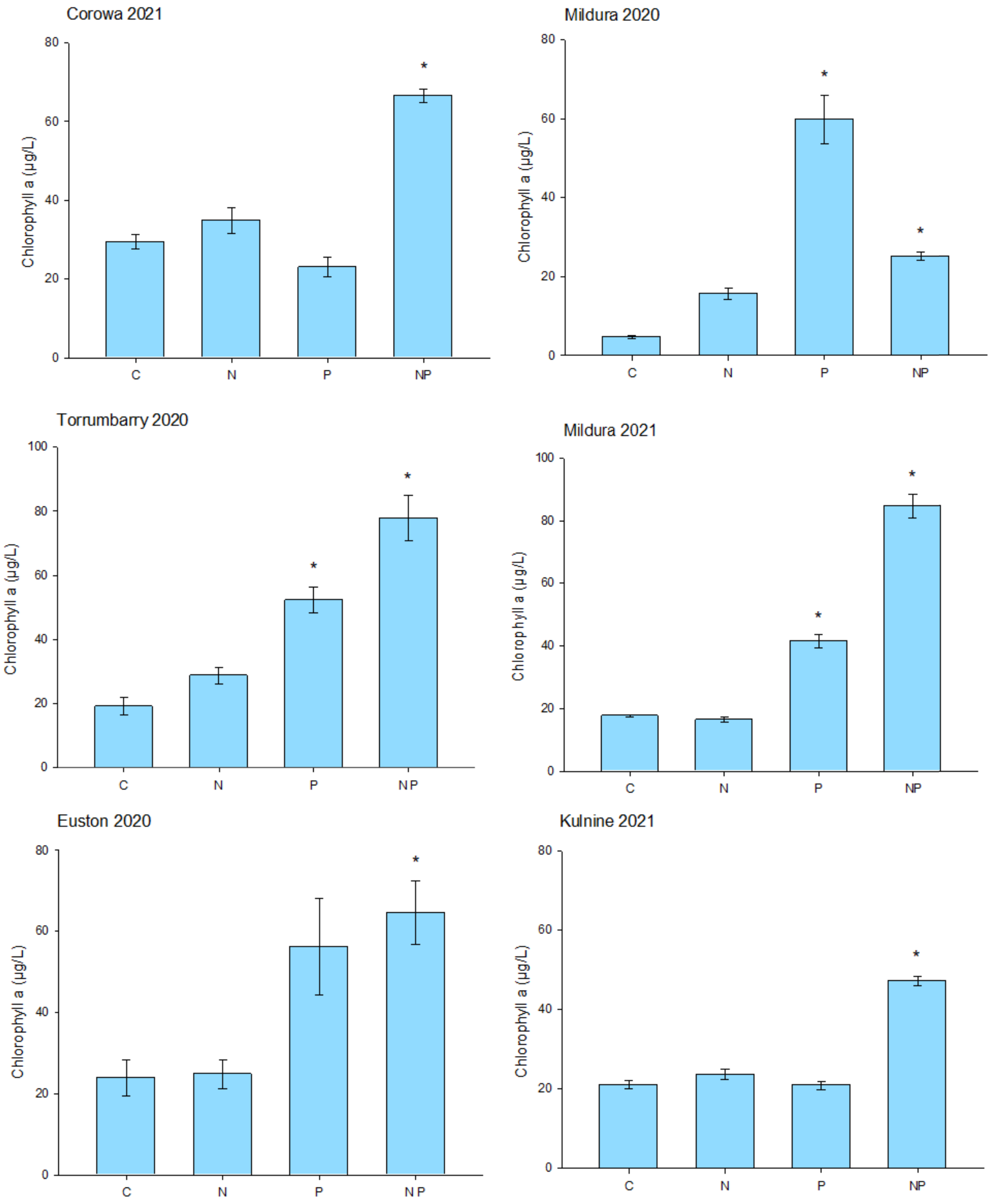


Figure 2.2 Chlorophyll-a concentrations from the microcosm assays conducted from January 2020, February 2020 and April 2021. Treatments were N (nitrogen alone), P (phosphorus alone) and NP (combination of nitrogen and Phosphorus), and C was the control. Asterisks indicates the samples significantly different to the control, determined by Bonferroni comparison (One-Way ANOVA, $p \leq 0.05$). All values are from day 8 of the experiment. Error bars are one standard error from the mean, n=3.

2.4.2 Biovolume of Major Algal Groups

At Torrumbarry, total algal biovolume increased notably with NP additions compared to the control (One-Way ANOVA: $p = 0.008$). Similarly, there was evidence of co-limitation observed at Kulnine with a significant response in the NP treatment ($p<0.006$), with no other treatment increasing significantly. Although there was an indication of co-limitation at Corowa, it was not statistically significant (Figure 2.3). At Mildura in 2021, there were significant differences in both the P and NP treatments when compared to the control ($p<0.003$, $p<0.001$ respectively), however no differences were observed between the P and NP treatments suggesting P limitation. This trend was not observed at Mildura or Euston in 2020, as phytoplankton biovolumes showed no significant response to nutrient additions (Figure 2.3).

Total cyanobacterial biovolume was different to the overall phytoplankton response. Across the three experiments in 2020 and at Mildura in 2021, there was a statistically significant growth in the P treatment when compared to the control suggesting P limitation (One-Way ANOVA: Mildura 2020 C vs P, $p = 0.002$, Euston C vs P, $p = 0.009$, Torrumbarry C vs P, $p = <0.001$, Mildura 2021 C vs P, $p = 0.012$) (Figure 2.3). At Kulnine and Corowa there was no observed response by the cyanobacterial community to any of the experimental treatments (Figure 2.3).

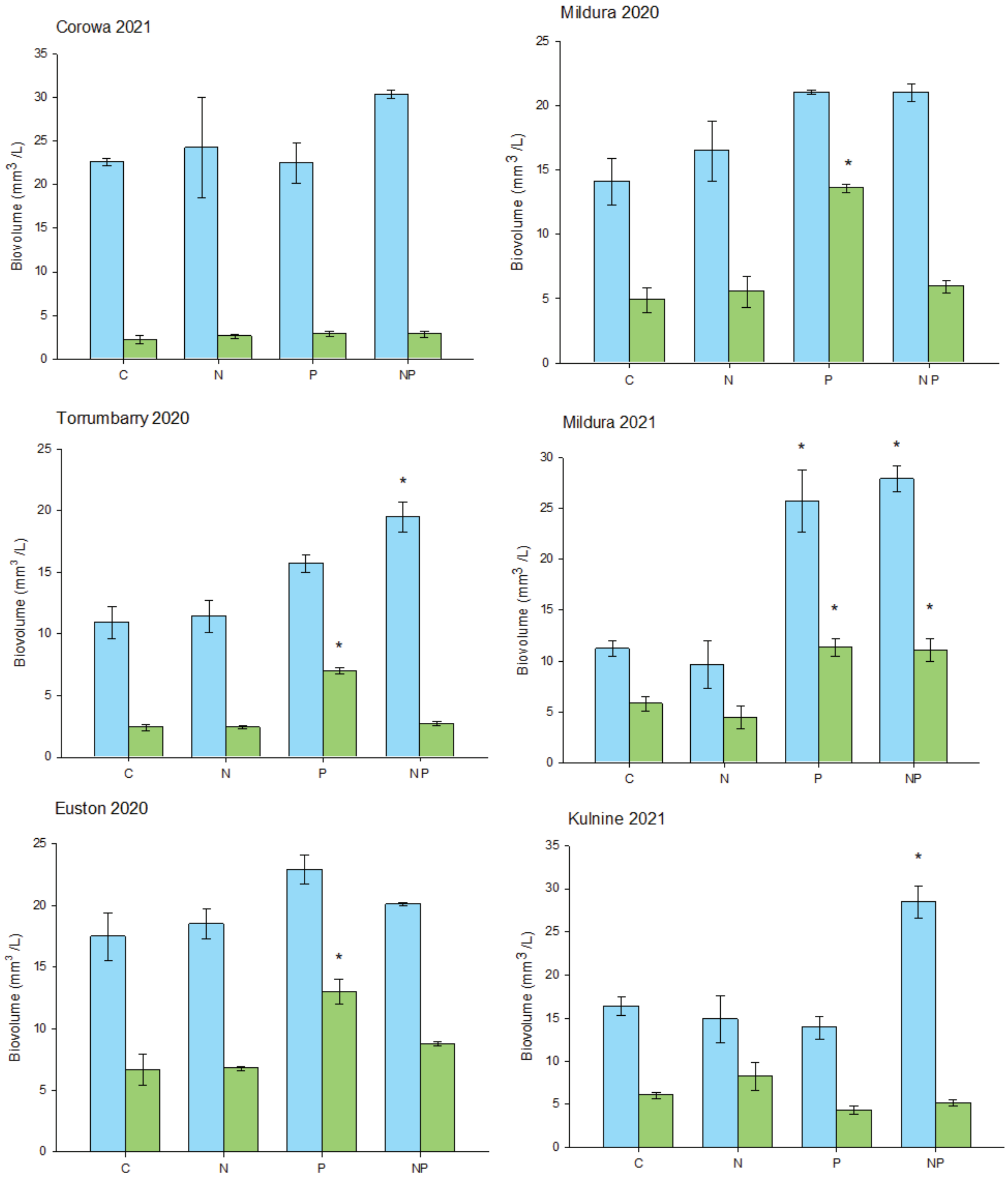


Figure 2.3 Total biovolume of phytoplankton (blue) and cyanobacteria (green) of all microcosm experiments. Phytoplankton biovolumes includes cyanobacteria. Treatment N and P were single concentration additions, while treatment NP was in combination, and C is the control. Asterisks indicates the samples significantly different to the control, determined by Bonferroni comparison (One-Way ANOVA, $p \leq 0.05$). Error bars are one standard error from the mean, n=3.

2.4.3 Community response

In 2020, community composition was heavily influenced by increased cyanobacteria in every P treatment across experiments. SIMPER analysis revealed that *D. crassum* and *D. planctonicum* were the largest contributors to differences at Mildura (~50%), Euston (~41%) and Torrumbarry (~42%) during that year. The only other experiment to show cyanobacterial community P limitation (Figure 2.3) was Mildura 2021, even though it showed no distinction between communities across the treatments (Figure 2.4). At Mildura 2021, SIMPER analysis revealed *D. planctonicum* as the primary driver in the P (~33%) and NP (~30%) treatments; however, in the N treatment, *C. ovalisporum* (~14%) contributed almost equally with *D. planctonicum* (~16%). In the Kulnine experiment, there was a greater proportion of cyanobacteria in the N and C treatments compared to green algae dominating the P and NP treatments. SIMPER showed the small increase in the N treatment when compared to the control was driven by *C. ovalisporum*. Green algae (Chlorophyta) were most abundant across NP treatments at all sites except Mildura in 2021, where Cyanobacteria was still most abundant (Figure 2.3). SIMPER revealed *Scenedesmus* as the largest non-cyanobacterial contributor across the remaining five experiments.

2.4.4 Cyanobacteria species responses to amendments

Species specific responses to nutrient additions followed similar trends to total cyanobacteria during the 2020 experiments (Figure 2.3, Figure 2.5); however, species showed differing responses through the 2021 experiments. Potentially toxic cyanobacterial species *D. crassum* and *D. planctonicum* were the primary phytoplankton species blooming during the 2020 experiments (Figure 2.5), with both species displayed clear P limitation. D. *planctonicum* was the only species to bloom across both years studied and at Mildura in 2021, followed a similar pattern to experiments in 2020 displaying clear P limitation. In the same experiment, *C. ovalisporum,* another potentially toxic species displayed no clear limitation by nutrients, although some response to the N treatment was observed. This was also reflected in the Kulnine experiment, again suggesting nutrient levels were sufficient for *C. ovalisporum* during the bloom. *Dolichospermum circinale* were present at both Kulnine and Mildura and despite low overall biomass showed a small, though not significant, response in the P treatment (Figure 2.5), suggesting P may be limiting growth. At Corowa, *Sphaerospermopsis aphanizomenoides* was observed at low biomass and the greatest response was in the control.

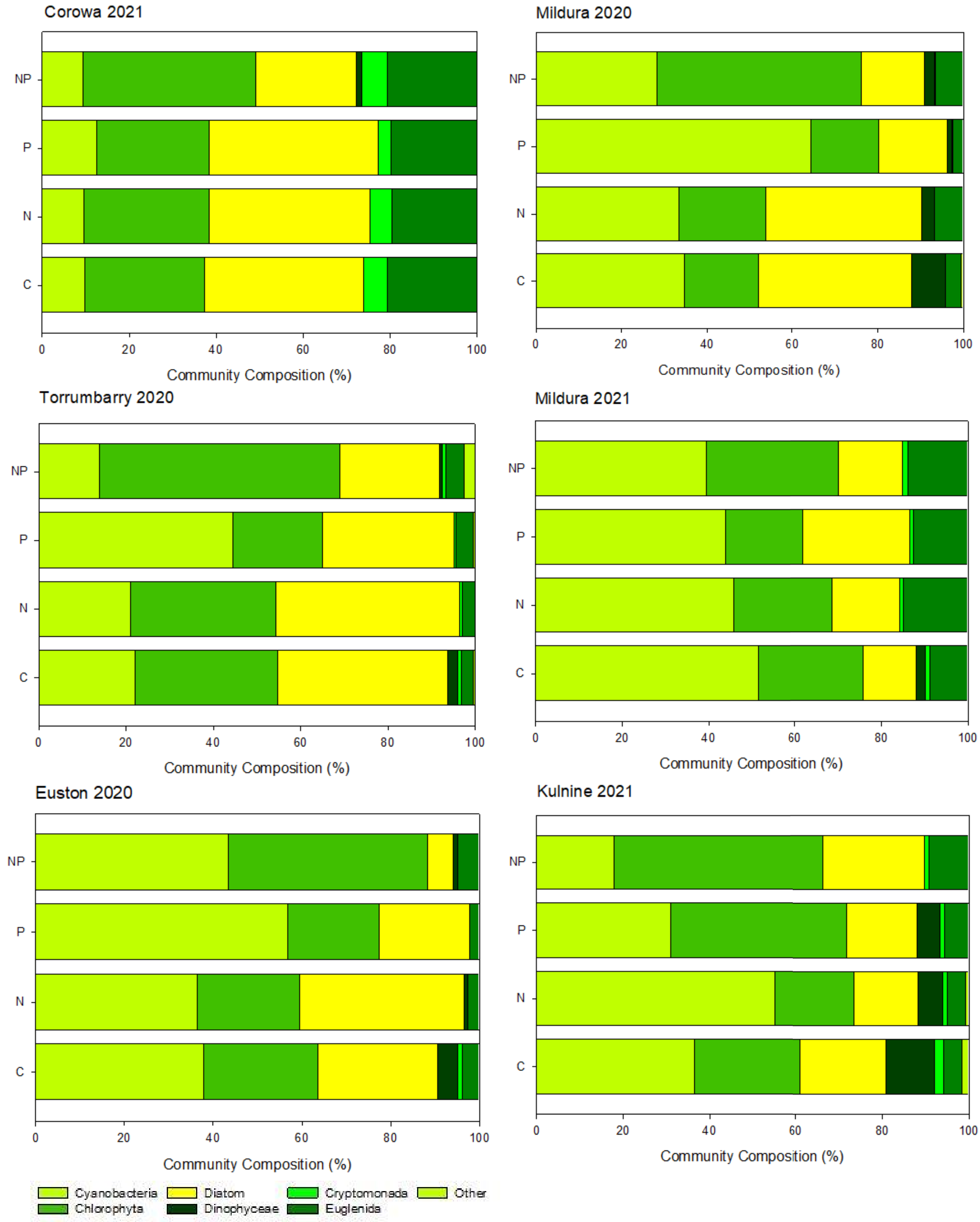


Figure 2.4 Proportions of the major Phytoplankton groups across all six experiments.

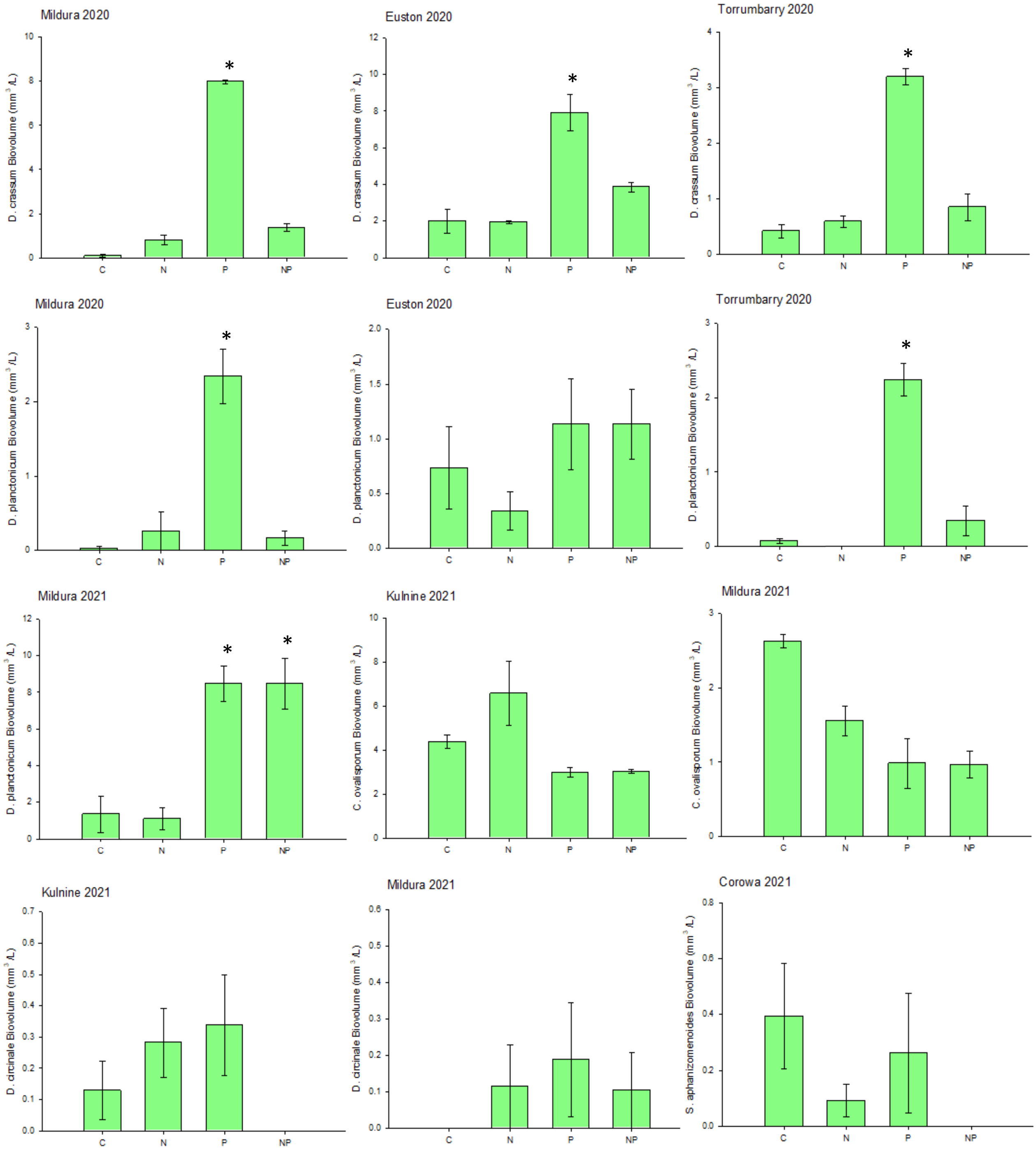


Figure 2.5 Biovolume responses of cyanobacterial species to nutrient additions. Treatment N and P were single concentration additions, while treatment NP was in combination, and C the control. Asterisks indicates the samples significantly different to the control, determined by Bonferroni comparison (One-Way ANOVA, $p \leq 0.05$). Error bars are one standard error from the mean, n=3.

Redundancy analysis (RDA) was performed on ambient and amended concentrations of N and P, which were used in the analysis for the environmental conditions experienced by the ten most abundant phytoplankton during all experiments. Redundancy analysis explained 19.3% of variation at Corowa, 57.1% at Torrumbarry, 47.4% at Euston, 55.5% at Mildura in 2020, 48.9% at Mildura in 2021 and 40.0% at Kulnine suggesting nutrients were more important at the lower 4 sites.

FRP had the largest effect on community structure at Torrumbarry (RDA: p-value = 0.014, λ = 0.34), Euston (RDA: p-value = 0.011, λ = 0.37), Mildura 2020 (RDA: p-value = 0.001, λ = 0.44), Mildura 2021 (RDA: p-value = 0.001, λ = 0.45) and Kulnine (RDA: p-value = 0.001, λ = 0.33). Generally a strong positive relationship was observed with FRP and *Dolichospermum crassum* and *Dolichospermum planctonicum* when present (Figure 2.6). Interestingly, during mixed blooms where *D. crassum* and *D. planctonicum* were present together (Torrumbarry, Euston and Mildura 2020), the relationship with FRP was weaker than during a single *Dolichospermum* sp. bloom (Mildura 2021) (Figure 2.6). Non-diazotrophic cyanobacteria such as *Aphanocapsa* sp. consistently showed a negative relationship with FRP. We also found *Chrysosporum ovalisporum*, was negatively correlated to FRP at both Mildura and Kulnine in April 2021 (Figure 2.6). This correlation was likely driven by the responses by *C. ovalisporum* in the N treatment evident in Figure 2.5.

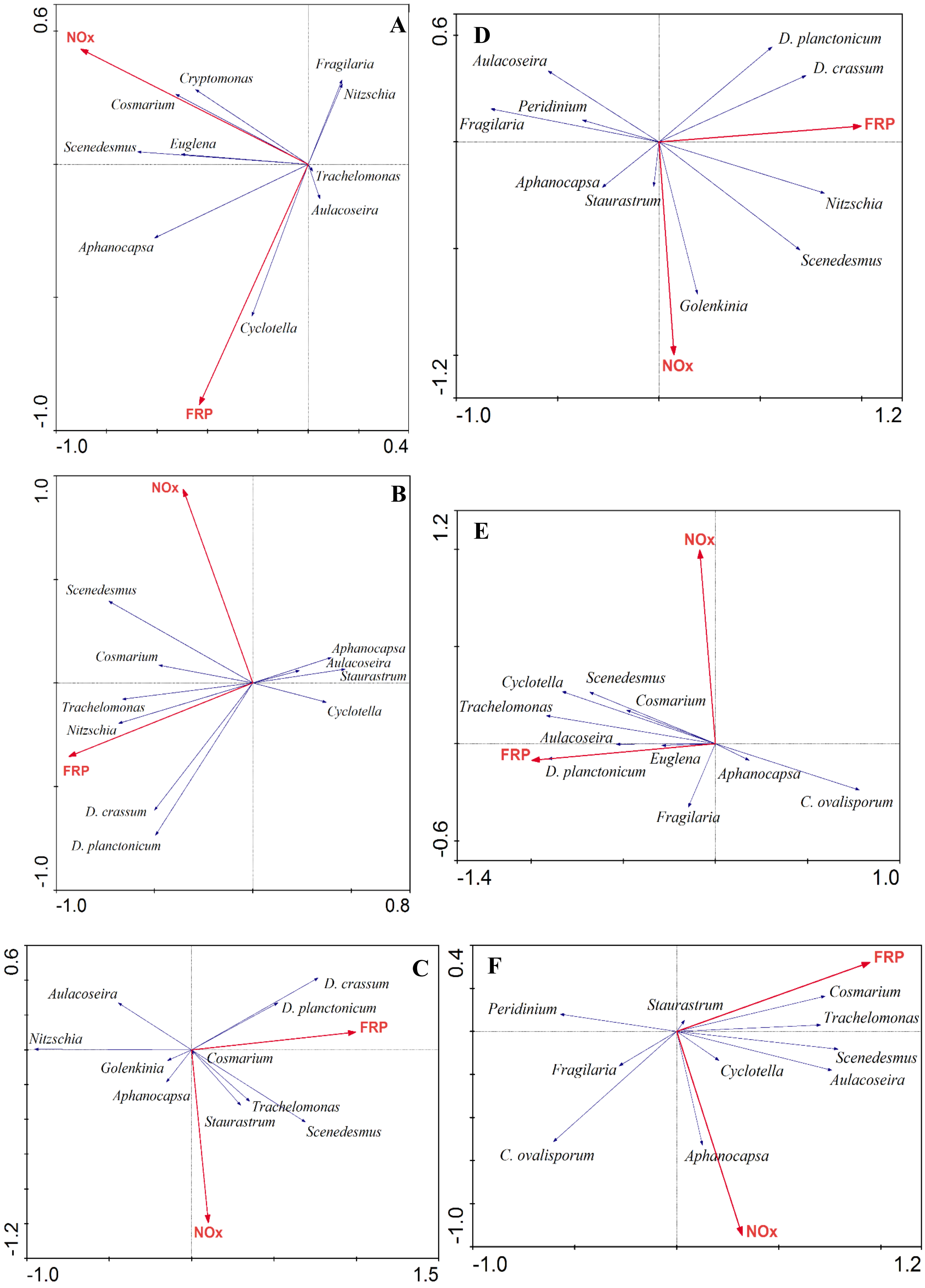


Figure 2.6 Redundancy analysis (RDA) ordination diagrams for each experiment. Environmental variables dissolved nitrogen (NOx) and phosphorus (FRP). A is Corowa 2021, B is Torrumbarry 2020, C is Euston 2020, D is Mildura 2020, E is Mildura 2021, and F is Kulnine 2021.

2.5 Discussion

In the Murray Darling Basin, little is known about the role of nutrients in limiting or promoting cyanobacterial blooms and influencing the phytoplankton community structure. In the Murray, only one study has previously explicitly looked at limiting nutrients, and the study ran over the austral winter period when few cyanobacteria were present (Walker and Hillman, 1982). This remains a major gap for understanding and potentially managing phytoplankton, and in particular cyanobacterial growth in the Murray River.

2.5.1 Chlorophyll-a and biovolume response to N and P

In our study each different site examined showed evidence of phytoplankton nutrient limitation based on chlorophyll-a concentrations, indicating that nutrient levels play an important role in regulating overall phytoplankton growth. Phosphorus was the key limiting nutrient at Mildura (both years) and Torrumbarry, while colimitation was evident at Euston, Kulnine and Corowa. Similarly, total algal biovolume was typically highest in either P and/or NP treatments at all sites. This suggests that phosphorus and nitrogen may be key nutrients limiting total phytoplankton growth on the Murray River, as suggested for other freshwater environments such as lakes and reservoirs (Schindler, 1977; Müller and Mitrovic, 2015). Ambient concentrations of NO_x were consistently low from Torrumbarry downstream to Mildura, whereas FRP was mostly low across the study (Table 2.1). Under conditions where both nutrients are low, it has been suggested that both N and P would be limiting overall growth (Harpole 2011). This was evident in the significant response of chlorophyll-a across all NP treatments in the study despite Corowa and Kulnine having higher than usual NO_x values (see Chapter 3), suggesting co-limitation overall (Figure 2.2). Evidence of co-limitation of the phytoplankton community has been observed in nutrient amendment experiments previously (Muller and Mitrovic 2015, Bratt 2020). However, we also found significant responses in the P treatments at Torrumbarry and Mildura sites due to the large proportion of nitrogen fixing taxa at the onset of experimentation (Figure 2.5) (RACC, 2022). This was confirmed in Figure 2.3, where the P limited sites show a clear significant response for the cyanobacterial biovolume in the P treatments. The low ambient concentrations of NO_x at these sites (Table 2.1) may be beneficial conditions for nitrogen fixing taxa, which may be why these cyanobacteria are so prevalent at these sites.

Cyanobacterial taxa such as those in the genus *Dolichospermum*, can utilize specialized nitrogen acquisition strategies (atmospheric nitrogen fixation) to meet their nitrogen demands (Hemida, 2014). This is particularly significant as it implies that reducing N loads and concentrations may not be effective at reducing the biomass of nitrogen-fixing cyanobacterial blooms. This is consistent with findings by Schindler et al. (2008) who observed that reducing N inputs did not decrease cyanobacterial biomass, instead shifting the cyanobacterial community to one dominated by nitrogen-fixing taxa. While there are many proponents of managing both N and P (Paerl et al. 2011), given the high abundance of nitrogen-fixing cyanobacteria in the Murray River, phosphorus reductions will likely be most effective in reducing cyanobacterial biomass in the Murray River.

2.5.2 Species responses to N and P

Growth of *Dolichospermum crassum*, a common nitrogen-fixing cyanobacterial species in the Murray River was highest in the P treatment at Mildura 2020, Torrumbarry and Euston (Fig 5). The response in the P treatment was notably higher than the NP treatment, and this may be caused by competition effects with other phytoplankton. Under low N (i.e P treatment) growth was supported perhaps as N demands were being met by nitrogen fixation, giving an ecological advantage. When exposed to high N availability (NP treatment), *D. crassum* growth was no longer favoured, possibly as nitrogen-limitation is alleviated in other phytoplankton giving them a growth advantage. As a result, we saw the proliferation of a non-diazotrophic genus, *Scenedesmus*. Therefore nitrogen-fixation may no longer be an ecological advantage under the higher N conditions (Hyenstrand et al. 2000; Paerl 2017). The response of *Dolichospermum planctonicum*, another nitrogen-fixing cyanobacteria, followed similar patterns to *D. crassum* in the Mildura 2020 and Torrumbarry experiments with greatest growth response in the P treatment (Figure 2.5). Additionally, *D. planctonicum* responses at Euston and Mildura 2021 also showed growth in both the P and the NP treatments (Figure 2.5). This response was again possibly due to the higher ambient NO_x levels (Table 2.1), which may also explain the slight elevation of *D. crassum* in the NP treatment at Euston (Figure 2.5). Under these conditions, we hypothesise that nitrogen-fixation was not utilised by these cyanobacterial species, as it is a known costly process (Berman-Frank et al. 2007; Vrede et al. 2009). The affinity of *D. crassum* and *D. planctonicum* with available P was reinforced by redundancy analysis, which showed a strong relationship with the two *Dolichospermum* spp. from Torrumbarry, Euston and

Mildura 2020 experiments. While the redundancy analysis revealed an even stronger correlation with *D. planctonicum* at Mildura 2021.

At the Corowa and Kulnine sites, where N and P alone were not limiting growth, increases in total phytoplankton biovolume appeared to be generally driven by Chlorophyta. Most cyanobacterial species did not respond notably to nutrient additions at Corowa and Kulnine; however, *Chrysosporum ovalisporum*, a known nitrogen-fixer, showed a slight response to the N treatment in the Kulnine experiment, with no response in the P or NP treatments. However, overall total cyanobacterial responses showed no significant response when compared to the control at this site (Figure 2.3). In these instances, cyanobacteria may again favour N-uptake rather than the more costly N-fixation as nutrients become available (Berman-frank 2007). The limited response of *C. ovalisporum* to nutrient additions may have been due to ambient levels being sufficient, which likely drove the negative correlation between it and FRP (Table 2.1 and Figure 2.5 and Figure 2.6). At the Corowa site, nutrients released from Hume Dam drove the higher ambient nutrient levels (Chapter 3). However, due to it being a riverine site, a smaller portion of cyanobacteria may have been present at the onset of experimentation despite a bloom of *Sphaerospermopsis aphanizomenoides* in Hume Dam (RACC 2022). At Kulnine, river discharge was increasing during the month prior to the experiment, which may have increased NO_x concentrations when experiments were setup compared to low flow periods. NO_x levels were typically low at this site, but concentration spikes seem to reflect increases in discharge (Chapter 3) (Mitrovic et al. 2022). The higher nitrogen concentrations may have favoured Chlorophyta, which may be exploiting faster growth rates than cyanobacteria to rapidly respond to the sudden increase in nutrients (Robarts and Zohary 1987).

At the Torrumbarry and Mildura 2020 sites where ambient NO_x was particularly low, the relative abundance of the cyanobacterial group in the P treatment, with respect to the rest of the phytoplankton community, was clear (Figure 2.4). These stark differences in phytoplankton communities between treatments were not reflected at Euston and Mildura 2021 where ambient NO_x was higher (Table 2.1; Figure 2.4). Despite this, biovolumes revealed a clear phosphorus limitation by diazotrophic species across these four sites. At the Kulnine site, ambient nutrient levels were greater than the Torrumbarry, Euston and Mildura sites. As a result they were not limiting the growth of diazotrophic species present (Figure 2.5). This reinforces that nitrogen-fixing cyanobacteria are particularly successful under high

P and low N conditions, and demonstrates the persistence of cyanobacteria under low N levels.

2.5.3 Management Implications

Due to the clear relationship of cyanobacterial growth with increased P concentrations, the data from this study suggests P reductions would be the most important for reducing the common blooms of N-fixing cyanobacteria. However, to establish an appropriate method for P reduction in the Murray, an in depth understanding of the sources of P would need to be established. Management may then be targeted at land use, nutrient rich agricultural developments, or sewage outfalls (Davis and Koop 2006, Mosely and Flemming 2010, Mainstone 2002). Alternatively, management may be directed at limiting P from being resuspended within the water column from sediments or from the breakdown of existing algal blooms and organic matter (Jung 2014, Pearl 2008). This could be achieved through maintaining a mixed and oxygenated water column (Pearl 2008).

Overall phytoplankton biomass is both limited by P and co-limited by N and P. Bloom management through only nitrogen reductions would likely shift the community to one dominated by nitrogen-fixing cyanobacteria. While dissolved concentrations of N are typically low (Harris 2001), increases can be seen in the Murray River system in flow events, even though they are typically short and sporadic (Chapter 3; Mitrovic et al. 2022). Another mechanism suggested to reduce blooms is to ‘invert’ the typical N to P ratio ideal for nitrogen-fixing cyanobacteria, by adding large amounts of N (Gophen 2015). Further research is needed on the feasibility of this approach, but this may have the risk of increasing overall phytoplankton biovolume/chlorophyll-a concentrations.

In summary, this study demonstrates that cyanobacterial blooms are typically limited by P in the Murray River, which supports the second part of our hypothesis. The large proportion of nitrogen-fixing cyanobacteria in the system is likely to be a response to consistently low N concentrations. The biomass of the overall phytoplankton community (using chlorophyll-a as a surrogate measure) is more likely to be co-limited by N and P. This did not support our hypothesis and was likely due to the observed low ambient N and high diversity of Chlorophyta, helping drive a NP response. Phosphorus reductions will likely be the most effective in reducing cyanobacterial biomass in the Murray River.

Chapter 3: Spatial and temporal nutrient dynamics in the Murray River

3.1 Abstract

Macronutrients such as nitrogen and phosphorus play an important role in shaping phytoplankton communities and the development of algal blooms. In many riverine environments, the source and behaviour or dynamics of macronutrients are poorly understood. Across two austral summers, we conducted temporal monitoring of total and dissolved inorganic or bioavailable nitrogen and phosphorus across five sites (Corowa, Torrumbarry, Euston, Mildura and Kulnine with downstream direction) along the Murray River, Australia. The Murray river has started to see yearly blooms of potentially toxic cyanobacteria, and nutrient dynamics are poorly understood during bloom periods. In this study, we sought to understand how total and dissolved inorganic nitrogen and phosphorus change longitudinally and seasonally, and whether those nutrients were greater concentrations in bottom waters implying that they are derived from the sediments. Longitudinally, we found evidence of a decrease in concentrations of dissolved inorganic nutrients with downstream direction but not in total nutrients. Seasonally, we found that during summer total nutrient concentrations were greater, but dissolved inorganic nutrient concentrations were very low. When looking at differences between the surface and bottom concentrations at each site, total nitrogen had peak differences during summer periods across all sites, but total phosphorus showed only weak summer peaks of difference downstream of Euston. Dissolved inorganic nutrient concentrations consistently showed minimal differences between surface and bottom waters across the study. The data collected in this study suggest that bioavailable nutrients are typically low in concentrations across bloom periods in the Murray River, and a large portion of the total nutrients seems to be associated within the phytoplankton present.

3.2 Introduction

Freshwater phytoplankton blooms in riverine environments are often dominated by potentially toxic cyanobacteria (Paerl 2014; Al-Tebrineh et al. 2012). As a requirement, these blooms uptake macro- and micronutrients which can fuel their growth to problematic levels (O'Neil et al. 2012). As a result, one approach in mitigating potentially toxic cyanobacterial blooms is to look at nutrient control (Schindler et al. 2016). To do this we must understand where the nutrients are originating. Nutrients can enter the system from allochthonous origins such as runoff from cleared land, sewage outfalls, forestry and agricultural land and irrigation (Davis and Koop, 2006; MDBA, 2022; Mosley and Fleming, 2010; Vink et al. 2005; Furst et al. 2019). However, a large portion can come autochthonously, from sediments or from bacteria and phytoplankton themselves (Miao et al. 2006; Roach et al. 2014).

The regulation of rivers though weirs and lower flows can impact the development of cyanobacterial blooms as it can increases the thermal stratification of the water column under warmer temperatures (Bormans et al. 2004; Mitrovic et al. 2011). When the water column is thermally stratified for extended periods, sediments may become anoxic, triggering the release of nutrients, such as nitrogen, phosphorus and trace metals, into hypolimnial waters (Covich et al. 1999). Nutrients derived from sediments can then be upwelled into the surface waters (Jung et al. 2014; Paerl, 2008) or transported downstream via low level offtakes (Westhorpe et al. 2015), where they may promote cyanobacterial blooms, or increase their magnitude and duration until the nutrients are depleted (Paerl et al. 2019).

Urbanisation and irrigation in Australia have put greater pressure on water resources in terms of both quantity and quality. As a result, many Australian rivers have seen considerable modification for water storage, navigation and recreation. Rivers, such as the Murray River, have many instream alterations in the forms of weirs, locks and dams built along their length which alter their flow regime from natural. Weirs and dams such as Hume dam in the Murray River capture and slowly release flows, dampening any seasonal floods whilst ensuring a steady flow across the year (Walker and Thoms, 1993). The resulting flows are often moderate and more consistent than natural, reducing lateral linkages with the floodplain and mobilisation of associated nutrients.

In the Murray River, the frequency of the occurrence of cyanobacterial blooms has increased (Bowling et al. 2018). Blooms can originate from upstream impoundments such as Lake Hume and Lake Mulwala (Scholz et al. 2003; Baldwin et al. 2008; Bowling et al. 2018) or from in-stream formations such as backwaters (Al-Tebrineh et al. 2012; Bowling et al. 2013). Harris (2001) suggested that the Murray River is N-limited due to de-nitrification processes, which may favour N-fixing cyanobacteria over other phytoplankton (Huisman et al. 2005). However, during cyanobacterial blooms in other parts of the Murray Darling Basin, bioavailable P has limited growth (Sherman et al. 1998; Webster et al. 2000). Further, Bowling et al. (2018) found indication of P-limitation during a bloom in the Murray. However, this was based only on total phosphorus concentration observations, and thus no insights were gained if the concentrations of different phosphorus species played a significant role in limiting the bloom.

It is important to understand the sources and concentrations of nutrients when seeking effective management plan to mitigate blooms. Nutrient sources, processing and dynamics in rivers may change longitudinally. It is also important to understand whether there are seasonal trends in nutrient concentrations within the river as this may interact with phytoplankton growth. It is also useful to compare surface and bottom of the water column concentrations to see if there is any influence of sediment nutrient release. This study aims to (1) monitor nutrient concentrations, including longitudinal and seasonal trends, in the Murray River over a 24-month period to understand their possible role in cyanobacterial blooms and (2) compare nutrient concentrations in surface and bottom waters to understand the role of sediment derived nutrients. I hypothesise that (1) nutrient concentrations would decrease longitudinally from upstream to downstream, with higher concentrations during summer bloom months and (2) that nutrient concentrations will be higher in bottom waters implying that sediments are a potential source of nutrients to the river.

3.3 Method

3.3.1 Study Sites

The Murray River has approximately fifteen impoundments along its length, including one large dam and fourteen weirs. To help understand how these impoundments have affected nutrient availability within the river, study sites were selected from both riverine and weir pool sections. Weirs 9, 11, 15 and 26, as well as a riverine (non-impounded) site near the township of Corowa were sampled during the summer of 2019–20 and 2020–21. All sample collection was conducted from a boat within 5 km upstream of the weir. However, some sites were only accessible from Victoria and were thus inaccessible some months due to the Covid-19 pandemic.

Kulnine weir or weir 9 sits 765 km from the mouth of the Murray River (Figure 3.1). The weir pool extends for ~63 km, while the river is mostly ~130 m wide and ~5 m deep along its length. The weir is a source of water for both Lake Victoria and Lake Cullulleraine, and it lies downstream of the Murray-Darling confluence, making it the only site to be potentially affected by the Darling river. Wallpollo Creek and the Great Darling Anabranch both meet the Murray in the Kulnine weir, however they very rarely flow and usually act as large backwaters. The riparian vegetation is quite heavy for most of the weir pool, with much of the riverbank untouched and unregulated. Samples were collected within 5 km upstream of the weir.

Mildura weir or weir 11 is situated 113 km upstream of the Kulnine weir (Figure 3.1). The weir has a lock which is separated by a small island that at capacity, creates a weir pool ~60 km long. Upstream of the weir, the river is ~176 m wide and ~5 m deep, and has very few tributaries. The weir pools riparian zone is subject to a large amount of urban land, such as the town of Mildura, which along with many farms and sparse riparian vegetation, are large contributors to allochthonous derived nutrients. Sampling occurred within 3 km of Mildura weir.

Euston weir or weir 15 is the next upstream weir, 232 km upstream of Mildura (Figure 3.1). The weir pool is typically ~120 m wide and ~5 m deep, creating a variable weir pool length of 60–100 km. The majority of the riparian zone for this weir pool is native vegetation, however both Euston and Robinvale share its banks close to the weir. The Murrumbidgee River and Edward/Wakool Anabranch are two major confluences within the Euston weir pool and are potentially important nutrient sources. Samples collected from Euston were always within 3 km of the weir.

Torrumbarry weir or weir 26, another 528 km upstream of Euston was the most upstream site, making it ~1638 km from the Murray rivers mouth (Figure 3.1). The weir pool close to the weir is ~70 m wide and ~6 m deep however the pool shallows to ~4 m a short distance upstream. The weir pools riparian zones are heavily dominated by vegetation, as it runs through Gunbower national park, with the only major urban influence being Echuca ~40 km upstream. The Campaspe and Goulburn rivers are two tributaries of the weir pool that are fed by large dams, with some of this water exiting the system through an irrigation channel and into Gunbower Creek.

Corowa is the only riverine site of the study. The area sampled is ~440 km upstream of Torrumbarry weir, and is typically ~70–80 m wide and ~3 m deep. The riparian zones are moderately vegetated with mostly agricultural land beyond the vegetation within the floodplain, while upstream is the tributary of the Kiewa River and the large Hume Dam.

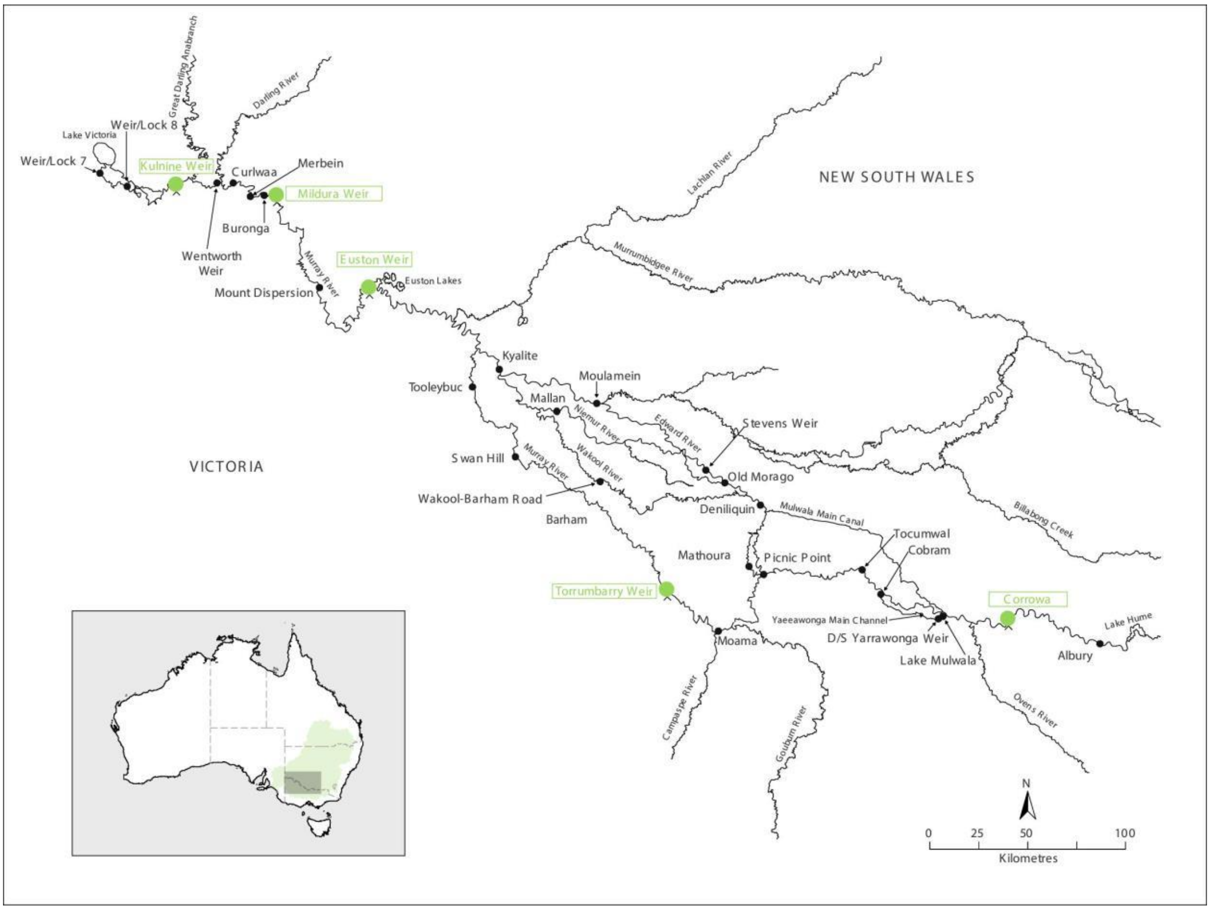


Figure 3.1 Detailed map of the Murray river with all five major sampling sites labelled in green during the study.

3.3.2 Nutrient Sampling and analysis

For all sites, water samples for total and dissolved nutrients were collected using a 4.2 L Van Dorn depth sampler, with replicate samples from the surface (1m below the surface) and bottom (at least 1m above the sediment) of the water column. 200 mL of unfiltered water were taken for total nutrients TN and TP. In addition, 200 mL of water was filtered through a pre-rinsed 0.45µm Sartorius cellulose acetate syringe filter into new 200 mL polyethylene bottles for filtered nutrients NO_x and filterable reactive phosphorus (FRP). All nutrient sample bottles were prerinsed with sample twice and then immediately frozen after sample collection. Analysis of the bioavailable nutrients FRP and the combined nitrate and nitrite

(NO_x) was determined photometrically using a QuikChem 8500 Lachat nutrient analyser (Loveland USA) following APHA (1995) methods. Phosphorus as FRP was measured with the molybdite blue method using ascorbic acid as the reductant (Murphy and Riley, 1962). NO_x was analysed with the sulphanilamide method after reduction by a cadmium column (APHA, 1995; Hosomi and Sudo, 1986). TP samples were first digested using a persulfate oxidation procedure to form orthophosphate, which was measured as above. TN was also digested using a persulfate oxidation procedure to form nitrate, the sample was then reduced and analysed as mentioned above. A subset of the nutrient samples was run externally by CSIRO at Charles Sturt University, Albury, following the same methods outlined above, with a detection limit of 2 µg/L. Additional nutrient data (NO_x, TKN, FRP, TP) was supplied by the MDBA, collected from sites A4260501, 414206, 414209, 409207B, and 409016A. TKN (Total Kjeldahl Nitrogen) (excluding inorganic N, except ammonia) data was used when TN was not available, and was reported with TN results.

3.4 Results

3.4.1 Seasonal site patterns

NO_x concentrations were highly variable between sites (Figure 3.2), with highest concentrations found at the most upstream site, Corowa (maximum NO_x = 420 µg/L). There was a distinct longitudinal trend of decreasing NO_x concentration with distance downstream. At sites Corowa-Mildura, NO_x concentration peaked between April and July. Interestingly, TN/TKN concentrations followed an inverse pattern to NO_x. There was a weak trend of increasing TN/TKN with distance downstream.

FRP concentrations were generally very low (<5 µg/L) at all sites throughout most of the study period (Figure 3.3). There were some brief spikes in FRP, particularly at the upstream sites Corowa and Torrumbarry, as well as the downstream site Kulnine, however these were intermittent and short-lasting (Figure 3.3). The highest concentrations of TP were observed at Torrumbarry (maximum TP = 110 µg/L). At Corowa and Torrumbarry, which had finer temporal sampling resolution, there was a general trend of increasing TP in winter months. They followed a broad trend of increasing TP concentration with distance downstream.

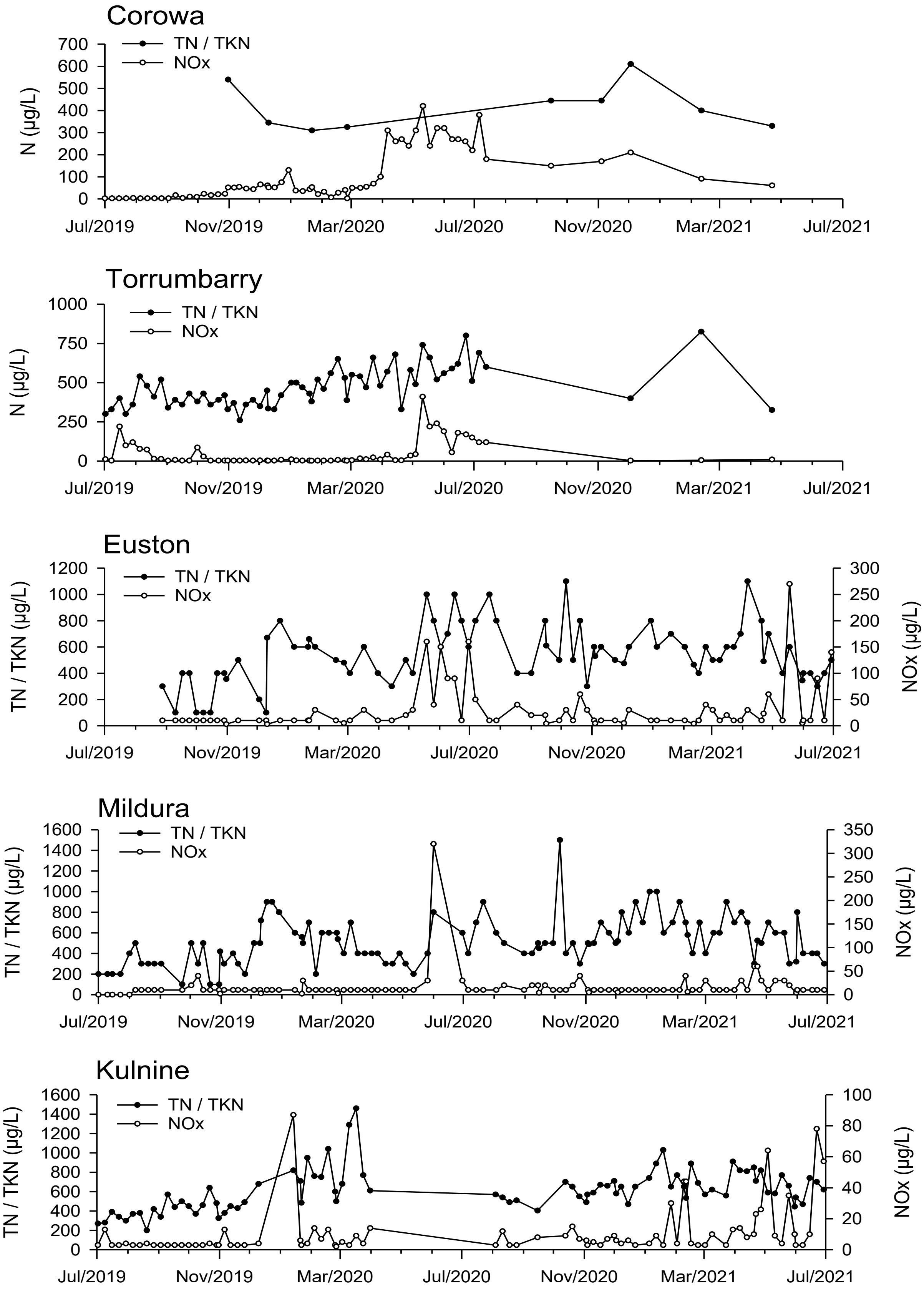


Figure 3.2. Time series graphs displaying combined TN/TKN, and NO_x concentrations across the 2-year study period. TKN values were used when TN was not available for a more complete dataset.

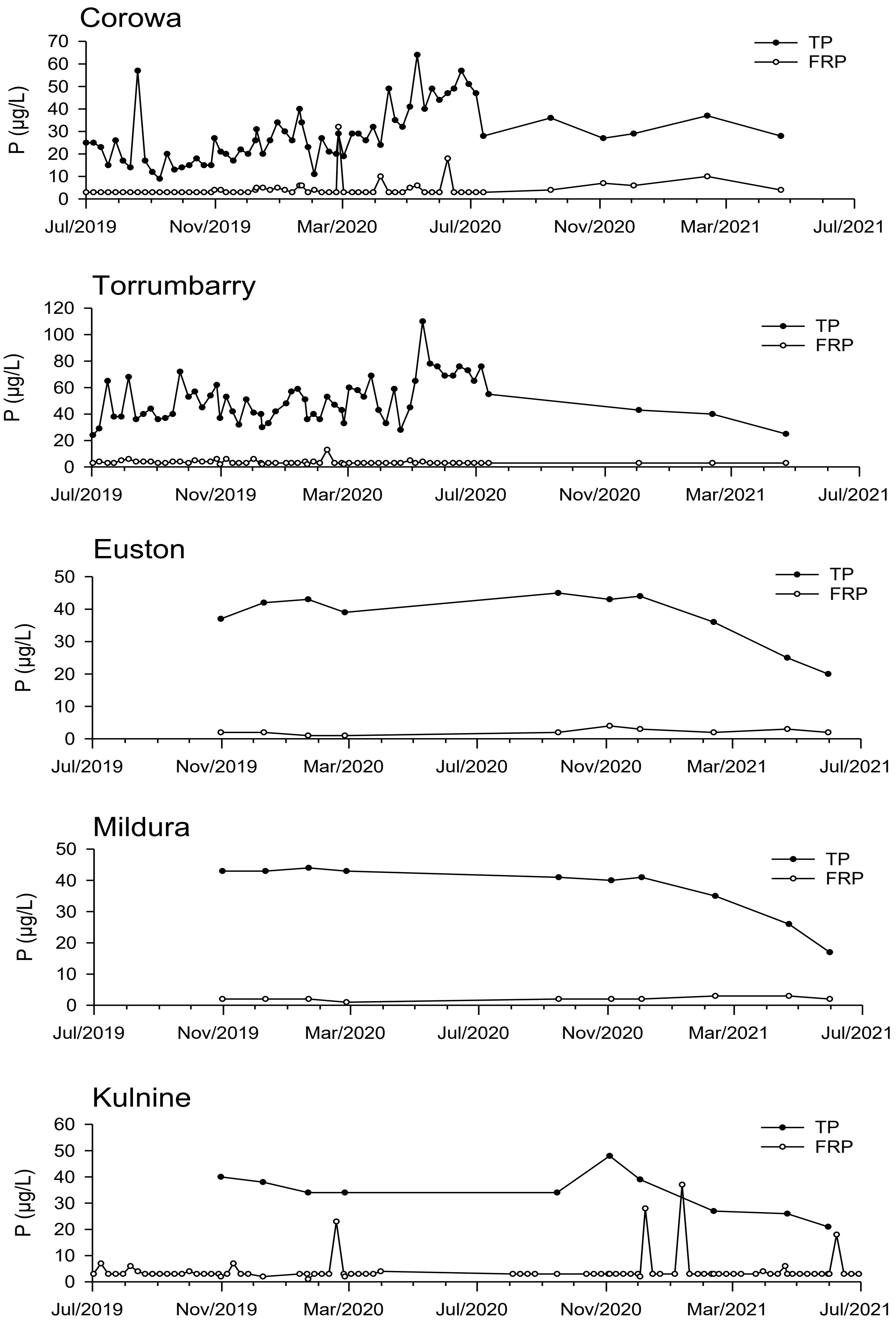


Figure 3.3. Time series graphs displaying TP and FRP concentrations across the 2-year study period.

Table 3.1 Values for mean and range of data at all sites across the period of sampling as µg L^{-1}*. Mean with ± SE. N=number of replicates.*

	Corowa	**Torrumbarry**	**Euston**	**Mildura**	**Kulnine**
FRP mean	4.4+/-0.5	3.6±0.2	2.2±0.3	2.1±0.2	4.3±0.6
FRP range	3 – 32 (n=64)	2 – 13 (n=62)	1 – 4 (n=10)	1 – 3 (n=10)	1 – 37 (n=84)
TP mean	28.5±1.6	50.2±2.1	37.3±2.7	37.2±2.8	34.1±2.5
TP range	9 – 64 (n=64)	24 – 110 (n=62)	20 – 45 (n=10)	17 – 44 (n=10)	21 – 48 (n=10)
NO$_x$ mean	100.25±14.2	47.2±10.1	29.3±5.2	15.9±3.1	10.8±1.8
NO$_x$ range	3 – 420 (n=64)	2 – 410 (n=62)	3 – 270 (n=76)	2 – 320 (n=105)	2 – 87 (n=84)
TN/TKN mean	416.7±-34.7	472.8±16.3	543.2±25.7	515.5±22.5	612.7±23.3
TN/TKN range	310 – 610 (n=9)	260 – 825 (n=62)	100 – 1100 (n=76)	100 – 1500 (n=105)	200 – 1460 (n=84)

3.4.2 Surface and Bottom concentration comparisons

There were generally higher concentrations of TN in bottom water compared to surface waters across all sites. Bottom concentrations of TN were typically greater during summer months as opposed to spring and autumn months at Kulnine and Mildura. There were peak differences between the surface and bottom concentrations in February 2021, at Kulnine and Mildura, which were 230 µg L^{-1} and 250 µg L^{-1} respectively, while Euston had peak differences of 395 µg L^{-1} in January 2020. In general surface and bottom concentrations of NO$_x$ were similar for sites Torrumbarry – Kulnine except for higher bottom concentrations in February 2020, and April 2021 at Torrumbarry. Similarly, a small difference was observed in Jan 2020 at Mildura, where bottom concentrations were 7 µg L^{-1} greater than the surface. Higher surface concentrations of NO$_x$ were found in April 2021 at Kulnine, Mildura and Euston. At Corowa, no differences between surface and bottom NO$_x$ were observed.

There were minimal differences in FRP concentrations between top and bottom samples across the study (Figure 3.7). The largest difference observed was 7 µg L^{-1} at Mildura in Jan 2020. In general, top and bottom concentrations had no clear distinction. However, Kulnine showed slightly higher bottom water concentrations in summer periods. During the January 2020 sampling at Euston there was a large difference in TP concentrations, where bottom concentrations were 33 µg L^{-1} greater than the surface (Figure 3.6).

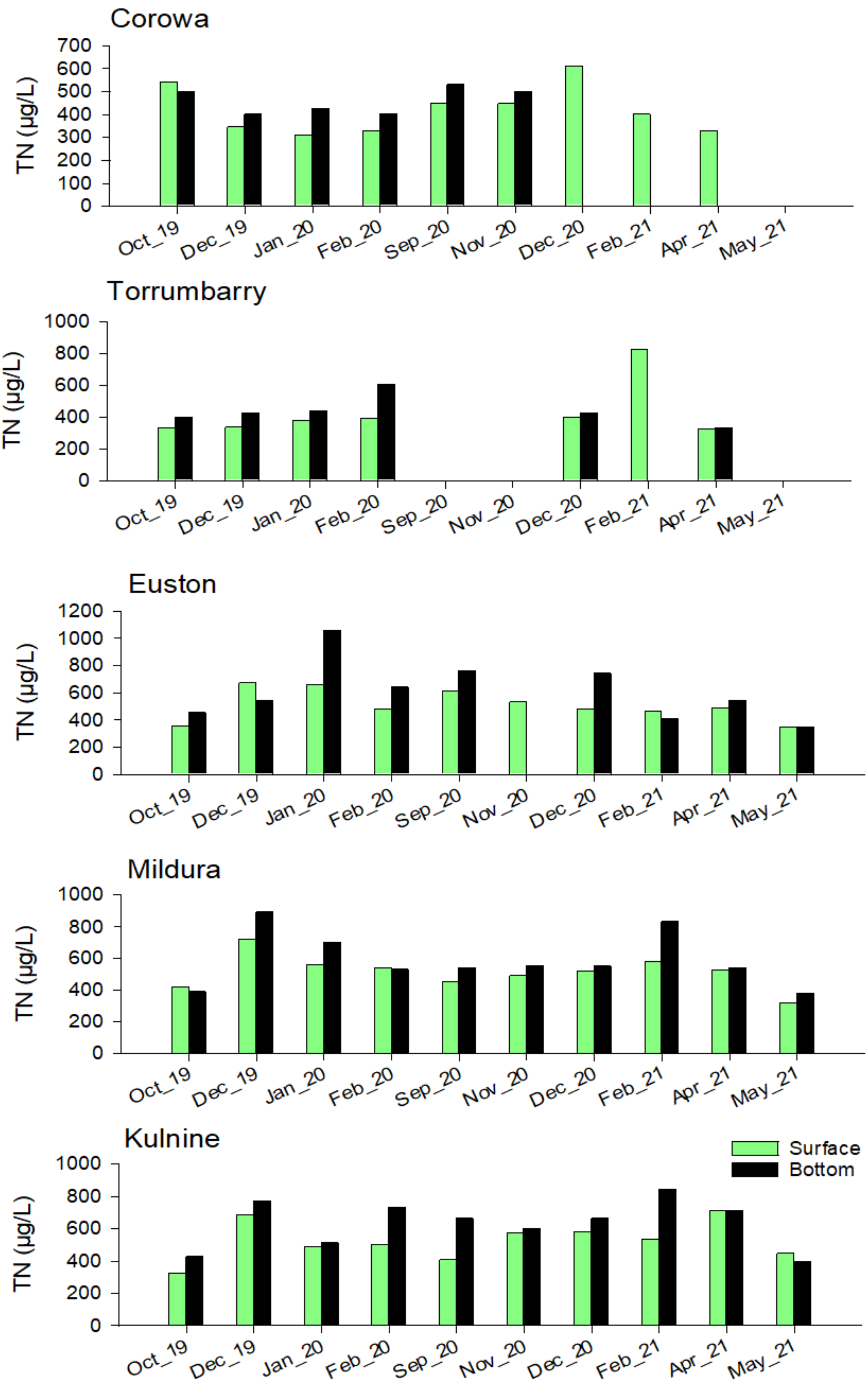


Figure 3.4 Surface and bottom concentrations of TN at all sites sampled across the 2-year sampling period.

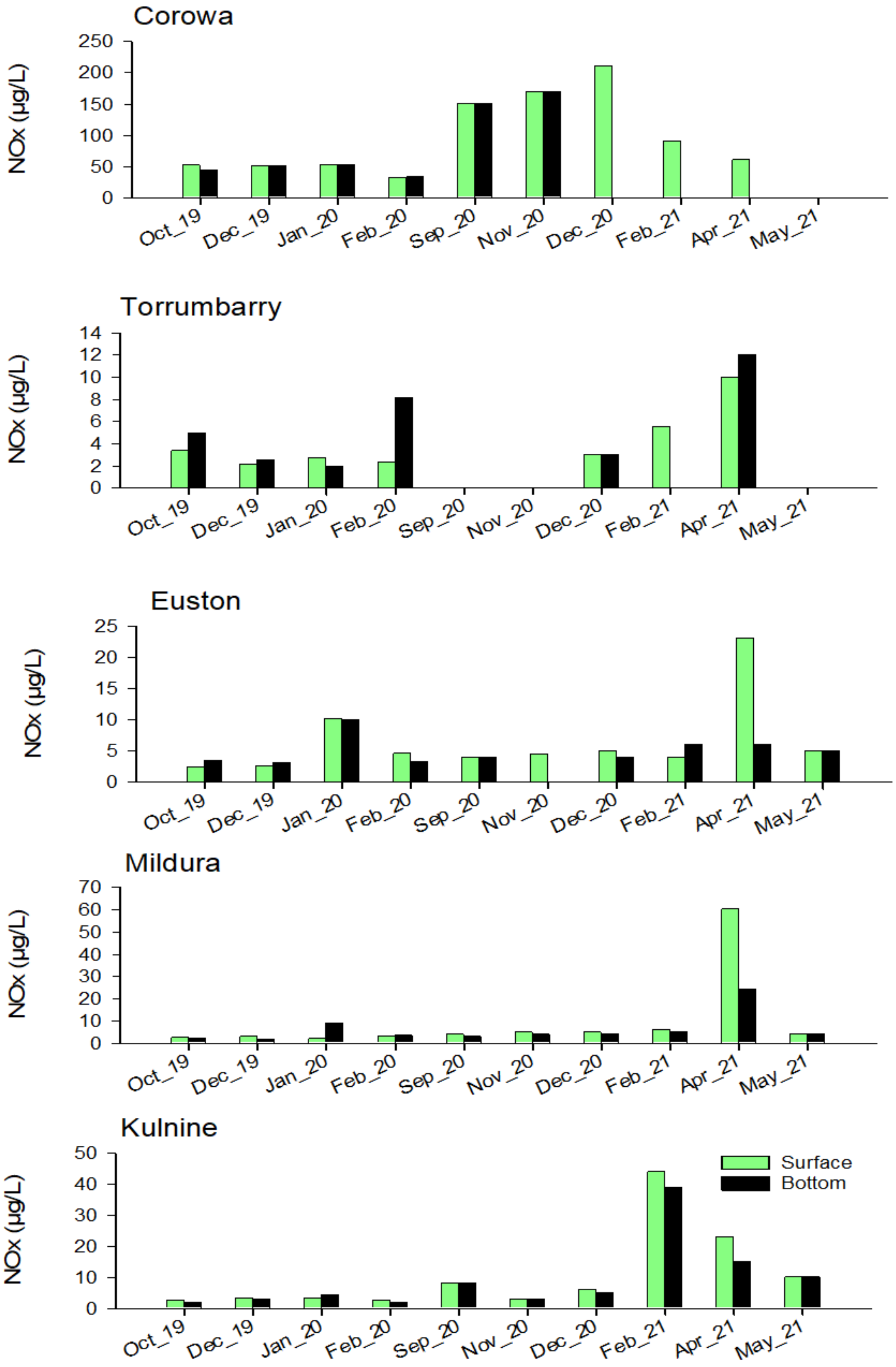


Figure 3.5 Surface and bottom concentrations of NO_x at all sites sampled across the 2-year sampling period.

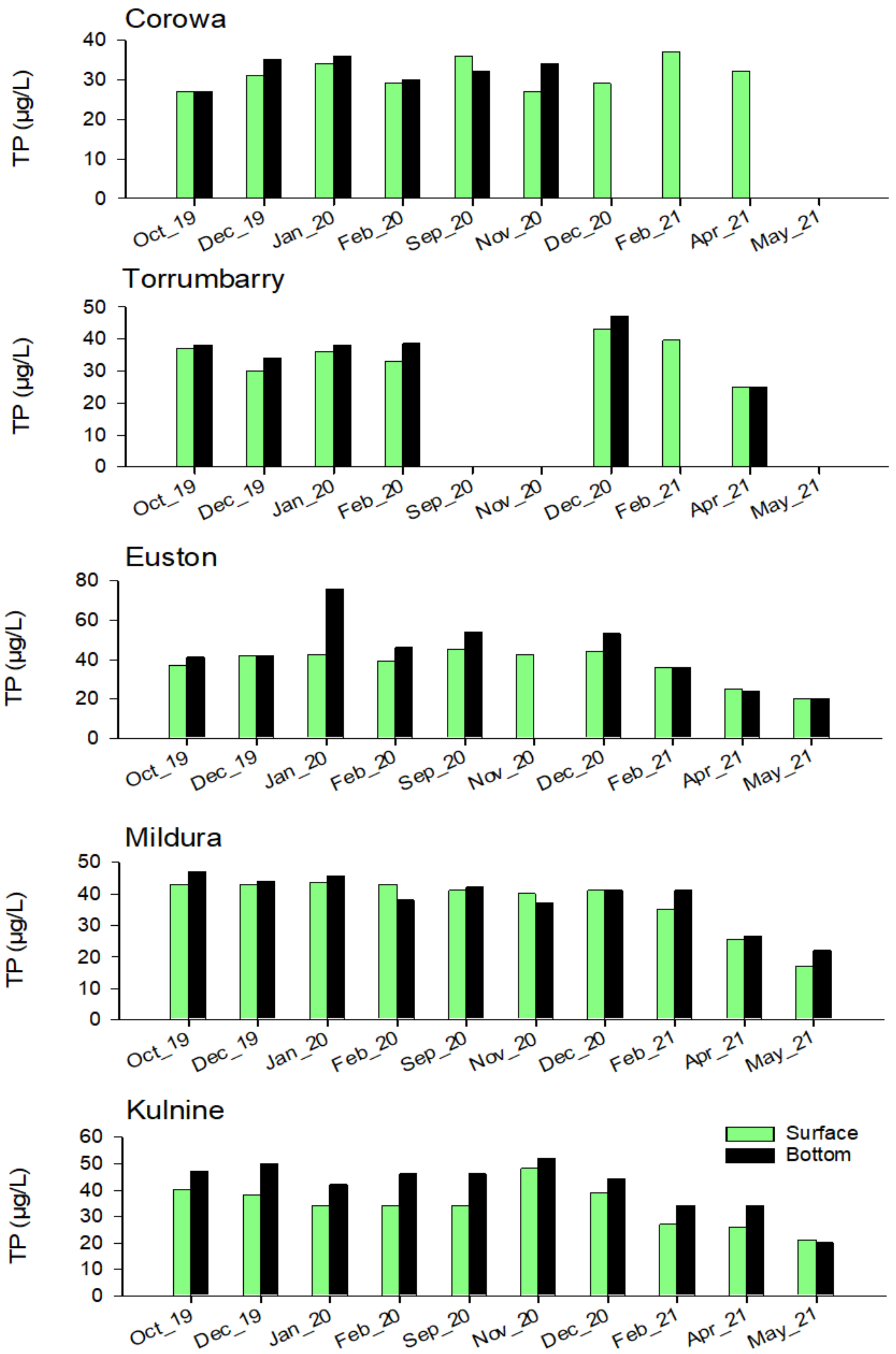


Figure 3.6 Surface and bottom concentrations of TP at all sites sampled across the 2-year sampling period.

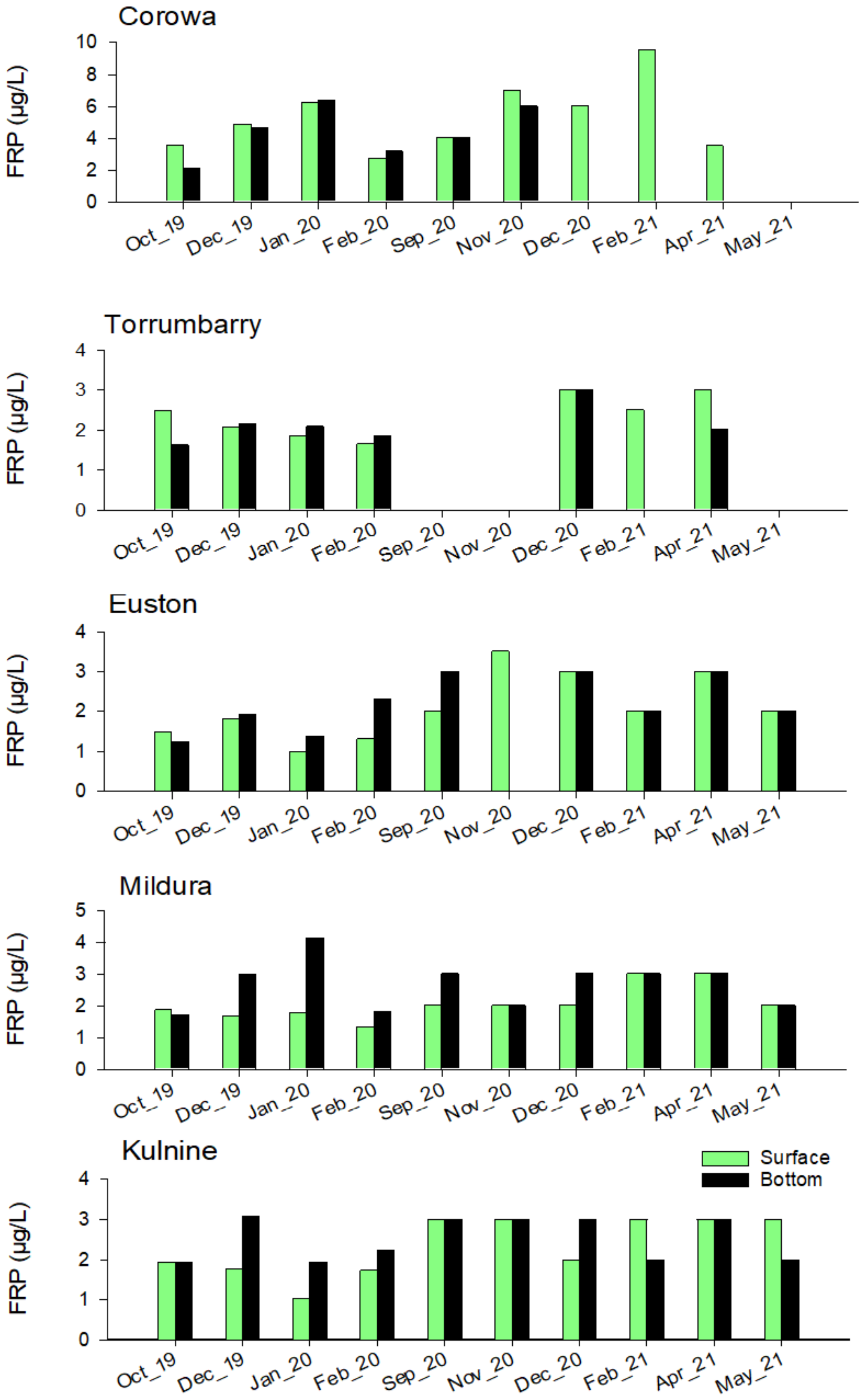


Figure 3.7 Surface and bottom concentrations of FRP at all sites sampled across the 2-year sampling period.

3.5 Discussion

The occurrence of potentially toxic cyanobacterial blooms has been widespread and problematic in the Murray River (Scholz et al. 2003; Baldwin et al. 2010; Al-Tebrineh et al. 2012; Whiterod and Sherman, 2012; Bowling et al. 2013, 2018). To determine the factors driving bloom events, it is important to understand nutrient dynamics within the river both spatially and temporally. Previous work on defining nutrient and cyanobacterial relationships across the Murray has focused on large impoundments such as Lake Hume and Lake Mulwala (Baldwin et al. 2010, 2006; Bowling et al. 2018). However, weir pools may play an important role in the formation of cyanobacterial blooms, particularly in warmer periods where sediment nutrient release may help promote and sustain cyanobacterial blooms. Therefore, it is important to understand the patterns of nutrients and their possible influence on cyanobacterial blooms along the Murray. During the two-year study, bioavailable nutrient concentrations were consistently low, with only NO_x concentrations following a general pattern of decreasing concentration as you move downstream. Total nutrient concentrations were generally highest in summer periods and showed a slight increase downstream. There were only small differences between surface and bottom nutrient concentrations across the study. Total nutrients had peak differences during summer periods across all sites for TN, however TP only showed weak summer peaks of difference downstream of Euston. Dissolved nutrient concentrations consistently showed minimal differences between surface and bottom waters across the study.

3.5.1 Seasonal Patterns of Total and Dissolved nutrients

Seasonal trends in nutrient concentrations can depend on a number of factors such as discharge, thermal stratification, localised point sources and biological uptake (Davis and Koop, 2006; MDBA, 2022; Mosley and Fleming, 2010; Vink et al. 2005). Hume Dam was previously identified as a potential source of nutrients to the Murray River as hypolimnial (or bottom-layer) nutrients can be transported downstream via bottom release offtakes (Baldwin et al. 2010). This is particularly likely during warmer stratified periods when anoxia may persist in the dam sediments. A general longitudinal trend of decreasing nutrients with distance downstream has been found in the Murray previously (Furst et al. 2019), so this may be expected in summer periods. Interestingly, in our study there was a pattern of decreasing NO_x concentrations going downstream (Figure 3.2). However, NO_x concentrations were low at all sites across the Austral summer and autumn months, including at Corowa, downstream of Hume Dam (Figure 3.2). This suggests that Hume Dam is not a significant source of NO_x to the lower Murray River. This is supported by Baldwin et al. (2010) who found that the dam was a net sink for NO_x through summer and autumn. The low NO_x concentrations were due to nitrate reduction processes or from increased denitrification rates, typically associated with stratification or hypolimnetic anoxia events (Baldwin et al. 2010).

The only clear summer peak in NO_x levels was at Kulnine in January 2020, which occurred across a period of increasing discharge. This may have been due to wetting of benches bringing in organic matter and associated nutrients (Baldwin et al. 2005). These low levels of NO_x in summer may be particularly favourable for N-fixing cyanobacteria, who are able to fix atmospheric nitrogen to meet their N demands (Harris, 2001, Wood, 2010). Across the study period, dense N-fixing cyanobacterial blooms of *Dolichospermum crassum*, *Dolichospermum planctonicum* and *Chrysosporum ovalisporum* occurred periodically in weir pools and reservoirs particularly during summer periods (RACC, 2022).

TN/TKN concentrations followed an inverse seasonal trend compared to NO_x (Figure 3.2). Generally, TN concentrations increased in summer months. It is possible that the increased TN concentrations were due to fixation of atmospheric nitrogen by diazotrophic cyanobacteria. For example, increased TN concentration coincided with a bloom of *Dolichospermum crassum* which moved from Euston down to Kulnine from January-February 2020 (RACC, 2022). A large proportion of the nitrogen budget during these bloom periods is likely held within cyanobacterial cells and transported downstream. Although on bloom breakdown, NO_x may be released, however this was not apparent (Figure 3.2). It is likely that any available nitrogen was quickly reused by non-diazotrophic species that can quickly uptake N. TN concentrations were not notably higher downstream of the dam at Corowa, suggesting Hume Dam was not a significant source of TN.

TP concentrations at upstream sites Corowa and Torrumbarry peaked in the winter months (Figure 3.3). These sites are the most likely to be influenced by Hume Dam. Downstream, TP levels were generally similar from Euston to Kulnine where TP concentrations showed weak peaks over the summer periods. These sites share similar physicochemical characteristics, such as turbidity, pH levels and typically experience the same blooms (Mitrovic et al. 2022), which can all influence TP levels (Baldwin et al. 2008; Bowling et al. 2013). FRP concentrations were consistently low at all sites across the study period, however some small spikes were observed at Kulnine (Figure 3.3). Instances of short stratification periods were observed at Kulnine (Mitrovic et al. 2022), although the small peaks of FRP at Kulnine did not match the stratification periods. FRP spikes may have been evident given a finer temporal sampling resolution, particularly at Kulnine. The quick reductions post nutrient spike are possibly due to uptake from the *Dolichospermum crassum* bloom (Figure 3.3) or potential re-adsorption to the sediment as experienced in Hume Dam (Baldwin et al. 2006).

3.5.2 Surface and bottom concentration comparisons

Generally, TN/TKN concentrations were greater in the bottom of the water column than the surface during the summer months. This pattern was consistent across all sites, although there were greater differences at sites from Euston downstream to Kulnine. Given that persistent thermal stratification was rare in the Murray River during our sampling periods (Mitrovic et al. 2022), the increased bottom water concentrations are unlikely to be caused by anoxia-based sediment nutrient release. It is more likely that increased bottom water N concentrations were caused by sediment resuspension or from ammonium accumulation at the sediment interface prior to oxidation (Aldridge et al. 2012). NO_x concentrations were not notably or consistently higher in bottom waters. The differences in trends between TN and NO_x suggests that TN trends are driven by another form of N, such as ammonia. Due to the results largely reflecting those in our riverine site where we would expect to see differences, it is unlikely that there are any bioavailable nutrients coming from the sediments in the weir pools under our observed conditions. We predict NO_x concentrations potentially influencing bloom development may be primarily driven by allochthonous inputs (Aldridge et al. 2012; Al-Tebrineh et al. 2012; Beversdorf et al. 2013).

There were rarely notable differences between TP concentrations in surface and bottom water across all sites during the study, perhaps with the exception of Kulnine. Phosphorus is often bound to iron compounds in sediments and their solubility is redox sensitive (Molot 2014). Under oxic and circumneutral conditions these compounds are predominantly insoluble (Molot 2014). This may explain why available phosphorus was not readily released from sediments, particularly as persistent thermal stratification was rare. However, at Euston in January 2020 TP was elevated in bottom waters, possibly due to a short change in redox chemistry at the water-sediment boundary resulting in the release of phosphorus, or from sediment resuspension. FRP concentrations were occasionally higher in bottom waters than surface waters, particularly at Euston, Mildura and Kulnine. However, given that TP concentrations did not follow this trend, this is more likely a result of utilization by cyanobacteria and other phytoplankton in surface waters.

As evident in Chapter 2, the particular *Dolichospermum* species blooming across the study period were limited by P, however available P was consistently low. This is interesting to note as these blooms may be persisting at the edge of a P threshold where blooms may no longer be supported. As cyanobacteria have the ability to scavenge and store P during periods of N and P stress, short pulses of P may be all that is required to sustain the blooms (Lu 2019). Alternatively, nutrients present may also be sourced from tributaries or anabranches along the river, however in previous years studies have shown tributaries to deliver minimal amounts of nutrients into the system (Furst 2019). Additionally, Bowling (2018) found any flows during the austral autumn period, led to the breakdown of the blooms present, however inflows were minimal during bloom periods in my study. Unfortunately, the sampling resolution was low for TP and FRP at Euston, Mildura and Kulnine (Figure 3.3), and sampling points did not match the periods of persistent stratification. However, as the influence of climate change on riverine systems grow, the likelihood of stratification in the Murray rivers weir pools is increasingly likely (Mitrovic et al. 2022).

3.5.3 Conclusion

This study has given insight into the potential sources of nutrients and their availabilities across the austral seasons. We hypothesised that nutrients would decrease as we move downstream and that overall concentrations would be higher during summer periods. When looking longitudinally, we found evidence of a decrease in available nutrients but not in total nutrient values. In support of this, seasonally we found that during summer total nutrients were greater, with low available nutrients, which also supports our hypothesis. We also hypothesised that sediments may act as a source of nutrients. We found little evidence of differences within the top and bottom of the water column, suggesting differences were not likely linked to sediment release. However, conditions rarely were suitable for sediment nutrient release and the onset of anoxia (Mitrovic et al. 2022), and sampling resolution was not ideal at sites likely to stratify. Due to this, it is likely sediments may still play a role in nutrient availability and thus bloom development, particularly as the threat of stratification increases. In the next chapter I will examine experimentally nutrient release under oxic and anoxic conditions for sediments taken from two sites on the Murray River.

Chapter 4: Nutrient release from weir pool sediments of the Murray River, Australia

4.1 Abstract

Riverine sediments are a potential source of macronutrients such as nitrogen and phosphorus, as well as micronutrients such as cobalt, iron, manganese and sulfate. These nutrients are vital for phytoplankton growth and bloom formation, however in the Murray River, Australia, whether they become available from sediments within the weir pools is still not understood. In the weirs of the Murray River, the threat of thermal stratification has increased, and thus the likelihood of hypolimnetic deoxygenation events. Under anoxic conditions, these nutrients may become readily available from the sediments, which may support problematic cyanobacterial blooms. In this study sediments were collected from two weir pools where potentially toxic cyanobacterial blooms are prevalent. Sediments were setup in anoxic and oxic incubation chambers for 21 days to assess nutrient release into overlying water. We found the sediments to be a source of both macronutrients and micronutrients. In anoxic treatments we found clear releases of NH_4^+ (Ammonium), Fe (Iron), Co (Cobalt), Mn (Manganese) and S (Sulfur). In oxic treatments we found NH_4^+, Co, Mn, and S to be released, while NO_x was also released at Kulnine. Interestingly, no P release was observed across the study. This suggests sediments may be a source of nitrogen and some micronutrients to weir pools of the lower Murray River.

4.2 Introduction

Riverine environments are increasingly becoming host to cyanobacterial blooms around the world (Bowling et al. 2018; Ha et al. 1999; Krogmann et al. 1986; Bowling and Baker 1996). However, understanding the main drivers of these riverine blooms has been problematic. The prevalence and accumulation of nutrients can dictate the severity of cyanobacterial blooms, however this is often overlooked in rivers (Wurtsbaugh et al. 2019). One major possible source of stored nutrients is sediments, and although this has been looked at often in lakes (Nowlin et al. 2005; Muller et al. 2016; Baldwin et al. 2007), there is limited information on sediment nutrient dynamics in rivers. In riverine environments, sediments can act as a sink for nutrients from upstream or allochthonous origins or under certain conditions may also become a source of nutrients (Baldwin et al. 2007). The main nutrients that affect phytoplankton growth are nitrogen (N) and phosphorus (P), as they are required in the greatest quantity. However, micronutrient trace metals such as iron, manganese and cobalt also play important roles in cyanobacterial bloom formation (Facey et al. 2019).

Structures such as dams and weirs are common in river systems, where they are built for the control of flow for anthropogenic use (Hong et al. 2016; Jones and Poplawski 1998). These impoundments can act as large sediment traps (Olive and Olley, 1997; McCarthy et al. 2004). In riverine environments, during times of lower or reduced flow, impoundments such as weir pools further reduce in channel flow velocity. This allows finer sediments suspended in the column to settle, leading to sedimentation of nutrient rich organic matter (Webster et al. 2001; McCarthy et al. 2004; Boström et al. 1988). Nutrients trapped may then become available from these sediments, particularly if the sediment-water interface becomes anoxic, typically when the waterbody thermally stratifies (Becker et al. 2009).

The micronutrients and macronutrients have different mechanisms of release from sediments, which in part, can be controlled by physicochemical properties such as oxygen, pH and temperature (Miao et al. 2006; Mortimer 1971; Bostrom 1988; Webster et al. 2001). Phosphorus release can arise either through the reversible adsorption and desorption process of phosphate with sediments (Webster et al. 2001). This typically occurs in a dynamic equilibrium, where available phosphate is used within the overlying water and this leads to increased phosphate being released from the sediment (Webster et al. 2001). Phosphorus is also made available in biotic nutrient cycling through extracellular enzymatic hydrolysis, which produces free bioavailable orthophosphate (Song et al. 2006). Similarly, under anoxia the redox potential is reduced, stimulating microbial activity which can release bound phosphate into the water column through reduction (Mortimer, 1971). This release can be due to various types of bacteria such as iron-reducing bacteria or sulfate-reducing bacteria (Baldwin 2002; Bostrom 1988).

The availability of nitrogen occurs as ammonium, nitrate, nitrite, and dinitrogen gas in a dissolved inorganic state (Sigee, 2005). Insoluble organic nitrogen is often released by plants and animals and settles in the sediments where bacteria and protozoans convert it to inorganic forms of nitrogen through remineralisation (Sigee, 2005). In oxygenated sediment, ammonium is microbially oxidised to nitrite and nitrate through nitrification, which release available nutrients and can also contribute to the onset of anoxia (Sigee, 2005). Under anoxic sediment conditions, ammonium will accumulate in the overlying water and won't nitrify (Saunders and Kalff 2001). However, ammonium and nitrite can be converted to dinitrogen gas under a process known as anaerobic ammonium oxidation (anammox) (Kartal et al.

2011). Also under anoxia, denitrification rates increase, and as a result nitrate and nitrite are microbially converted to dinitrogen gas which exits the aquatic system into the atmosphere (Saunders and Kalff, 2001; Sigee, 2005). Sediments can also act as a source for micronutrients such as iron, cobalt and manganese which are significantly important for cellular function (Facey et al. 2019; Huerta Diaz et al. 1998). Similarly, other sediment derived micronutrients such as sulfate and iron can also influence nutrient availability through microbial reduction (Baldwin et al. 2007).

The role of sediment derived micronutrients and macronutrients in phytoplankton and cyanobacterial growth is well known (Molot et al. 2014; Facey et al. 2019; Huerta Diaz et al. 1998; Bostrom 1988; Nowlin 2005). In the Murray-Darling basin, the influence of sediments and nutrients has been examined but these studies have focussed on sediment and nutrient origins, primarily through the northern parts of the basin (Moran et al. 2005; De Rose et al. 2004; Oliver 1999). These studies have not explicitly looked at the release and concentration of nutrients from sediments under a controlled environment. In Australian waters, controlled sediment incubation experiments have been conducted in large water bodies (Muller et al. 2016; Baldwin et al. 2007) such as Lake Hume. On the Murray River, Lake Hume is the largest impoundment on the river that was looked at for the release of nutrients under a controlled environment (Baldwin et al. 2007). Sediment studies within the river channel have not been examined to our knowledge.

In this study we are looking to fill the knowledge gap of sediment nutrient release from weir pools in the Murray River. In weir pools, the likelihood of stratification and a drop in dissolved oxygen has increased in recent years, thus the likelihood of problematic cyanobacterial blooms has increased (Bowling 2018; Mitrovic et al. 2022). The aim of this study was to determine which nutrients are released from sediments under oxic and anoxic conditions and their respective concentrations in the overlying water. This experiment was carried out in a laboratory to specifically see the influence of oxygen on riverine sediments. We hypothesised that sediments would release higher amounts of nutrients under anoxic conditions than oxic conditions.

4.3 Methods

4.3.1 Study Sites

Study sites chosen included Kulnine and Mildura, along the Murray River. Sites were chosen based on within weir pool location, their relative likelihood to stratify and cyanobacterial bloom prevalence. The Mildura weir pool has no major tributaries, and extends 60 km upstream from the weir. It is directly downstream of Euston Weir which is influenced by the Murrumbidgee River as well as the Edward-Wakool Anabranch. The weir pool itself has no major inflows, however it is readily frequented by recreational boaters. The weir pool is also known to interact readily with saline groundwater (Fitzpatrick et al. 2007; Mccarthy et al. 2004). The Kulnine weir pool lies downstream of the Darling River confluence, making it the only site to be influenced by the Darling River. The weir is a fixed weir and is controlled by SA Water. Some of the water is diverted 14 km upstream through Frenchmans Creek into Lake Victoria, an important water supply for South Australia. Additionally, there is a major irrigation diversion upstream in the weir pool, supplying water to Lake Cullulleraine. Having a gently sloping river-bed, the weir pool extends 63 km upstream to the Wentworth Weir.

4.3.2 Sediment collection and Incubation

Sediments were collected on the 3rd of June 2021, and were brought back to the university for experimentation. Incubations commenced on the 4th of June and ran for 21 days. Sediments were collected from Kulnine (M1) and at Mildura (M2) using an Ekman grab sampler collecting the top 5cm of sediment. Approximately 500 mL of the sediment was then carefully placed into the bottom of the containers to minimise disturbance of layers. The plastic incubation containers were made almost identically to Müller et al. (2016). Containers were then filled with ultrapure water and anoxic containers were sealed. Samples were kept cool (~18°C) for overnight transport to the laboratory.

The containers were setup under controlled laboratory conditions, with water temperatures consistently at 18°C for the study. The containers were left in coolers and water was filled in the coolers to the lids of containers. All treatments were kept under dark conditions using loose fitting lids on the coolers. Treatments included incubation under anoxic and oxic environments with 4 replicates per treatment. Anoxic container lids were sealed airtight, and had a butyl rubber septum glued into it for sampling with a pre-rinsed syringe and hypodermic needle. Lids also had an opening with an airtight rubber nitrile glove fixed for pressure equalization, similar to Muller et al. (2016). Oxic chambers had fixed lids with two holes, one was for sample collection and the other had a fixed oxygen line that kept the water oxygenated. The collection of samples was taken on days 0, 2, 5, 9, 14, and 21 for all samples. Dissolved micronutrient and macronutrient samples were taken (20 mL) from all incubation containers on sampling days. Sub-samples were then filtered through pre-rinsed 0.45 µm Sartorius cellulose acetate syringe filters, into acid washed falcon tubes and immediately frozen. Physicochemical conditions, pH and oxygen were collected on all sampling days for oxic treatments, and just day 21 in the anoxic treatments. Dissolved oxygen, pH, and temperature was measured using a Hydrolab field hand-meter Surveyor and MS5 minisonde probe.

4.3.3 Nutrient analysis

Dissolved macronutrients measured include filterable reactive phosphorus (FRP), ammonium (NH_4^+) and the combined nitrate and nitrite (NO_x) were determined photometrically using a QuikChem 8500 Lachat nutrient analyser (Loveland USA) following APHA (1995) methods. Phosphorus as FRP was measured with the molybdite blue method using ascorbic acid as the reductant (Murphy and Riley, 1962). NO_x was analysed with the sulphanilamide method after reduction by a cadmium column (APHA, 1995; Hosomi & Sudo, 1986). Micronutrient concentrations were determined using US EPA methods U.S. EPA. (1994), and U.S. EPA. (2014), on an Agilent 5110 ICP-AES.

4.3.4 Statistical analysis

Graphs were made using SigmaPlot 12.5. All statistical analyses were carried out using the software Primer 6 and R. Pearson's regression analysis was performed on R with *car* (Fox and Weisberg 2019) and correlation plots were created using *ggplot2* (Wickham 2016). PERMANOVA (with Euclidian distances) was performed using the software PRIMER 7.0.

4.4 Results

4.4.1 Incubation conditions

In both oxic and anoxic treatments, pH levels were consistently low <6 (Table 4.1). However, ambient pH levels were consistently around 7 when sediments were collected. Dissolved oxygen was consistently high in oxic treatments during the 21-day incubation, while readings could only be taken from anoxic treatments at the experiments end. Anoxic treatments were confirmed to be hypoxic <2.0 mg/L, however readings below 2.0 mg/L in these treatments are labelled and assumed anoxic, as oxygen readings always occurred after to exposure to oxygen. In the oxic treatments of both sites, pH and dissolved oxygen dropped slightly across the study.

Table 4.1 pH and dissolved oxygen (mg/L) in experiments on day 2 and 21. N=4

		pH	DO (mg/L)
M1 – Anoxic	**Day 2**	-	-
	Day 21	**5.8**	**<2.0**
M1 – Oxic	**Day 2**	**5.3**	**9.5**
	Day 21	**4.5**	**8.9**
M2 – Anoxic	**Day 2**	-	-
	Day 21	**5.0**	**<2.0**
M2 – Oxic	**Day 2**	**4.3**	**9.6**
	Day 21	**3.7**	**9.2**

4.4.2 Nutrient release from the sediment

NH_4^+ release was large at day 2 of the experiment across all treatments. Concentrations consistently rose in unison across sampling days and plateaued after day 14 (Figure 4.1). There were no statistical differences between the oxic and anoxic treatments at both sites (PERMANOVA: p-value <0.05). The overall concentrations rose from ~5000 µg/L to ~11000 µg/L.

NO_x release was quite variable between oxic and anoxic treatments (Figure 4.1). At both sites, NO_x levels in the anoxic treatments rose rapidly at day 5, whereas there was no response in the oxic treatments. By day 5 the anoxic treatments were significantly greater than the oxic (PERMANOVA: p-value <0.05). Anoxic treatments were 817.0 ± 65.9 µg/L and the oxic treatments 23.8 ± 15.3 µg/L at Kulnine, and at Mildura reached 1323.3 ± 60.6 µg/L in the anoxic and 12.5 ± 6.3 µg/L in the oxic. NO_x levels at both sites then started to decrease in anoxic treatments till day 14, where it then plateaued at levels similar to day 2 for the last week of incubation (Figure 4.1). In oxic treatments, NO_x levels at Mildura were consistently below 12.5 ± 6.3 µg/L across the study, whereas at Kulnine levels started to rise from day 5 to day 14 where they peaked at 620.0 ± 465.9 µg/L. At Kulnine, only days 2 and 5 were statistically different, whereas at Mildura there were differences on all sampling days (PERMANOVA: p-value <0.05).

FRP release was below the detectable limit (<5 µg/L) across the study except for one replicate on day 2 (Figure 4.1). As a result, FRP was not included in the PERMANOVA analysis.

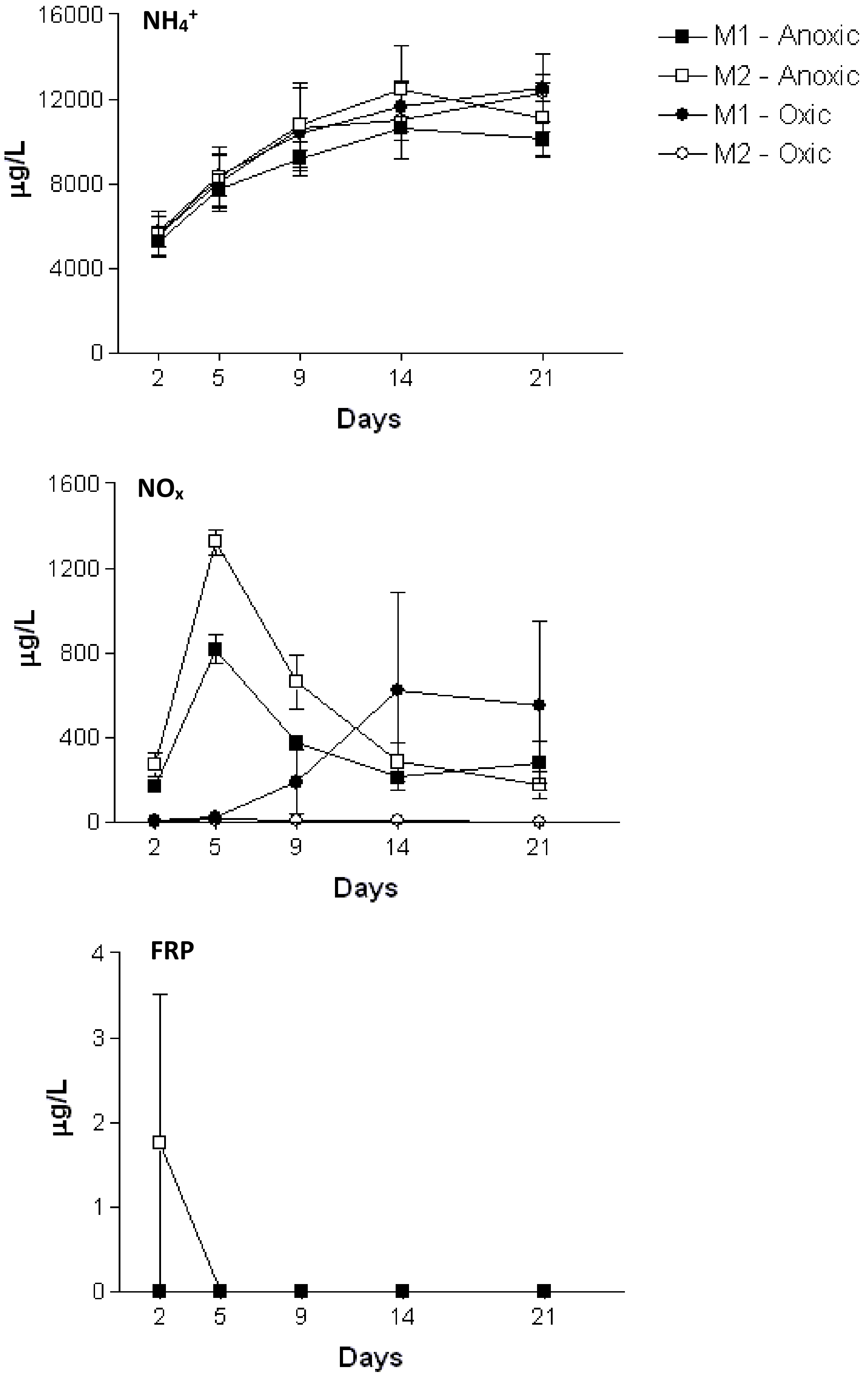


Figure 4.1. Ammonium (NH_4^+), nitrate and nitrite (NO_x) and phosphorus (FRP) concentrations in each treatment from both sites across the 21-day study. Sites are Kulnine (M1) and Mildura (M2). Concentrations are averages of four replicates per treatment at each site. Error bars are one standard error from the mean, n=4.

Fe release was not apparent until after day 5 (Figure 4.2). After day 5, anoxic treatments at both sites released significant amounts of Fe till day 14 (PERMANOVA: p-value <0.05). At Kulnine, anoxic treatments peaked on day 14 at 7349.0 ± 452.2 µg/L, while Mildura also peaked on day 14 at 8218.0 ± 4771.8 µg/L in anoxic treatments (Figure 4.2). From day 14 to day 21 levels fell slightly in the anoxic treatments. By day 21, the anoxic treatment at Kulnine reached 5667.0 ± 1453.1 µg/L, and Mildura reached 6944.0 ± 3606.7 µg/L, which were both significantly greater than the oxic treatment (PERMANOVA: p-value <0.05). The response by the anoxic treatments did not differ based on site (PERMANOVA: p-value >0.05). The oxic treatments at both sites followed patterns similar to FRP treatments, showing little to no Fe release.

Co release followed similar trends in both the oxic and anoxic treatments at both sites. At Kulnine, there was a steady increase in both oxic and anoxic treatments till day 9, where the oxic treatment continued to rise and the anoxic plateaued until the experiments end. Co release rose from 7.1 ± 1.3 µg/L on day 2 to 23.8 ± 8.5 µg/L on day 21 in the oxic treatments. Interestingly, the oxic treatment is only significantly greater than the anoxic treatment on day 21 (PERMANOVA: p-value <0.05), where the response of the oxic treatment was approximately double the anoxic (Figure 4.2). Mildura followed trends similar to Kulnine in both anoxic and oxic treatments. However, the release in the oxic treatment at Mildura was always slightly greater than Kulnine and unlike Kulnine, the oxic treatment at Mildura was significantly greater than the anoxic treatment on day 14 and day 21 (PERMANOVA: p-value <0.05). On day 21 the oxic treatment was 29.1 ± 2.1 µg/L, whereas the anoxic treatment was 13.0 ± 2.6 µg/L. The two sites did not differ significantly on any day across the study.

Mn release consistently rose in all treatments along the length of the study (Figure 4.2). At Kulnine, release of Mn was greater in the anoxic treatment till after day 9 when release slowed, although the oxic treatments continued to rise. By day 21 the oxic treatment was 7198.3 ± 1172.0 µg/L which was significantly greater than the anoxic treatment at 4506.7 ± 422.2 µg/L (PERMANOVA: p-value <0.05). At Mildura, the oxic treatment released slightly more Mn than the anoxic treatments however the treatments did not differ significantly (PERMANOVA: p-value >0.05). At Mildura there was an overall release of 4270.2 ± 405.3 µg/L in the oxic treatment and 3126.6 ± 145.2 µg/L in the anoxic treatment by day 21 (Figure 4.2).

S release was different in the anoxic to oxic treatments across the 21 days, however both sites displayed similar patterns. At Kulnine release of S was greater in the anoxic treatment till after day 9 when it plateaued, while the oxic treatments continued to rise. By day 21 the oxic treatment was 1406.3 ± 300.1 µg/L, and the anoxic was 675.3 ± 16.7 µg/L, however differences were not significant (PERMANOVA: p-value >0.05). Mildura had a significantly greater release of S in the anoxic treatments on days 2 and 5 (PERMANOVA: p-value <0.05), however after day 9 the anoxic release plateaued and the oxic release continued to increase. By day 14 the oxic treatment was significantly greater than the anoxic (PERMANOVA: p-value <0.05), however by day 21 this was no longer the case (PERMANOVA: p-value >0.05). By day 21, the oxic treatment had released a maximum amount of 836.8 ± 26.9 µg/L, which was only 117.6 ± 151.1 µg/L greater than the anoxic treatment.

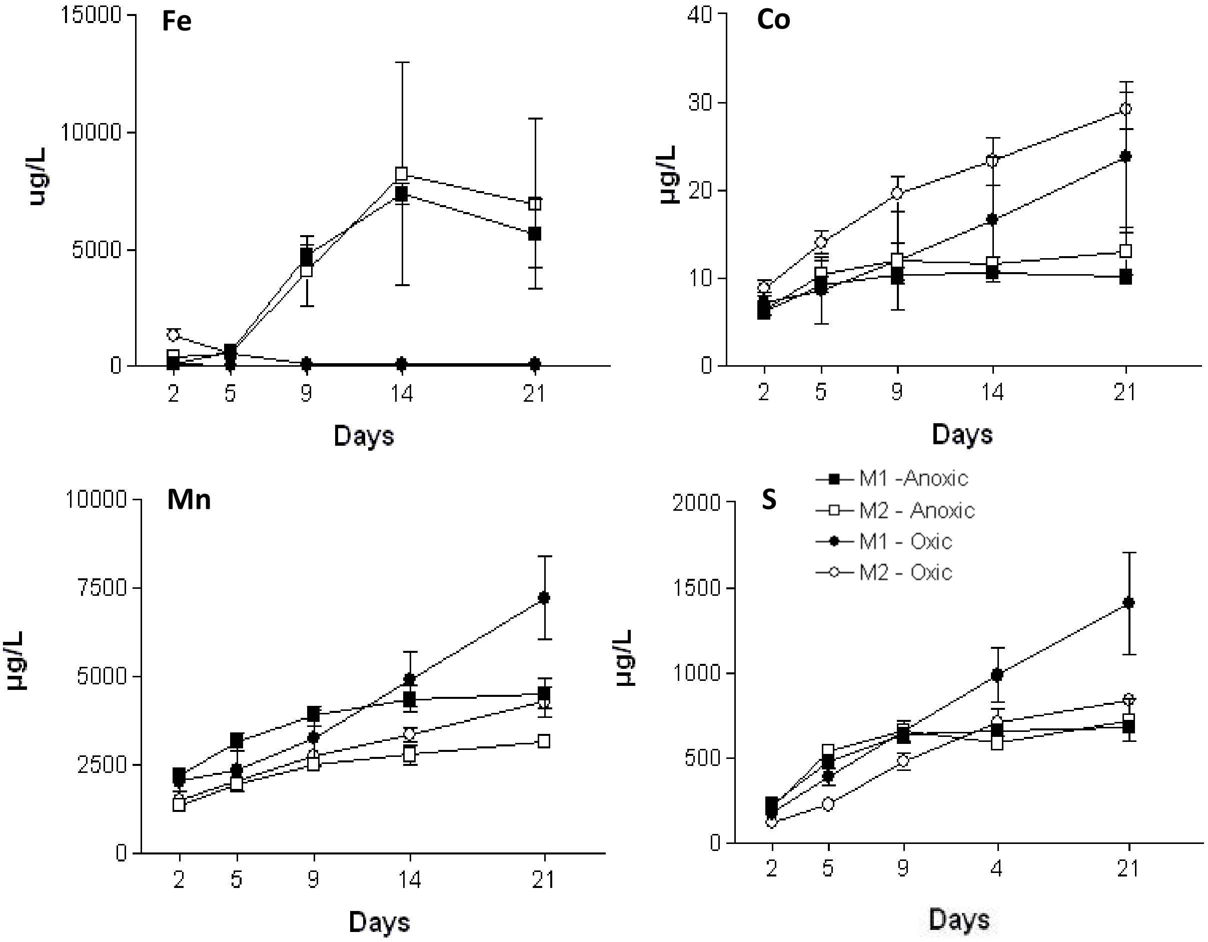
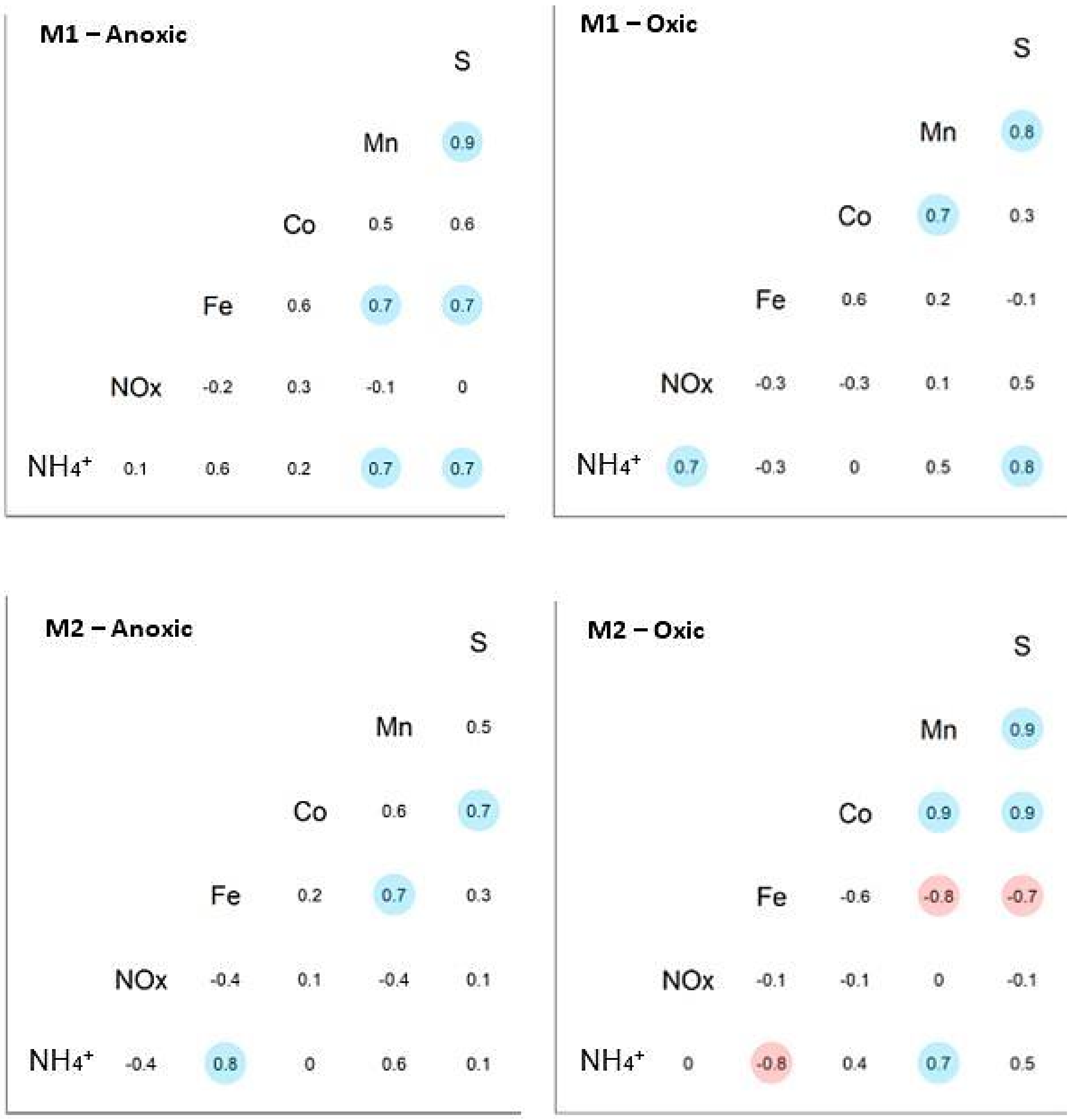


Figure 4.2 Concentrations of micronutrients iron (Fe), cobalt (Co), manganese (Mn), and sulfate (S) in each treatment from both sites across the 21-day study. Sites are Kulnine (M1) and Mildura (M2). Concentrations are averages of four replicates per treatment at each site. Error bars are one standard error from the mean, n=4.

4.4.3 Nutrient interactions

In the anoxic treatments, at Kulnine there were strong positive relationships of both Fe and NH_4^+ with Mn and S (Figure 4.3). The strongest relationship at this site however was between Mn and S. At Mildura, there were also strong positive relationships between Fe and Mn, however we also observed positive relationships between Co and S, as well as Fe and NH_4^+. In oxic treatments, at Kulnine NH_4^+ had a strong positive relationship with both NO_x and S. S also had a strong positive relationship with Mn, and Mn had a strong positive relationship with Co. At Mildura however, there were very strong positive correlations between Co, Mn, and S, as well as between Mn and NH_4^+. Finally, Fe at Mildura had a strong negative correlation with NH_4^+, Mn and S (Figure 4.3).



M1 – Anoxic

	NOx	Fe	Co	Mn	S
Mn					0.9
Co				0.5	0.6
Fe			0.6	0.7	0.7
NOx		-0.2	0.3	-0.1	0
NH_4^+	0.1	0.6	0.2	0.7	0.7

M1 – Oxic

	NOx	Fe	Co	Mn	S
Mn					0.8
Co				0.7	0.3
Fe			0.6	0.2	-0.1
NOx		-0.3	-0.3	0.1	0.5
NH_4^+	0.7	-0.3	0	0.5	0.8

M2 – Anoxic

	NOx	Fe	Co	Mn	S
Mn					0.5
Co				0.6	0.7
Fe			0.2	0.7	0.3
NOx		-0.4	0.1	-0.4	0.1
NH_4^+	-0.4	0.8	0	0.6	0.1

M2 – Oxic

	NOx	Fe	Co	Mn	S
Mn					0.9
Co				0.9	0.9
Fe			-0.6	-0.8	-0.7
NOx		-0.1	-0.1	0	-0.1
NH_4^+	0	-0.8	0.4	0.7	0.5

Figure 4.3 Correlation plots of the R values of different dissolved nutrients and macronutrients in each treatment across the study. Sites are Kulnine (M1) and Mildura (M2). Blue circles indicate a positive relationship ≥0.7, and red circles indicate a negative relationship ≤-0.7.

4.5 Discussion

This study examined the release of different macronutrients and micronutrients from weir pool sediments of the lower Murray River, NSW Australia and the influence of oxic and anoxic conditions. Overall, we found that in environments where oxygen was lacking, the sediments became a source of NH_4^+, NO_x, Fe, Co, Mn and S. However, when sediments were oxygenated they were a source for NH_4^+, Co, Mn and S at both sites, as well as NO_x at just Kulnine. Interestingly, across both sites and within each treatment of the 21-day study, no FRP release was observed. Relationships were highly variable between the nutrients found in anoxic and oxic treatments, particularly NO_x and Fe. Additionally, we found a clear positive relationship between Fe and both NH_4^+ and Mn in the anoxic treatment. Whereas in the oxic treatment, there were strong negative relationships between Fe and both NH_4^+ and Mn (Figure 4.3).

4.5.1 Nitrogen release from sediments

The release of NO_x was highly variable across the 21-day experiment. In the anoxic treatment there was a large release of NO_x by day 5 of the experiment. This response was somewhat unexpected as typically under anoxic conditions, denitrifying processes occur (Saunders and Klaff, 2001). I predict that this is a case of denitrifying bacteria succeeding nitrifying bacteria present as anoxia establishes. As the river system was mixed at the time of sample collection for both sites, and samples were possibly agitated during transport, it is very likely that some oxygen was present in the containers at the start of the incubations. It is likely that nitrifying bacteria present quickly utilised the available oxygen to nitrify ammonium to nitrite and nitrate. Then when sealed, oxygen supply was depleted and denitrifying bacteria likely dominated the bacterial community. Similarly, anoxic ammonium and nitrite can be converted to dinitrogen gas under a process known as anaerobic ammonium oxidation (anammox) (Kartal et al. 2011). From day 5 it is also likely this was occurring, possibly at the same time, as there seemed to be a slight decrease in the rate of NH_4^+ release, which coincided with the drop in NO_x. However, NH_4^+ levels were high at the onset of the experiment, and NH_4^+ release was still occurring till day 14 so it is hard to distinguish between what process was truly occurring (Figure 4.1). A similar response has been observed in incubation experiments of lake sediments in eastern Australia (Muller et al. 2016).

The lack of NO_x response in oxic treatments at the Mildura site compared to Kulnine was interesting. It is very likely that at both sites, the rate of denitrification was in equilibrium with the rate of nitrification. Seitzinger (1988) found rates of denitrification to be higher than the rate of nitrification, and studies have found denitrification to occur in aerobic sediments (Chen et al. 2006). Therefore, it is possible that at Mildura they stayed in equilibrium, whereas at Kulnine after day 5, nitrification took over (Figure 4.1). Similarly, the rate of nitrification could have been limited by the low pH in oxic containers across the study (Table 4.1). Nitrifying bacteria have slower growth rates and smaller yield rates as opposed to denitrifying heterotrophic bacteria and are also known to prefer an alkaline environment for nitrification (Shammas 1986). As a result, they are more susceptible to physicochemical changes. The variable response in the Kulnine oxic treatment may have been due to the slightly elevated pH when compared to Mildura (Table 4.1), perhaps being above a threshold required for the bacterial release of NO_x under oxic conditions. Additionally, the Kulnine sediments were collected downstream of the Darling River confluence, thus they could have contained slightly more organic nitrogen, which could have driven the nitrification process to occur. The release of NH_4^+ in both the oxic and anoxic treatments was expected. NH_4^+ can be released through mineralization of organic components of nitrogen, under both oxic and anoxic conditions. As values for all treatments were above 5000 µg/L on day 2, it was clear the release of NH_4^+ was rapid (Figure 4.1). These responses have been found before in lake sediments (Nowlin et al. 2005; Muller et al. 2016; Baldwin et al. 2007), however the initial values of our study were double that found by the end of a 30-day incubation experiment on a large dam upstream in the same river system (Baldwin et al. 2007). Additionally, the ratio of sediments to overlying water may have differed between studies, thus producing different overall concentrations. This release of NH_4^+ may be particularly problematic as cyanobacteria are known to prefer the uptake of ammonium for growth (Flores 2005).

4.5.2 Metals release from sediments

Fe and FRP release were not similar in anoxic treatments, however showed similarities in oxic treatments. The lack of a response by Fe and FRP to the oxygenated treatments was expected, as phosphate is known to bind with iron hydroxides adsorbed to sediment readily (Forsgren et al. 1996; Bostrom et al. 1988). Similarly, the release of Fe into the overlying water in the anoxic treatments was expected. However, the lack of FRP release was unexpected. Typically, it is thought FRP release from sediments is due to anaerobic processes and conditions, however this was not the case. In some situations, nitrate has been found to increase the redox potential in the water column (Anderson 1982). It is possible that the redox potential was too high for FRP to be released in the anoxic treatments, however low enough still for Fe release to occur. Alternatively, the sediments may still contain oxidized Fe minerals, where any P released is quickly reabsorbed (Baldwin et al. 2002). This response was hypothesized by Baldwin and Williams (2007), who also suggested the lack of FRP response was due to the lack of sulfate reducing bacteria. This however was not the case for us as sulfate was being produced from the sediments.

The sedimentary release of the micronutrients Co, Mn and S was interesting and were not influenced greatly by oxygen. We predict that in both oxic and anoxic treatments, the reduction of sulfate was occurring, producing sulfide as a result. Interestingly, even when the overlying water is oxic, increased concentrations of sulfide in sediment porewaters can lead to the release of Co and Mn into the overlying water (Lenstra et al. 2022). We predict this was the case in our treatments as Co and Mn are bound with S to sediments, creating similar responses by all three. This was supported by all three being highly correlated, particularly in oxic treatments (Figure 4.3). Typically, during this process however, Fe (and thus P) would also be released, although in the oxic containers we did not see this. We suspect that this is due to Fe being more redox sensitive than Mn and Co, as typically Fe also requires anoxia and a low pH. Despite these processes potentially occurring, we still did not see any response by FRP in any treatments at both sites, suggesting perhaps that P was depleted in the sediments.

4.5.3 Management implications

We have found in Chapter 2, that potentially toxic species of cyanobacteria are limited by the availability of available phosphorus. Also in the Murray River, the prevalence of potential stratification events, and thus hypolimnetic deoxygenation events is likely under lower flow conditions (Mitrovic et al. 2022). However, the lack of a release of phosphorus in the anoxic (and oxic) treatments in this study was unexpected, and suggests that sediments are not a major source of P. It is possible that the sediments in these weir pools are sinks for P, however further research on the nutrient composition of the rivers weir pools would be needed. As cyanobacteria have been found to be limited by P, future research should be aimed at understanding alternate routes for P availability in the river.

My experiment suggests that under anoxic conditions such as may occur after periods of thermal stratification considerable amounts of nitrogen may be released as either NH_4^+or NO_x. This may support further phytoplankton growth, as suggested by the co-limitation of chlorophyll-a response seen in Chapter 2. Higher concentrations of nitrogen may not favour the N-fixing cyanobacteria and move the community to non N-fixing cyanobacteria and other phytoplankton groups such as the Chlorophyceae.

4.5.4 Conclusion

Overall, I found sediments to be a source of macronutrients and micronutrients. In anoxic treatments there were clear releases of NH_4^+, Fe, Co, Mn, and S. Similarly, NH_4^+, Co, Mn, and S were released under oxic conditions, however NO_x was also released at Kulnine. The results of this study suggest that the release of P is not significantly related to the oxygen concentration at the sediment interface. Therefore, the sedimentary release of P likely plays a small part of the overall sources of P in the system. However, future experimental works should consider closely monitoring oxygen levels to ensure absolute anoxia. Only Fe levels reflected our hypothesis, with all other nutrients not reflecting our prediction, this may have been due to the oxygen levels present.

Chapter 5: General discussion and conclusion

5.1 Overview and conclusions

The general aim of this thesis was to understand more about how nutrients may influence cyanobacterial growth in the Murray River. This was achieved by a series of field and laboratory experimental studies and a field monitoring campaign. My initial *in-situ* microcosm experiments were conducted across four weir pools and one riverine site to see how the macronutrients N and P would influence cyanobacterial and phytoplankton growth. In Chapter 3, we looked at nutrients at the top and bottom of the water column, as well as their spatial and temporal changes. And finally, in chapter 4 we looked at how P might enter the river system for possible phytoplankton use through sediment incubation experiments.

In the limiting nutrient study, the experiments indicated that the phytoplankton community as a whole, using chlorophyll-a as a surrogate, are either phosphorus limited or co-limited by nitrogen and phosphorus. We found evidence of co-limitation by P and N at Corowa, Euston and Kulnine, while P was found to be limiting at Torrumbarry and Mildura. The experiments also revealed that dominant cyanobacterial species present during the study, mainly N-fixing cyanobacteria, were limited by phosphorus. When ambient nutrient concentrations of N were low, cyanobacterial species responded significantly to the phosphorus amendments. As a result, we suggest that phosphorus dynamics are important, and understanding their influence may inform management actions to aid in remediation of nuisance blooms.

To look at dissolved nutrient concentrations along the Murray River we carried out a monitoring program across two austral summer periods when blooms were prevalent. Our data was supplemented by the Murray Darling Basin Authority’s data to achieve a finer temporal resolution. We found that across most of the Murray River dissolved nutrient levels were very low for N and P, with small winter peaks. We also observed a pattern of decreasing NO_x concentrations as you moved downstream from Corowa. This may have been due to a release of nutrients from Hume Dam as observed by Baldwin (2007). Conversely, TN increased with distance downstream, perhaps indicating uptake into phytoplankton biomass. I also examined differences in nutrient concentration between the top and bottom of the water column to help inform whether sediments were contributing notable concentrations of nutrients. Little to no difference for available nutrients was observed between top and bottom samples. However, total nutrients showed some evidence of higher bottom water concentrations at our downstream site of Kulnine. This observation was stronger during summer periods when blooms were present, suggesting that nutrients may be becoming available from the sediments. This led me to examine sediments as a potential source of nutrients in greater detail, through sediment incubation experiments.

The release of sediment bound nutrients is often looked at in lakes or dams, but is largely overlooked in riverine settings. The sediments may be a source of N, P, and trace metals such as cobalt, iron, manganese and sulfur, which are all known to influence cyanobacterial growth (Facey et al. 2019). If the water column stratifies and the hypolimnion becomes hypoxic or anoxic, these conditions may further lead to the release of sediment bound nutrients (Muller et al. 2016). The likelihood of stratification events to occur in weir pools of the Murray River may increase under lower flow conditions during summer (Mitrovic et al. 2022). Therefore, I carried out sediment nutrient release studies under oxic and anoxic conditions. Sites were chosen based on the greatest differences between top and bottom nutrients observed in Chapter 3, as well as from the monthly algal reports which provide a snapshot of the blooms present in the Murray River across the two-year period (RACC, 2022). We found the sediments to be a source of both macronutrients and micronutrients. In anoxic treatments we found clear release of NH_4^+, Fe, Co, Mn and S. Whereas in oxic treatments we found NH_4^+, Co, Mn, and S to be released, while NO_x was also released at Kulnine. It is possible the Darling River influenced the sediments of Murray River downstream of its confluence. It is likely this response was from nitrification processes utilizing a potentially greater organic nitrogen load from the Darling Rivers influence. Interestingly, and significantly, no P release was observed across the study. We predict that P was either quickly re-adsorbed to available iron hydroxides or that the sediments were not high in P levels.

5.2 Management recommendations

In this study we found that N-fixing cyanobacteria were P limited, and if phosphorus concentrations increase in the Murray it is likely that blooms will become more prolific and have higher biovolumes. Therefore, the control of P would be the most effective management strategy in the Murray River for the reduction of cyanobacterial blooms (Schindler 2008). However, first understanding where the phosphorus fuelling blooms are originating from would be required. This would require sampling over finer spatial and temporal scales and include major tributary inflows and possible point sources. Specific weir pools could be targeted to determine key sources of nutrients. This is an essential step in reducing inputs into the Murray River to control cyanobacterial biomass.

5.3 Research recommendations

One area of research that could be explored further is the availability of phosphorus. Other studies in addition to ours have found low overall dissolved phosphorus concentrations across the Murray River (Baldwin et al. 2007). However, cyanobacteria require phosphorus for growth, hence there is still a significant source of available phosphorus for the cyanobacterial blooms observed in the Murray. Therefore, future research should look into where the phosphorus fueling the blooms is originating from.

Another area of research could look at understanding the impact of sediment derived N and micronutrients on cyanobacteria. As we found significant release of N and the micronutrients iron, cobalt, manganese and sulfur released in our container experiments, it would be worth exploring what implications these may have on phytoplankton and cyanobacterial growth. Similarly, other trace metal micronutrients not looked at in this study may contribute to bloom formation and should be looked at and considered.

One more recommendation for research would be to look at the chemical and bacterial processes occurring in sediments, particularly when carrying out sediment incubation experiments. A greater insight into what processes are occurring may help understand why certain nutrients are or are not being released from sediments.

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