The importance of tributary inflows on productivity. A study of the Barwon-Darling River

Taskforce MER Plan: Project 08.6 Report

A research collaboration between UTS and DPE – Water

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Acknowledgement of Country

The Department of Planning and Environment acknowledges that it stands on Aboriginal land. We acknowledge the Traditional Custodians of the land and we show our respect for Elders past, present and emerging through thoughtful and collaborative approaches to our work, seeking to demonstrate our ongoing commitment to providing places in which Aboriginal people are included socially, culturally and economically.

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1. Executive Summary

Flow events are important to promote the productivity of lowland river food webs. They create connectivity between the river and the surrounding floodplain landscapes (e.g., billabongs & flood runners), mobilising allochthonous organic matter and nutrients such as nitrogen and phosphorus. These resources can stimulate the basal food web and may also have consequences to higher trophic levels such as native fish. Tributary inflows play an important role in delivering resources to the mainstem of a river and also improving water quality and ecological functions in the rivers main stem.

To determine the importance of tributary flows into the Barwon-Darling River for river productivity we conducted a study which addressed two important questions. Firstly, what is the role of major tributaries in delivering basal resources to the main channel of the Barwon-Darling River? Secondly, do basal resources mobilised during flooding support increased food web productivity? To answer these questions, we conducted a two-part study following a flood event between April and July 2021. The first part was a monitoring study examining resource and productivity changes in two tributaries (the Namoi River and Mehi River) of the Barwon-Darling River. We sampled physico-chemical parameters, total and filtered nutrients, dissolved organic carbon, phytoplankton and zooplankton on both tributaries as well as up and downstream of the tributaries on the Barwon River mainstem. The second part of the study experimentally tested how floodplain leachates from three tributaries (Namoi, Mehi and Macquarie) influenced the microbial food web over three time periods post flood event. We used microcosms to test the effects of two concentrations of leachate (2mg/L and 6mg/L DOC concentration) from each tributary floodplain on the microbial food web of the Barwon mainstem. These occurred immediately after the April flood had receded, then 3 and 6 weeks after the flood had receded, examining changes in nutrients, DOC, phytoplankton (Chlorophyll-*a* and community) and bacteria.

The results from the monitoring study showed discharge was important for mobilising nutrients and increasing productivity. In particular, the large flood event in early April 2021 mobilised very high loads and concentrations of nutrients, DOC and zooplankton, however, suppressed phytoplankton growth likely due to turbidity and dilution. During and after the flood event, the Mehi River appeared to contribute loads of nutrients to the main stem much higher than the contribution to total discharge. Once flood conditions had receded, phytoplankton concentrations boomed on both tributaries while bacterial concentrations were also an order of magnitude higher on the Namoi and Mehi Rivers compared to the Barwon main stem. Further, flow events on tributaries mobilised concentrations of nutrients and DOC similar to that of the Barwon despite being 15% of the flow size of the Barwon, suggesting smaller tributary flows may mimic the results of very large mainstem floods. Despite lower concentrations of DOC, bacteria were generally much higher on the Namoi and Mehi than the Barwon River, suggesting the organic carbon present in the tributaries was of a higher quality than that of the main stem.

Phytoplankton and bacterial responses to leachates in the microcosms in the Barwon River varied across experiments. The C:N:P ratio of the leachates appeared to influence food web

responses as the Namoi leachate (lowest concentration of nutrients to carbon) always had the lowest bacterial and phytoplankton growth across the experiments. Furthermore, phytoplankton and bacterial growth in the microcosm experiments was generally highest in the 6mg/L leachate additions from the Macquarie and Mehi floodplains, which had higher concentrations of nutrients to carbon. The results from the microcosms therefore supported those of the monitoring study, suggesting flows that mobilise more floodplain materials will result in higher microbial productivity in the riverine food web.

Our data suggests flow events down either tributary may result in high nutrient and DOC concentrations in the Barwon Darling, leading to greater basal production in the tributaries and subsequently, the Barwon River. Tributary flows entering the Barwon-Darling River when the main stem is at relatively low flow, such as after localised tributary flow events, will likely have a much greater effect on the main stem, both in terms of the biota imported and the utilisation of resources delivered. Overall, this study supports the importance of tributary flows to the main stem Barwon-Darling River. Flow rules that protect flows in tributaries entering the main stem should see ecological improvements in river productivity through increased resources and biota such as algae and zooplankton.

This study provided some preliminary evidence of the importance of tributary inflows to supporting the main stem food web on the Barwon-Darling River. A longer study period encompassing different flow periods and inflow volumes would offer far clearer results on the influence of tributaries on production within the Barwon River. Further, understanding differences between tributaries such as bioavailability, nutrient loads and phytoplankton community composition would allow for better understanding of the influence from specific tributaries.

2. Introduction

Flow events play an important role in the productivity of lowland river food webs (Junk et al 1989; Poff and Zimmerman, 2010). Large flow events, such as floods, create connectivity between the river and the surrounding floodplain, mobilising high loads of allochthonous organic matter as dissolved organic carbon (DOC) and nutrients such as nitrogen and phosphorus (Westhorpe and Mitrovic, 2012). Medium sized flow events or 'freshes' may also increase organic carbon and nutrient concentrations from within channel, benches and flood runner sources, but often at lower total loads compared to high flow events which inundate the floodplain (Sheldon and Thoms 2006; Woodward et al., 2015). These resources brought in by flow events (Westhorpe and Mitrovic 2012) can strongly influence primary production and energy transfer to higher trophic levels. During low flow conditions in-stream photosynthesis dominates production (Oliver and Merrick, 2006) and phytoplankton/algae often form the base of the food web (Bunn et al., 2003).

The alternating dynamics between high and low flow conditions can lead to distinctly different groups of producers at the base of the food web and shift rivers from being net autotrophic at low flow to net heterotrophic during floods (Gawne et al., 2007; Carney et al., 2016). Despite mobilising organic carbon and nutrients, flow events may initially suppress primary production in many rivers (Townsend and Douglas, 2017) due to higher in-stream turbulence, dilution of phytoplankton and reduced light for photosynthesis from increased turbidity (Allan and Castillo, 2007; Devercelli, 2010). Under these conditions bacterial production and heterotrophic protists may form the base of the food web, utilising DOC transported from the floodplain to outcompete phytoplankton for nutrient resources (Karlsson, 2001; Carney et al. 2016). However, as carbon diminishes and light availability increases following a flow event, residual nutrient loads mobilised by the flow may increase phytoplankton production leading to net increases in primary production. These changes between basal producers may lead to a significant bottom-up effects as different secondary consumers (e.g. zooplankton) dominate according to shifts in the amount and source of food resources (Hunter and Price, 1992).

Zooplankton are the major consumers of planktonic organisms in freshwater lowland river systems and may greatly increase in abundance and diversity following flow pulses that result in increased phytoplankton and bacterial production (Kobayashi, 1996;1998; Shiel et al., 2006). Consequently, zooplankton are a crucial link in transferring energy from producers to higher trophic levels (Kobayashi and Church, 2003; Ning et al., 2010). Zooplankton groups such as rotifers, copepods and cladocerans are particularly important for the recruitment of Australian native fish, making up a significant part of fish diets during their larval and juvenile stages (Rowland, 1996; Humphries, 1999; King et al., 2009). Flow events that produce high concentrations of zooplankton may therefore offer considerable boosts to resources for juvenile fish (Humphries, 1999).

Tributary inflows are an important source of organic matter, nutrients and biota to the main stem of a river (Kiffney et al., 2006). The comparatively small channels of tributaries tend to be more closely connected to surrounding floodplains and riparian vegetation than the main channel of rivers, resulting in higher hydrologically-driven terrestrial organic matter inputs (Agren et al., 2007). These inflows can improve water quality and affect ecological functions in the rivers main stem such as planktonic community structure and food web production (Rice et al., 2008). However, the influence of a tributary on main stem water quality is dependent on the relative contribution of tributary discharge compared to that of the main stem (Benda et al., 2004). Further, the catchment

characteristics and geomorphology of different tributaries may influence the quantity and types of carbon and nutrients that are mobilised during flow events. Using tributaries to deliver water to the main stem of a river may result in higher organic matter and nutrient loads compared to those of dam releases (Rohlfs et al., 2016) and more natural ecosystem responses (Rohlfs et al. 2018).

The aim of the study was to determine the importance of tributary flows into the Barwon-Darling River for river productivity. The study addresses two important questions. Firstly, what is the role of major tributaries in delivering basal resources to the main channel of the Barwon-Darling River? Secondly, do basal resources mobilised during flooding support increased food web productivity? To answer these questions we conducted a two-part study following a flood event between April and July 2021. The first part was a monitoring study of the Barwon-Darling River and two of its tributaries, the Namoi and Mehi Rivers. It examines contributions of the tributaries to the main channel of the Barwon River in terms of nutrients and carbon, and food web components, and how this influences productivity in the Barwon- Darling, comparing upstream and downstream of the tributaries. The second part was an experimental study using microcosms to test the effects of terrestrial leachates made from the floodplains of the Mehi, Macquarie and Namoi Rivers on bacterial and phytoplankton growth. These microcosms offer the ability to simulate the carbon and nutrient deliveries during different sized flow events to understand their effects on basal food webs of the Barwon River. By assessing carbon and nutrient limitation at different post-flood intervals, this also provides insight into how long after a large flow event these resources may persist in the river.

This research was undertaken in the Barwon-Darling Water Sharing Plan area under the 'Improved Water Management in NSW (Treasury) funding'. The outcomes of this study will help assess the benefits to riverine productivity of relevant end-of-system and active management flow rules within the Water Sharing Plans. The results will provide ecological information to help:

- Inform northern Basin WSP rules related to active management, and flow targets, thus allowing more confidence in deciding which tributary flows are worth protecting and
- Guide section 324 orders and the new resumption of flow rule, by showing the importance of tributary inflows in supporting productivity requirements for these flow rules.

3. Monitoring study: The effects of discharge and tributary inflows on the basal resources and lower food web of the Barwon River.

3.1 Experimental design and sampling

Six sites were monitored in total: four on the main channel of the Barwon River and two on its major tributaries the Namoi and Mehi Rivers (Figure 1). Sites upstream and downstream of each tributary were sampled to measure the impact of tributary inflows on the lower food web of the Barwon River and their contribution to loads of carbon and nutrients. Samples were taken on four occasions during and after a large flood event in April 2021. Samples were taken every third week starting from the 7th of April and ending on the 10th of June (Figure 2). Due to flooding we were unable to access the Barwon site upstream of the Mehi on the 7th of April. Discharge was obtained from gauging stations located at or near each site (Table 1) operated by the NSW Department of Primary Industries (waterinfo.nsw.gov.au).

Table 1. Sampling site names with corresponding river gauging stations

Site*	Gauge name	Gauge number
Barwon U/S Mehi (B-U/SM)	Barwon at Mogil	422004
Mehi	Mehi near Collarenebri	418055
Barwon D/S Mehi (B-D/SM)	Barwon at Collarenebri	422003
Barwon U/S Namoi (B-U/SN)	Tara U/S Namoi Jnct	422025
Namoi	Namoi U/S Walgett	419091
Barwon D/S Namoi (B-D/SN)	Barwon at Dangar Bridge	422001

* B-U/SM= Barwon upstream of Mehi, B-D/SM= Barwon downstream Mehi, B-U/SN =Barwon upstream of Namoi, B-D/SN=Barwon downstream of Namoi.

At each site, water samples were collected for the determination of water quality and concentrations of phytoplankton and zooplankton. All instruments and sample bottles were rinsed three times with in-situ river water to minimize contamination. Samples were taken using buckets and sub-sampled for nutrients (total nitrogen, total phosphorus, soluble reactive phosphorus and nitrite/nitrate), DOC, Chlorophyll-a (Chl-a) and phytoplankton. All samples were taken in polyethylene containers, stored in a portable fridge/freezer and frozen. Dissolved oxygen, water temperature, electrical conductivity and pH were measured in situ using a Hydrolab probe and Surveyor field hand-meter. Zooplankton samples were taken directly from the river by pouring buckets through a 35 µm net.

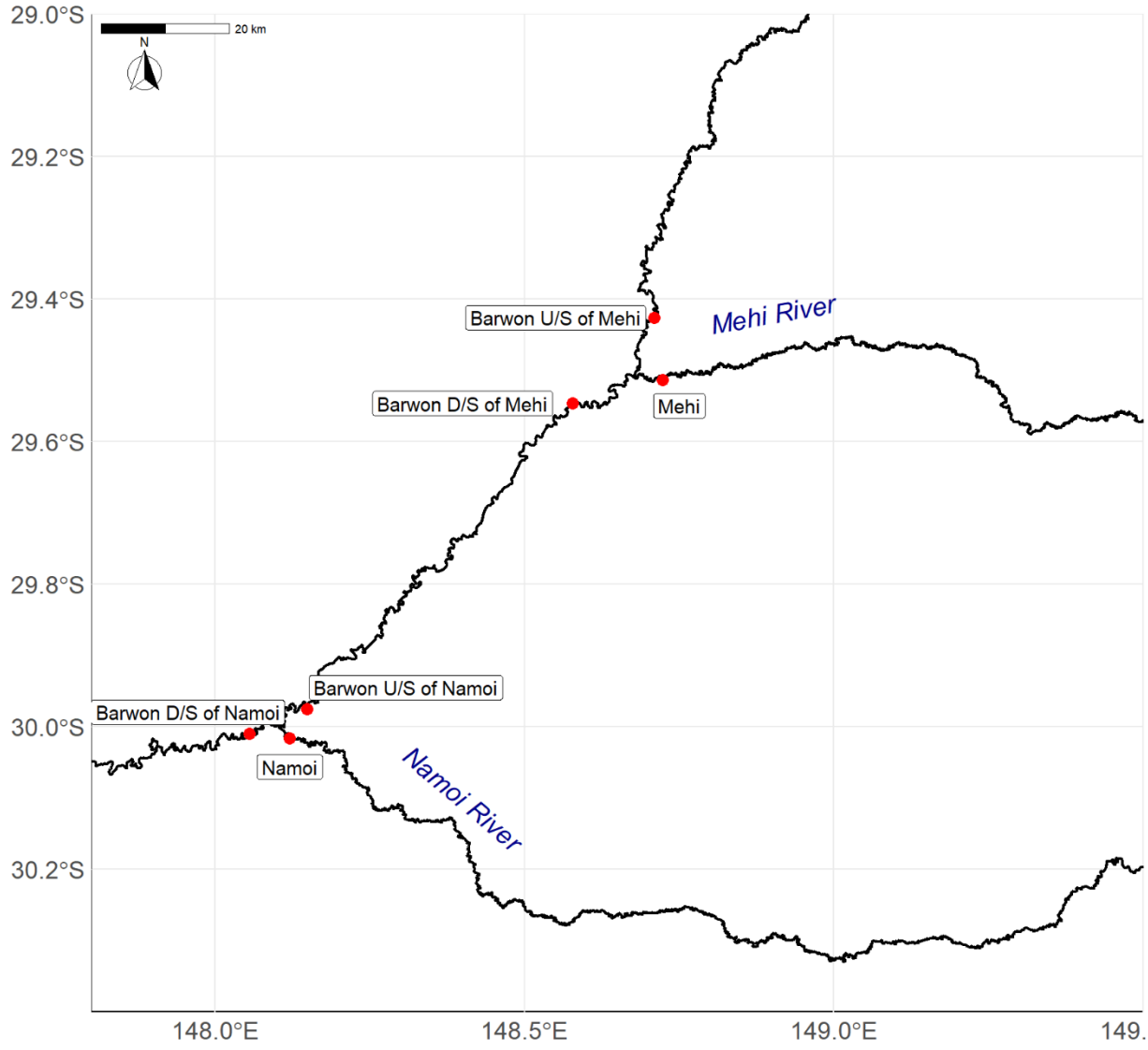

Figure 1. Map showing the 6 sites (red dots) examined in the monitoring study on the Barwon-Darling River and its tributaries.

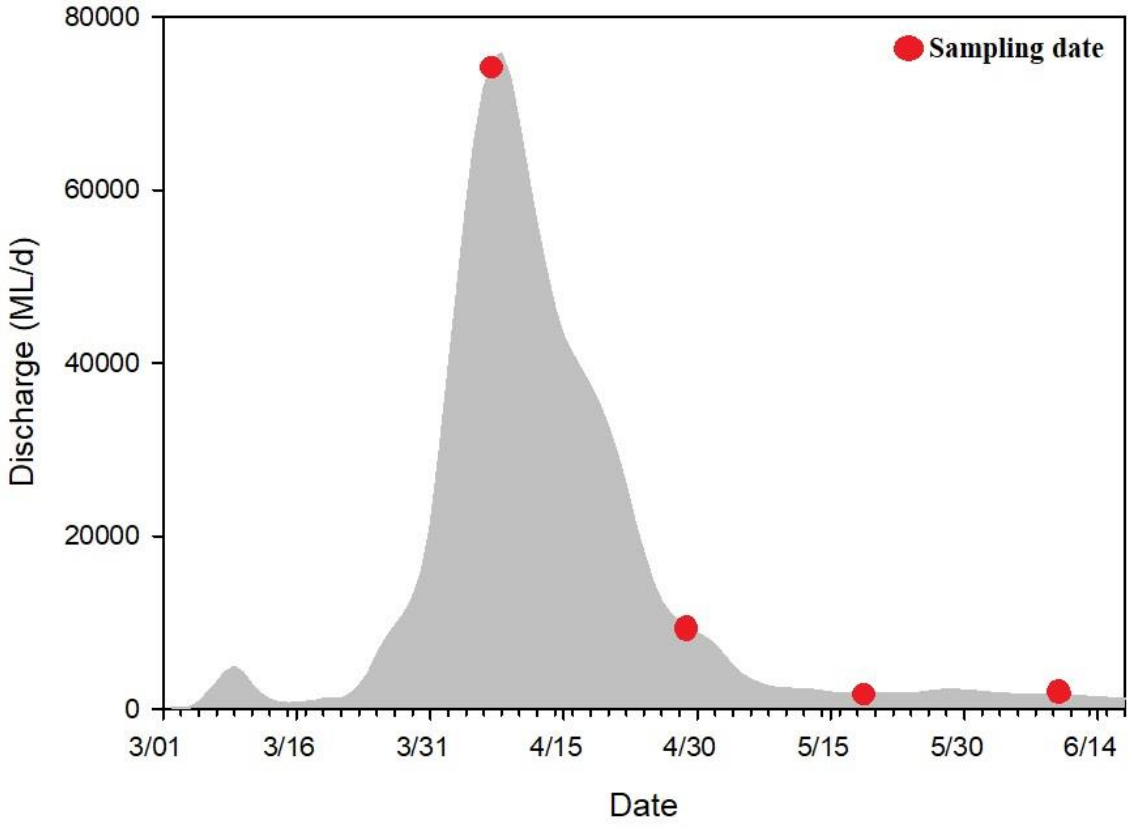

Figure 2. Hydrograph showing discharge in the Barwon River at Dangar bridge with sampling days marked

3.2 Results

3.2.1 Discharge and physico-chemical parameters

Heavy rain during the first 3 months of 2021 led to a large flood event on the Barwon River in early April. The flood peaked at 78,000 ML/d at the most downstream site as measured at Dangar Bridge near Walgett (Figure 3) with overbank flows persisting until mid-late April. Tributaries peaked slightly earlier and with lower maximum discharge; approximately 12,000 ML/d on the Namoi directly upstream of Walgett and 14,600 ML/d on the Mehi upstream of Collarenebri. By mid-May discharge on the Barwon had receded to relatively low flow conditions (950-2,250 ML/d) where it remained for the rest of the study period. Similarly, discharge on the Namoi River reduced to low flow conditions (120-300 ML/d) by late April where it remained for the rest of the study. The Mehi followed a similar pattern with the flood receding by 30th April. Following this, flows were very low (10-200 ML/d) for the remainder of the study period. The contributions of tributaries to main stem discharge were highest in early and mid-April (Figure 4). On the 1st of April the Namoi and Mehi tributaries contributed >70% of discharge to the Barwon River at Dangar Bridge (Mehi River >50% at Collarenebri, Namoi River >20% at Dangar Bridge). However, this reduced quickly as upstream flows increased in the Barwon main channel, reducing the relative contribution of tributary inflows. A second large flow event on the Namoi caused proportional inflows to increase to >40% on the 22nd of April. By the end of the study the Namoi River generally contributed around 10% of discharge to the Barwon River whereas the Mehi was much lower at <1%.

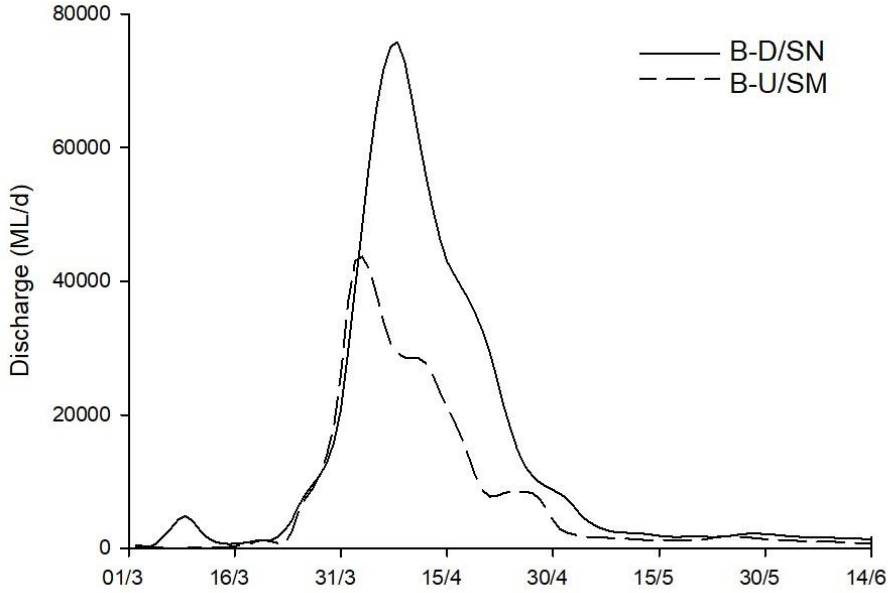

Figure 3. Discharge (ML/d) at the most upstream site (Mogil Mogil: B-U/SM, dashed line) and most downstream site (Dangar Bridge: B-D/SN, solid line) throughout the sampling period.

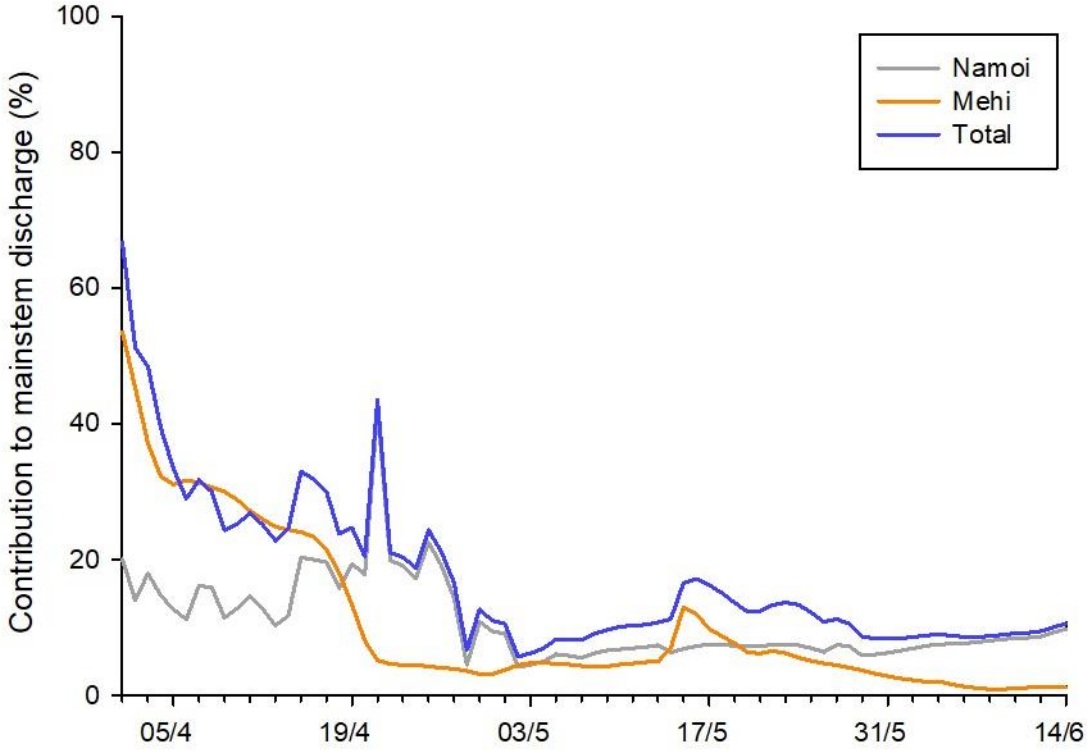

Figure 4. Percentage contribution of tributaries to downstream main stem discharge (ML/d). Namoi data is compared to Dangar Bridge, Mehi to Collarenebri and Total (discharge from both tributaries) is compared to Dangar Bridge.

3.2.1.1 Dissolved Oxygen

Dissolved oxygen (DO) concentrations followed similar trends across all sites (Figure 5). DO concentrations were the lowest during the April flood event (<40%) and increased notably by April 28th to ~80%. The Namoi was an exception, increasing to ~140% on the 28th April. DO was stable at slightly above 80% at all sites for the remainder of the study period.

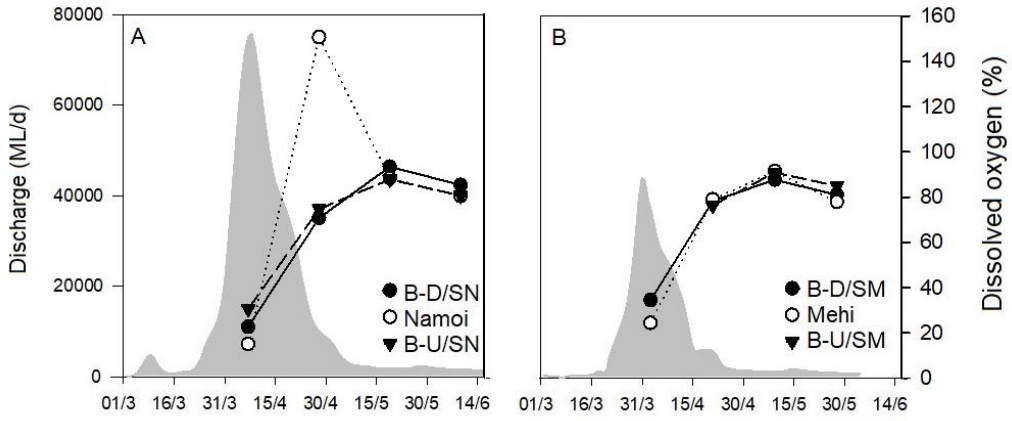

Figure 5. Dissolved oxygen (%) on the Namoi and its up/downstream sites on the Barwon (A) and on the Mehi and its up/downstream sites on the Barwon (B). White circles indicate tributaries, Black triangles: upstream sites and black circles: downstream sites. The grey shaded area represents discharge at B-D/SN in A and discharge at B-U/SM in B.

3.2.1.2 Turbidity

Turbidity (Figure 6) followed a similar trend at most sites, peaking during the April flood event (>500 NTU at all sites except the Mehi), then decreasing at all sites until turbidity was below 200 NTU by the end of the study period. Turbidity on both tributaries was consistently lower than the Barwon main stem.

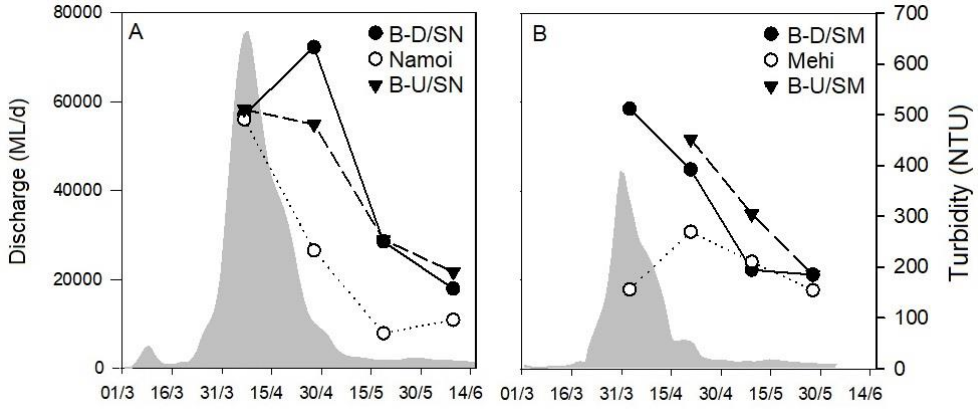

Figure 6. Average turbidity on the Namoi and its up/downstream sites on the Barwon River (A) and on the Mehi and its up/downstream sites on the Barwon River (B). White circles indicate tributaries, Black triangles: upstream sites and black circles: downstream sites. The gray shaded area represents discharge at B-D/SN in A and discharge at B-U/SM in B.

3.2.2 Dissolved Organic Carbon

Dissolved organic carbon concentrations (DOC) (Figure 7) followed similar patterns across all study sites throughout the study period. DOC concentrations ranged from 10.01-13.10 mg/L during the April flood event and peaked at all sites (range: 15.51-17.81 mg/L) on the 28th of April once flood conditions had receded, excluding the Mehi which declined to 11.06 mg/L. DOC concentrations

declined at all sites from the 19th of May until the end of the study period with concentrations on the Barwon upstream of the Namoi as well as up and downstream of the Mehi remaining >10mg/L for the entire study. DOC concentrations on the Barwon downstream of the Namoi were lowest (6.52 mg/L) on the 9/6, DOC was also low on the Namoi (4.9 mg/L) and Mehi Rivers (6.99 mg/L) in June.

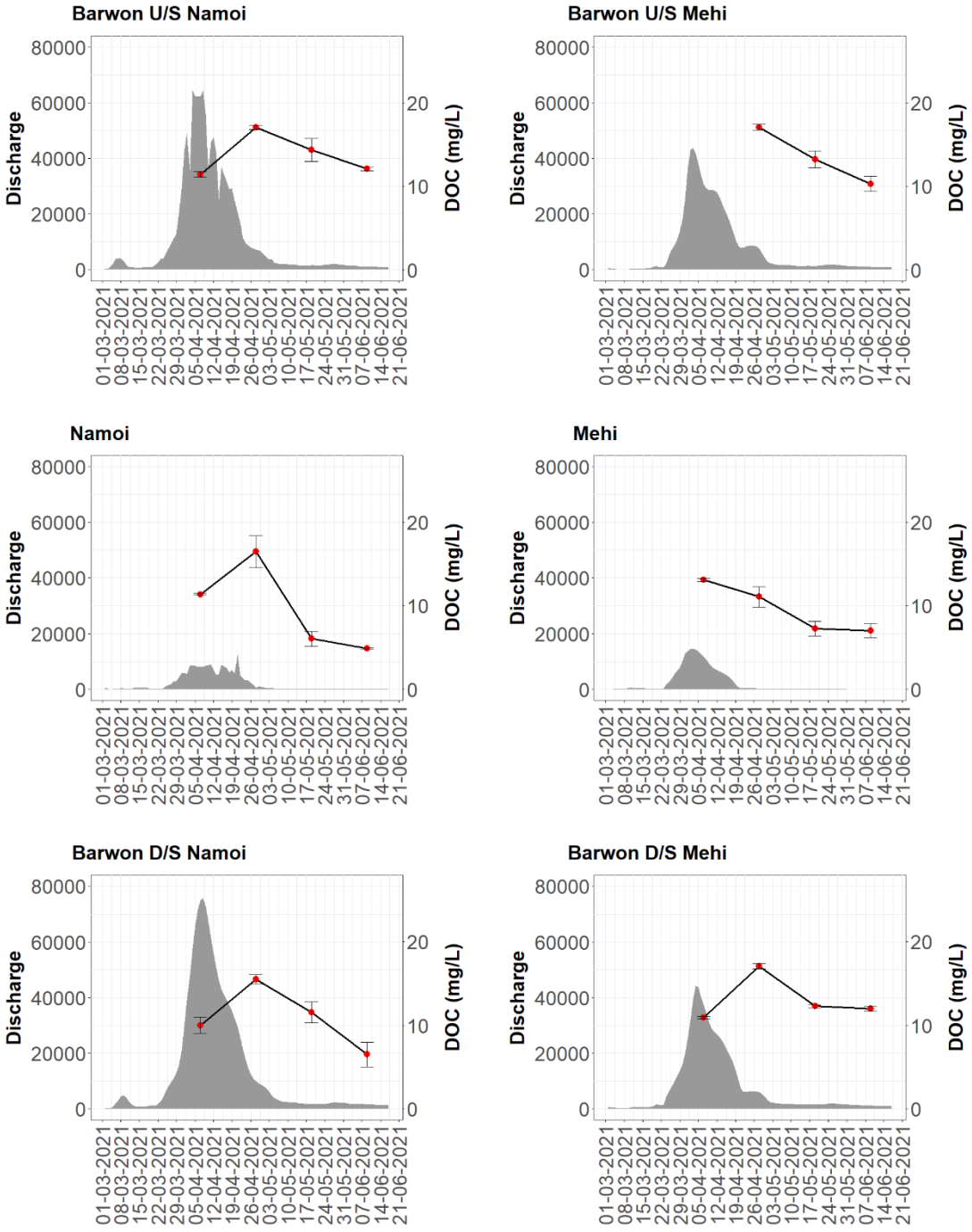

Figure 7 Dissolved organic carbon concentrations (mg/L) over time at each sample site with standard error of the mean. Gray shaded area shows discharge (ML/d) at each site.

3.2.3 Nitrogen

Average ammonia concentrations followed a similar pattern at all sites (Figure 8), peaking during the April flood event (0.39 mg/L at Mehi) before declining to low levels (<0.09 mg/L, all sites) for the remainder of the study period. In contrast, nitrate concentrations were lowest at all sites during the April flood. Once flood conditions had subsided nitrate concentrations on the Barwon main stem increased to high levels (>0.5 mg/L) where they remained for the duration of the study. Nitrate concentrations were much lower on both tributaries compared to the main stem (Namoi: <0.1 mg/L, Mehi: 0.2 mg/L). The Mehi exhibited one small increase in nitrate levels on the 28th April however returned to lower levels (<0.1 mg/L) for the remainder of the study. Similarly to nitrate, total nitrogen (TN) concentrations were much higher on the Barwon main stem than both tributaries (Figure 8). TN concentrations on the main stem were relatively stable, remaining high (range: 0.9-1.2 mg/L) throughout the study period. TN concentrations on the tributaries peaked during the April flood event (Namoi: 1.1 mg/L, Mehi: 1.0 mg/L) then declined to the lowest levels for the study period on the 10th of June (both sites: <0.5 mg/L).

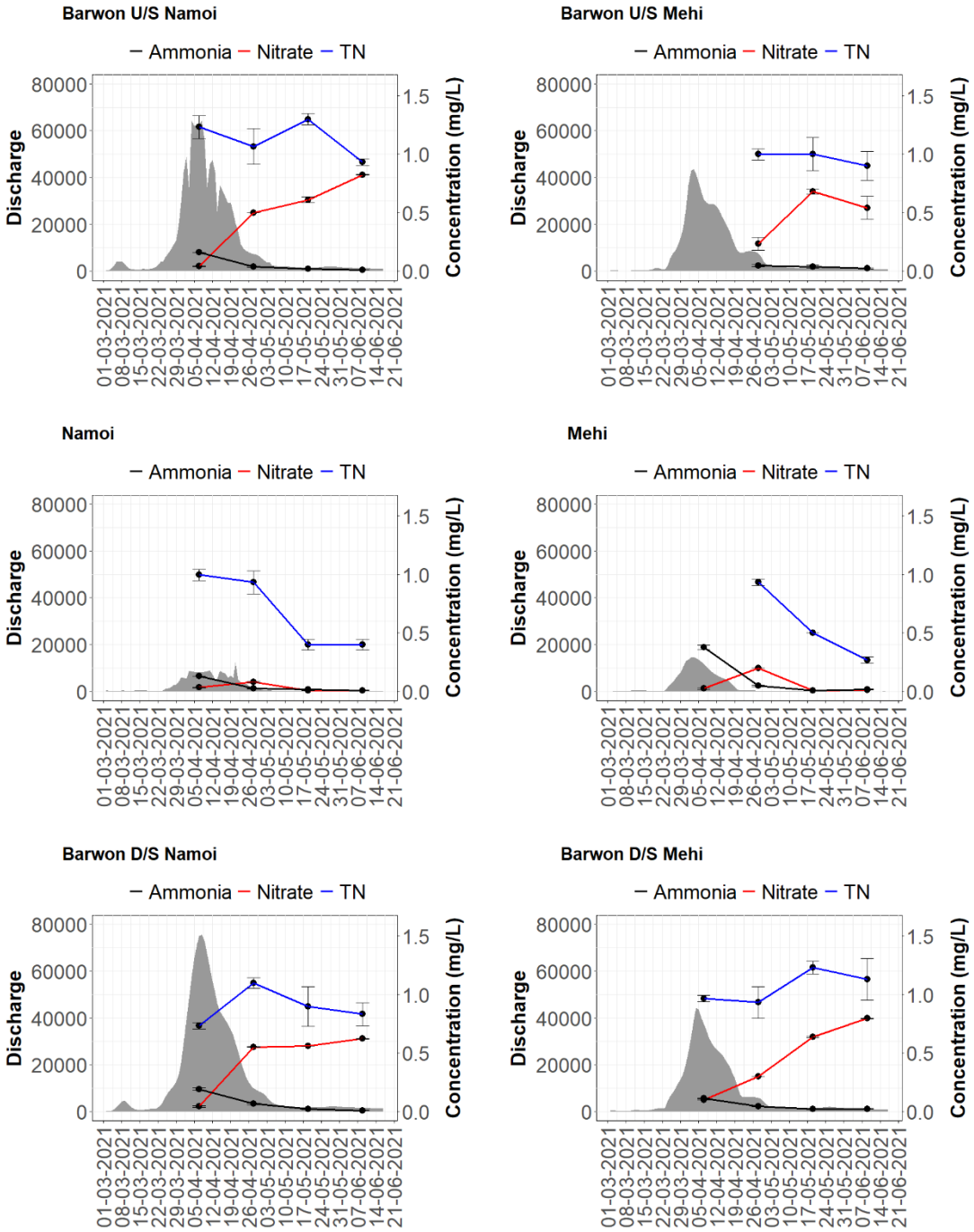

Figure 8. Average nitrate, ammonia and total nitrogen concentrations (mg/L) with standard error. Gray shaded area shows discharge (ML/d) at each site.

3.2.4 Phosphorus

Average phosphate concentrations (Figure 9) followed a very similar pattern across all sites, peaking during the April flood event then gradually declining to the lowest levels in the study period on the 10th of June. Phosphate concentrations were higher on the Namoi (0.2 mg/L) and Mehi (0.22 mg/L) rivers during the flood than on the Barwon main stem (range: 0.18 - 0.15 mg/L). Total phosphorus concentrations (Figure 9) followed a similar pattern to phosphate, peaking during and immediately after the April flood at all sites (range: 0.59 - 0.7mg/L) then decreasing to the lowest

concentrations (0.35 mg/L) for the sampling period by 10/6/21. Concentrations were generally similar at all sites.

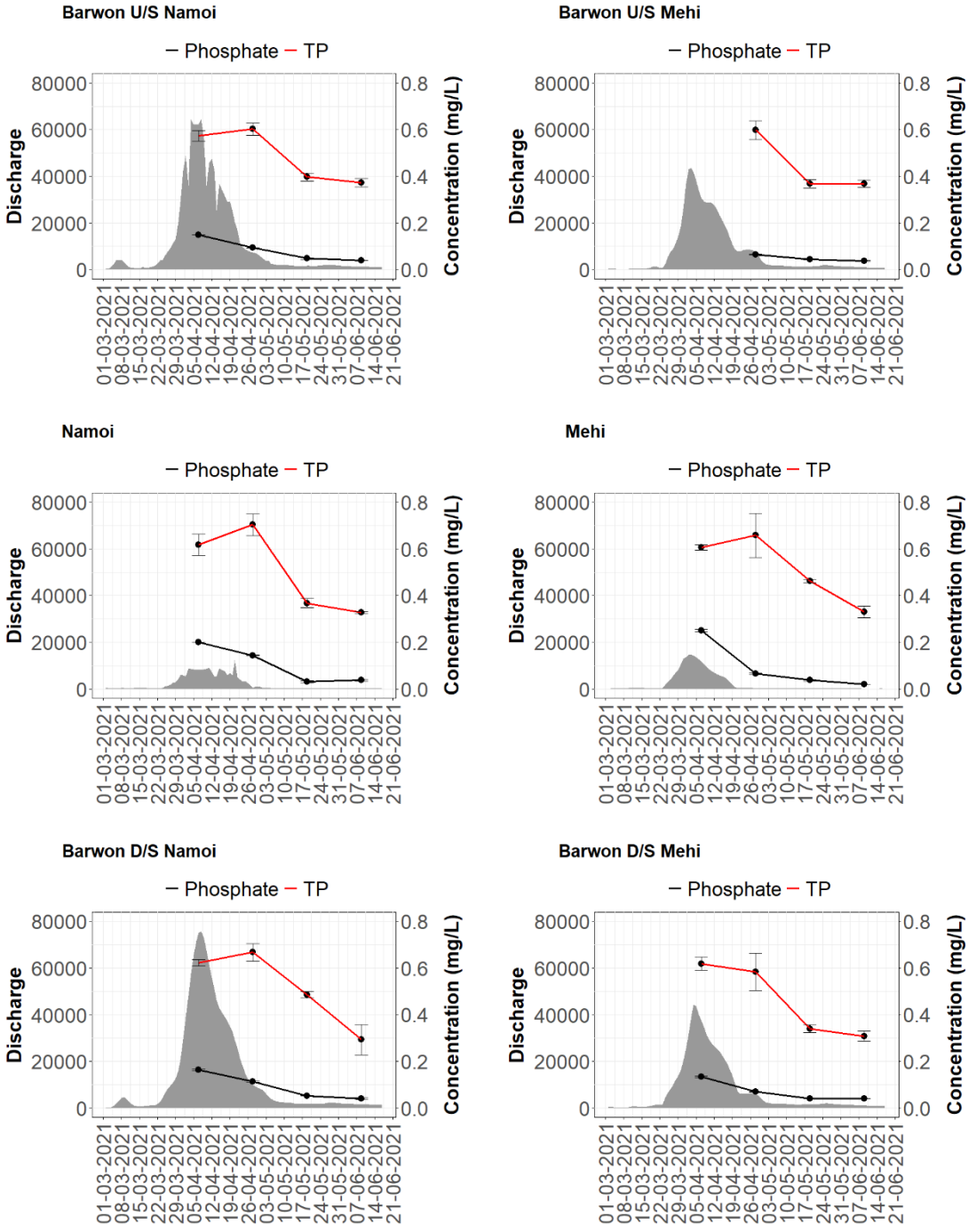

Figure 9. Average phosphate and total phosphorus concentrations (mg/L) during the study period at all sites. Gray shaded area shows discharge (ML/d) at each site.

3.2.5 Bacteria

Average concentrations of bacteria cells at all sites (Figure 10) were generally lowest during the April flood event and increased over time, peaking on the 10th of June. Bacteria concentrations followed a similar trend up and downstream of the Namoi River, with low concentrations from the April flood until the 18th of May (both sites <300,000 cells/mL) after-which they increased 10-fold to peak concentrations for the sampling period (B-U/SN: 1,288,592 ± 432,696 cells/mL, B-D/SN: 765,554 ± 221,231 cells/mL). Bacteria concentrations up and downstream of the Mehi River followed different patterns from each other. Upstream (B-U/SM) bacteria concentrations remained steady across the sampling period (range: 598,271 – 401,412) whereas the downstream site followed a pattern more similar to that of the Mehi River; with concentrations initially low (217,708 ± 61,181 cells/mL) during the April flood and gradually increasing to peak concentrations (947,490 ± 374,706 cells/mL) in June. On the Namoi River concentrations were low during the April flood (358,440 ± 91,648 cells/mL) but increased 10-fold by the 28th of April where they remained high (2,644,875 – 6,070,854 cells/mL) for the remainder of the study. Bacteria concentrations on the Mehi River were higher than all other sites during the April flood event (953,057 ± 219,432 cells/mL) after-which they continued to increase, peaking at 4,468,560 ± 89,901 cells/ML in June. Bacteria concentrations were typically an order of magnitude higher in the tributaries than on the Barwon main stem.

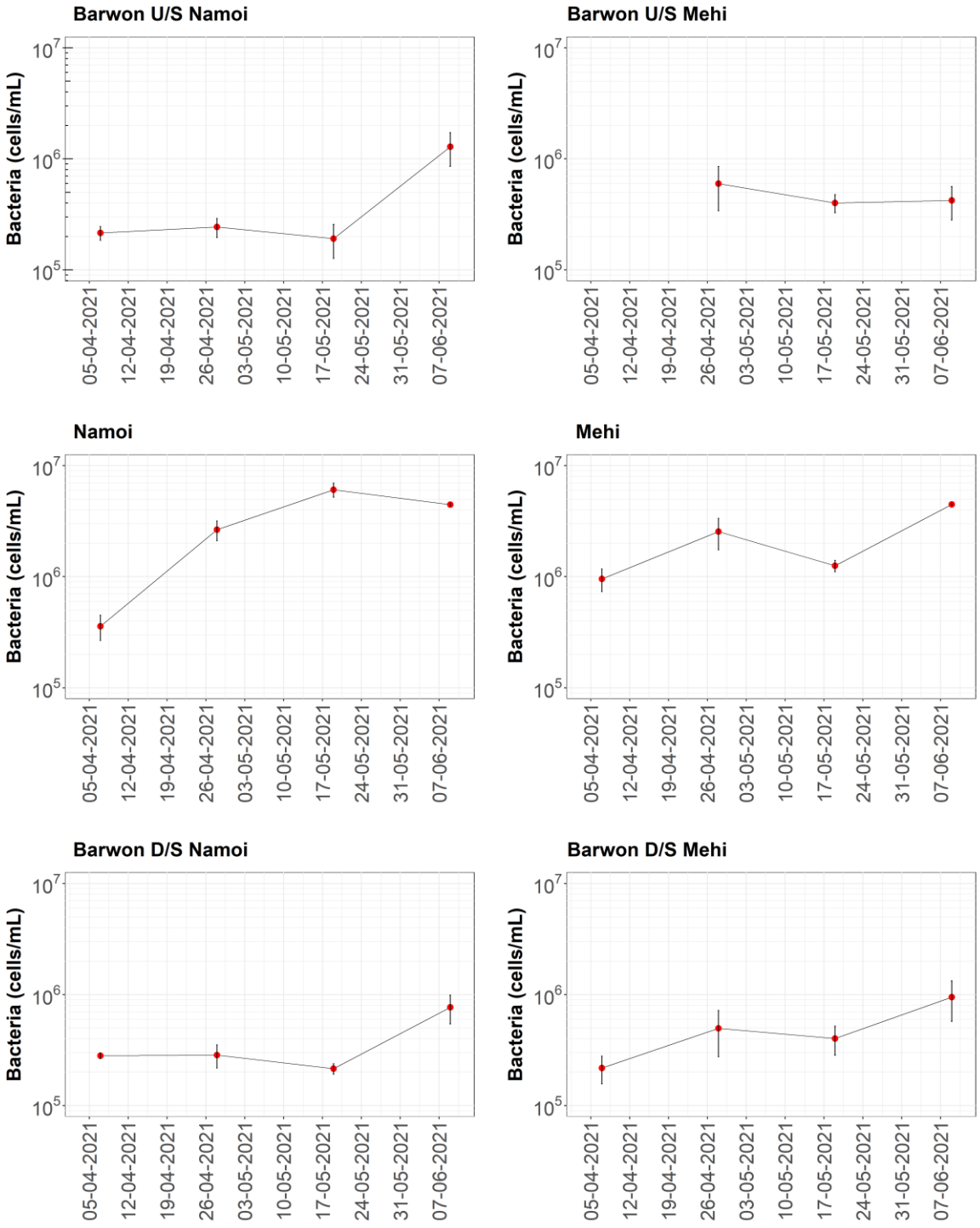

Figure 10. Average bacteria concentrations (cells/mL) with standard error. The Y axis is in logarithmic scale

3.2.6 Phytoplankton

Chlorophyll-*a* concentrations followed similar trends at all sites on the Barwon main stem (Figure 11 A,B,E,F). Typically, concentrations were lowest (<10 µg/L) during the flood event and gradually increased to more consistent levels (15-20 µg/L) for the remainder of the sampling period. Chl-*a* concentrations on the tributaries (Figure 11 C-D) were distinct from those on the main channel, increasing to very high levels at both sites following the flood event (Namoi: 140 µg/L, Mehi: >55 µg/L), before reducing to levels similar to that of the main stem by the 19th May.

Phytoplankton community composition showed broadly similar trends at all sites (Figure 12), with mixotrophic taxa (dinophyceae, cryptomonads, euglenoids) contributing the highest biovolume during and immediately after the flood event (Figure 12 A-B), including the bloom on the Namoi River observed on the 28th April (Figure 12 B). By 19th May the phytoplankton communities were predominantly of a low-biovolume, autotroph dominated composition. Diatoms contributed the greatest proportion to overall phytoplankton biovolume post-flood (Figure 12 C-D).

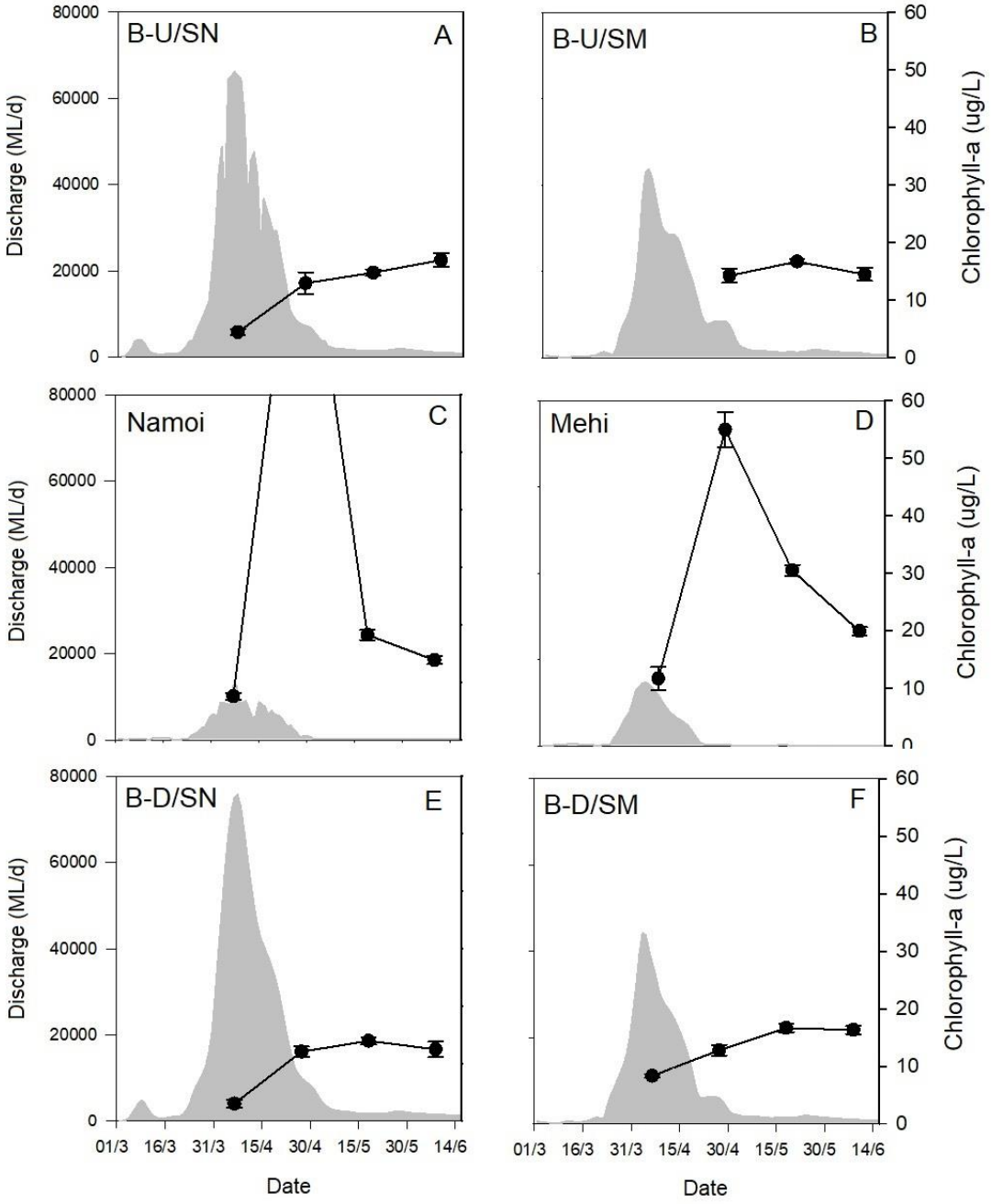

Figure 11. Average chlorophyll-a (ug/L) concentrations with standard error of the mean. The high reading above the scale on the Namoi is 140 ug/L. The Namoi and its up/downstream sites are in the left column (A, C, E) and the Mehi and its counterparts in the right column (B, D, F). Upstream sites are in the top row, tributaries in the middle and downstream sites are in the bottom row. B-U/SN =Barwon upstream of Namoi, B-D/SN=Barwon downstream of Namoi, B-U/SM= Barwon upstream of Mehi, B-D/SM= Barwon downstream Mehi. Gray shaded area shows discharge (ML/d) at each site.

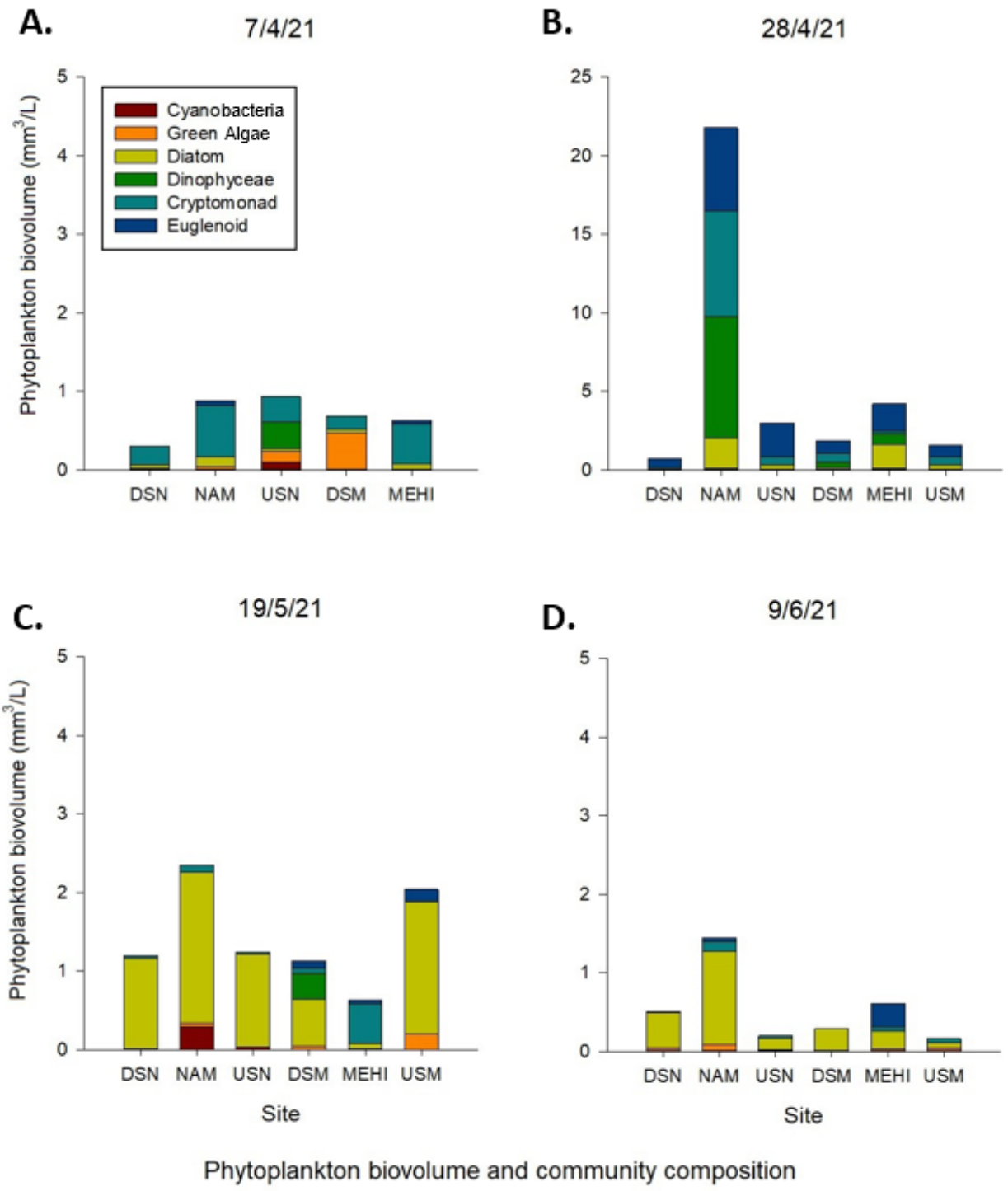

Figure 12. Phytoplankton biovolume and community composition at each site separated by sampling date. Different colours indicate different phytoplankton taxa. The Y axis on panel B is higher than those of all other panels to account for an algal bloom on the Namoi River

3.2.7 Zooplankton

3.2.7.1 Nauplii and Rotifers

Rotifer and nauplii concentrations followed a similar pattern at all sites on the Barwon main stem, however, were distinct from the tributaries. On the main stem, concentrations of both nauplii and rotifers at all sites peaked during the April flood event and gradually declined until reaching the lowest concentrations for each site by June 10 (Figure 13 A,B,E,F). Nauplii and rotifer concentrations on the Barwon River up and downstream of the Namoi River were generally more than double that of the Mehi River counterparts.

Nauplii and rotifer concentrations on the Namoi River peaked after the April flood (Nauplii: 400 ind/L, Rotifers 2700 ind/L, Figure 13 C). Rotifers remained high (>2000 ind/L) until mid-May after which they reduced to levels similar to all other sites. Rotifers on the Mehi River followed a similar trend, peaking after the flood event (1000 ind/L) from late April to mid-May. Nauplii on the Mehi peaked during the flood event before sharply decreasing in late April followed by a slight increase in May and June (Figure 13 D). Generally, nauplii and rotifer concentrations were much higher on the Namoi and its associated sites on the Barwon (Figure 13 A, C, E) than the Mehi sites.

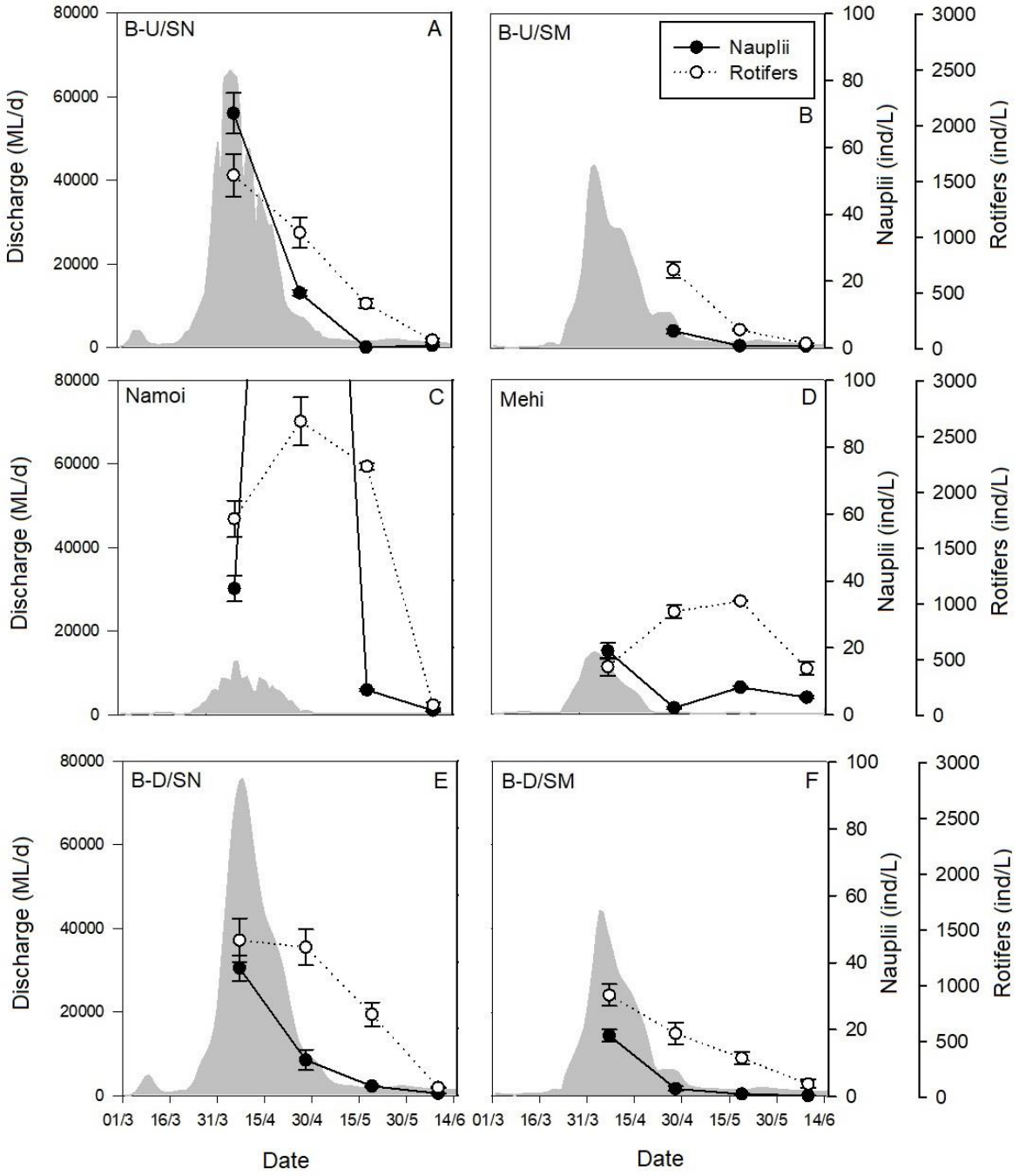

Figure 13. Average nauplii (black circles) and rotifer (white circles) at each site. The Namoi and its up/downstream sites are in the left column (A, C, E) and the Mehi and its counterparts in the right column (B, D, F). Upstream sites are in the top row, tributaries in the middle and downstream sites are in the bottom row. Nauplii concentrations on the Namoi above the scale are 400 ind/L. Gray shaded area shows discharge (ML/d) at each site.

3.2.7.2 Mesozooplankton

Copepod and cladoceran concentrations followed a very similar pattern across every site except the Mehi, peaking during the April flood event (Figure 14). Once flood conditions had subsided mesozooplankton concentrations quickly reduced to low levels across all sites (downstream sites <3ind/L, upstream sites <1 ind/L). Concentrations of all mesozooplankton were close to 10 fold higher on the Namoi and its up/downstream sites (Figure 14 A,C,E) compared to those of the Mehi.

An increase in Calanoid concentrations (>3 ind/L) on the Mehi (Figure 14 D) occurred in mid-May; these concentrations remained higher than all other sites until the 10th of June. Cladoceran concentrations in this same time period at Mehi only showed a very small increase.

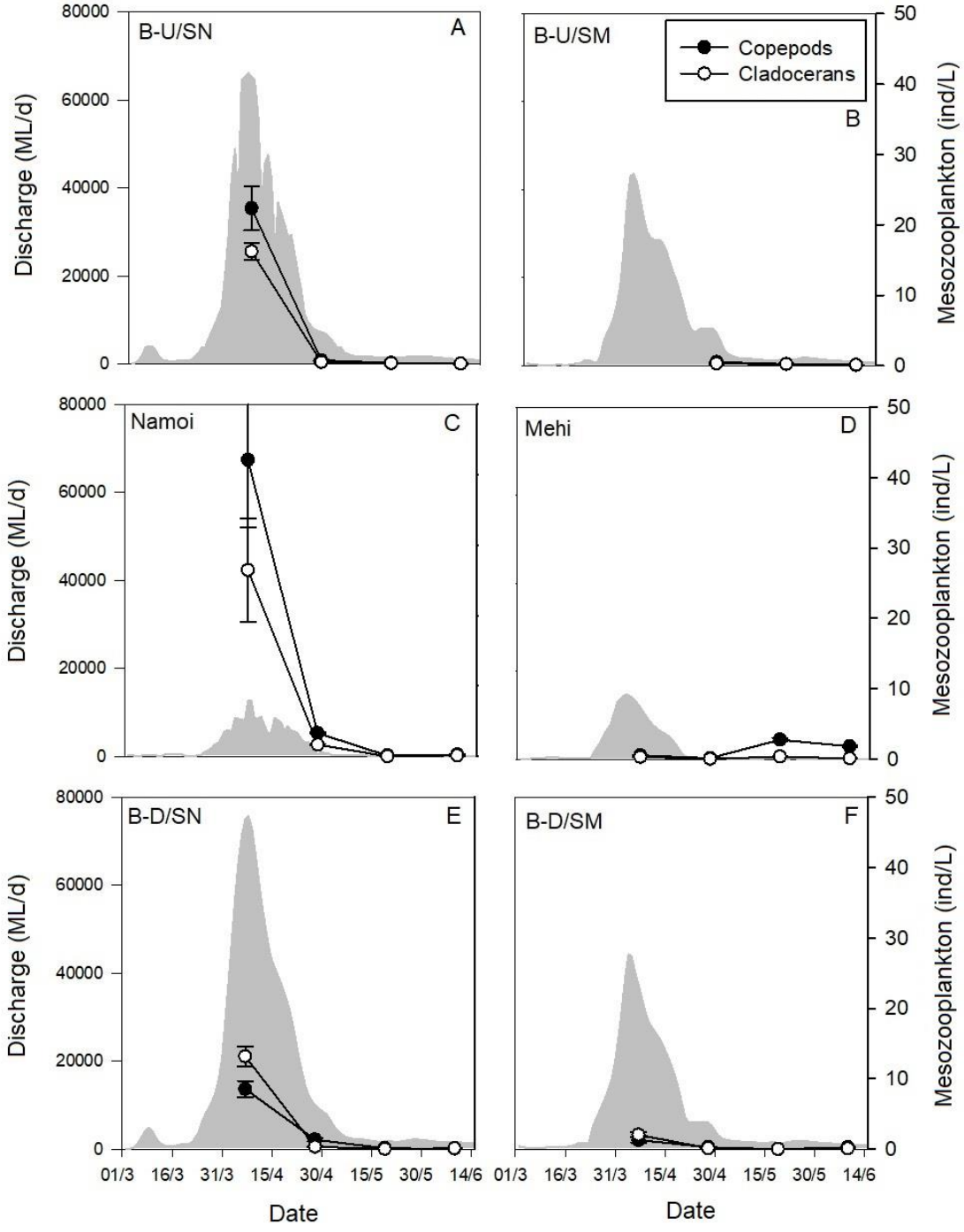

Figure 14. Average copepod (black circles) and cladoceran (white circles) concentrations at each site. The Namoi and its up/downstream sites are in the left column (A, C, E) and the Mehi and its counterparts in the right column (B, D, F). Upstream sites are in the top row, tributaries in the middle and downstream sites are in the bottom row. The y axis for mesozooplankton on the Namoi and its Barwon sites is higher than that of the Mehi. Gray shaded area shows discharge (ML/d) at each site.

3.2.8 Tributary contributions to main stem loads

3.2.8.1 Nutrients

Figure 15 shows the relative contribution of the Namoi and Mehi Rivers to main stem nutrients. The contribution of nutrients from tributaries typically increased with greater contribution of discharge to the main stem, leading to both tributaries contributing the largest proportions to main stem nutrients during the April flood event (Figure 15). Nutrient contributions from the Namoi River (Figure 15A-B) appeared closely related to discharge contribution with a range of 5-15% in ammonia and TN, 1-10% in nitrate and 10-15% for phosphate and TP. Nutrient contribution appeared less related to discharge contribution on the Mehi River (Figure 15C-D) during the April flood with TN (53%), ammonia (>100%) and phosphate (59%) all contributing more to main stem nutrient loads than discharge (30%). Similarly to the Namoi, Mehi contributions of nitrate to the main stem were much lower (>10%) than discharge. Total tributary contributions (Figure 15E-F) to nutrients at Dangar Bridge (B-D/SM) followed a similar pattern to the Mehi River with ammonia, TN and phosphate making up 39%, 50% and 37% of total main stem contribution, respectively, during the April flood event. Nitrate contributions were consistently low, particularly once flood conditions had subsided (29/4-9/6).

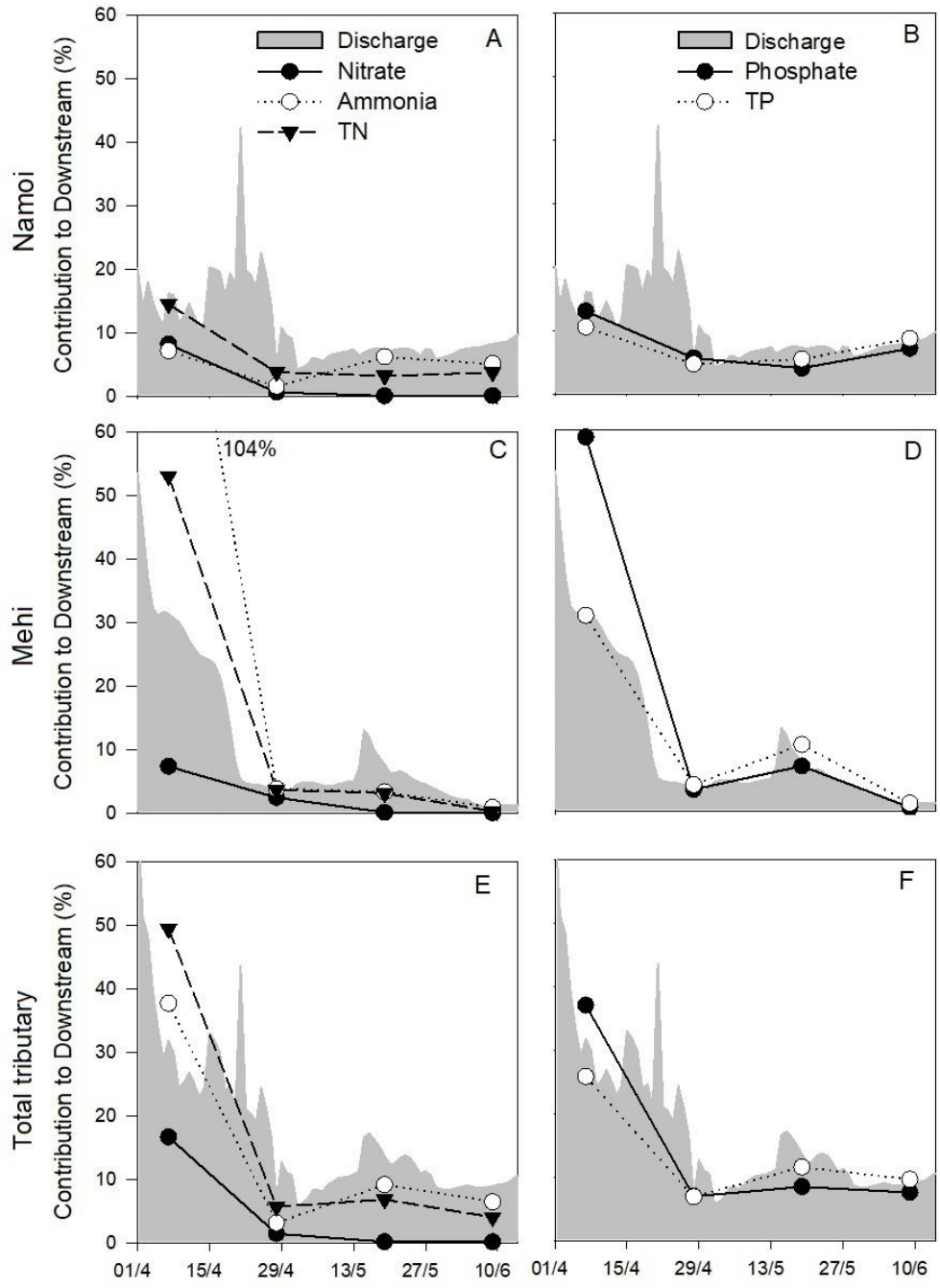

Figure 15. Percentage contribution of nutrients from tributaries to main stem loads. Namoi (top) is compared to nutrients and discharge at Dangar Bridge, Mehi is compared to Collarenebri and total contribution is the combined total of the Namoi and Mehi Rivers compared to nutrients and discharge at Dangar Bridge. Gray area indicates percentage contribution of discharge at each site to main stem.

3.2.8.2 Phytoplankton and bacteria

Contributions of Chlorophyll-a (Chl-*a*) and bacteria to the Barwon main stem (Figure 16) were proportionally higher compared to tributary discharge to the main stem throughout the study period. On the Namoi River Chl-*a* contributions (Figure 16A) were >37% of main stem until the 19th of May where they declined to less than 10% and remained at levels similar to discharge for the remainder of the study. Bacteria loads on the Namoi River (Figure 16B) were similar to discharge during the April flood event however contributions to the main stem increased once flood conditions had receded and remained above 40% of main stem loads for the remainder of the study, peaking at 206% of main stem loads on the 19th of May. On the Mehi River, contributions of Chl-*a* to the main stem (Figure 16C) loads were similar to that of the Namoi River, contributing the highest percentage loads during the April flood event (43.7%) and decreasing as discharge declined. Contributions of bacteria from the Mehi (Figure 16D) to main stem loads peaked during the April flood event at 137% and decreased once flood conditions had receded, however, remained consistently higher than contributions of discharge until the end of the study. The total tributary contributions of Chl-*a* to main stem loads at Dangar Bridge (Figure 16E) were highest during the April flood event (98%) and remained higher than discharge until the 10th of June. The combined contribution of bacteria to main stem loads (Figure 16F) was always much higher than discharge (bacteria: 50-243%, discharge 9-34%), peaking at 243% greater than main stem bacterial loads on the 19th of May.

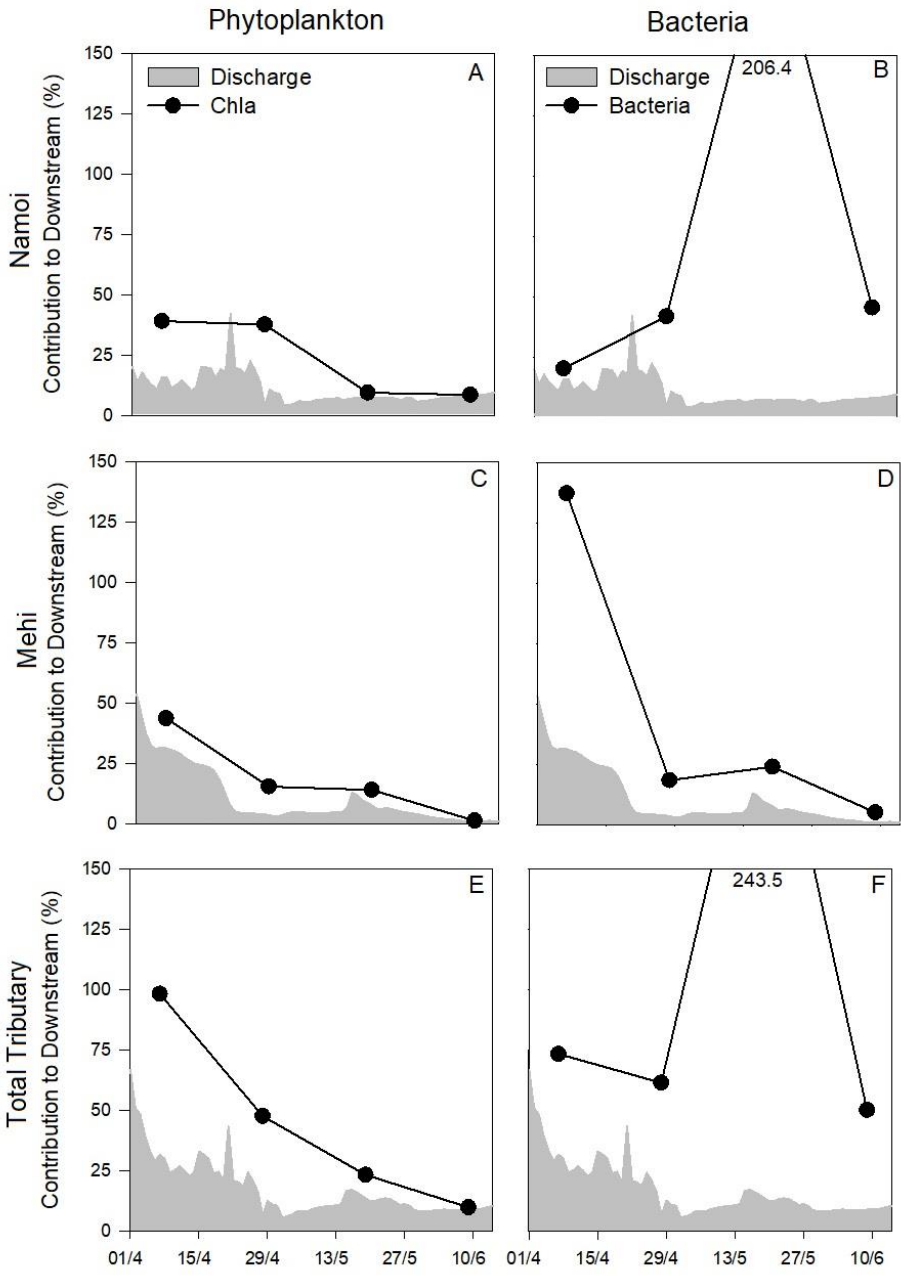

Figure 16 Percentage contribution of chlorophyll-a (left) and bacteria (right) from tributaries to main stem loads. Namoi (top) is compared to nutrients and discharge at Dangar Bridge, Mehi (middle) is compared to Collarenebri and Total contribution is the combined total loads of the Namoi and Mehi River compared to Dangar Bridge. Gray area indicates percentage contribution of discharge at each site to main stem.

4. Experimental Microcosm Study: The effects of dissolved leachate additions from three different tributary sources on growth and production in the Barwon River

4.1 Experimental design and sampling

In-situ microcosm experiments were conducted to observe changes in the basal food web resulting from the addition of carbon and nutrient loads derived from the floodplains of three tributaries on the Barwon River. Microcosm experiments were run three times; during high flow conditions (28-30th April), in post-flow conditions (19-26th May) and during lower basal flow conditions (10-17th June) (Figure 17). The first microcosm experiment (started on the 28th of April) was set up at the most downstream monitoring site (Barwon D/S of Namoi River; Figure 1) however was only run until day two as the microcosms were vandalised and removed from the river making day seven sampling impossible. Consequently, the following experiments were run at the Barwon site U/S of the Mehi River which was considerably more remote and allowed the experiments to run for their full duration without risk of vandalism.

River water was filtered through a 65 µm plankton net to remove large zooplankton and collected in a 70 L prewashed plastic tub. Samples for total and filtered nutrients (NOx, SRP), bacteria, zooplankton, physico-chemistry, dissolved organic carbon, Chlorophyll *a* and phytoplankton enumeration were taken in triplicate from the filtered water at the onset of the experiment. Day zero zooplankton samples were taken from the initial sample bucket (filtered to 65 µm) then filtered to 35 µm. Dissolved organic carbon and filtered nutrient samples were also taken from three surrogate bottles containing river water and an approximately 6 mg C/L addition from each leachate source (Namoi, Mehi, Macquarie). These surrogates are used to determine the total carbon/nutrient concentrations of the addition plus the ambient concentration.

The filtered water was then homogenised and poured into 5 L clear plastic microcosm bottles. All bottles were filled to 4.5 L to ensure an airspace for photosynthesis and to minimise anoxia. Leachates were added in the concentrations outlined in Table 2. Following the leachate additions, bottles were mixed by rotation and tied together in random order. Styrofoam floats were used at even points along the rope to ensure microcosms were suspended at the same depth and within the euphotic zone (~90% surface irradiance). Cinderblocks were used to secure the experiment in place.

Microcosms were sampled again on days two and seven. On each sampling day dissolved oxygen (%), total and filtered nutrients (NOx, SRP), dissolved organic carbon, bacteria, Chlorophyll-*a* and phytoplankton were sampled according to the methods outlined in Appendix A. All microcosms were gently mixed before sampling but prior to oxygen readings. Zooplankton samples were taken from microcosms on day seven only, due to the large volume required. They were sampled by pouring 3 L of microcosm water through a 35 µm plankton net and preserved with ethanol (>50% v/v).

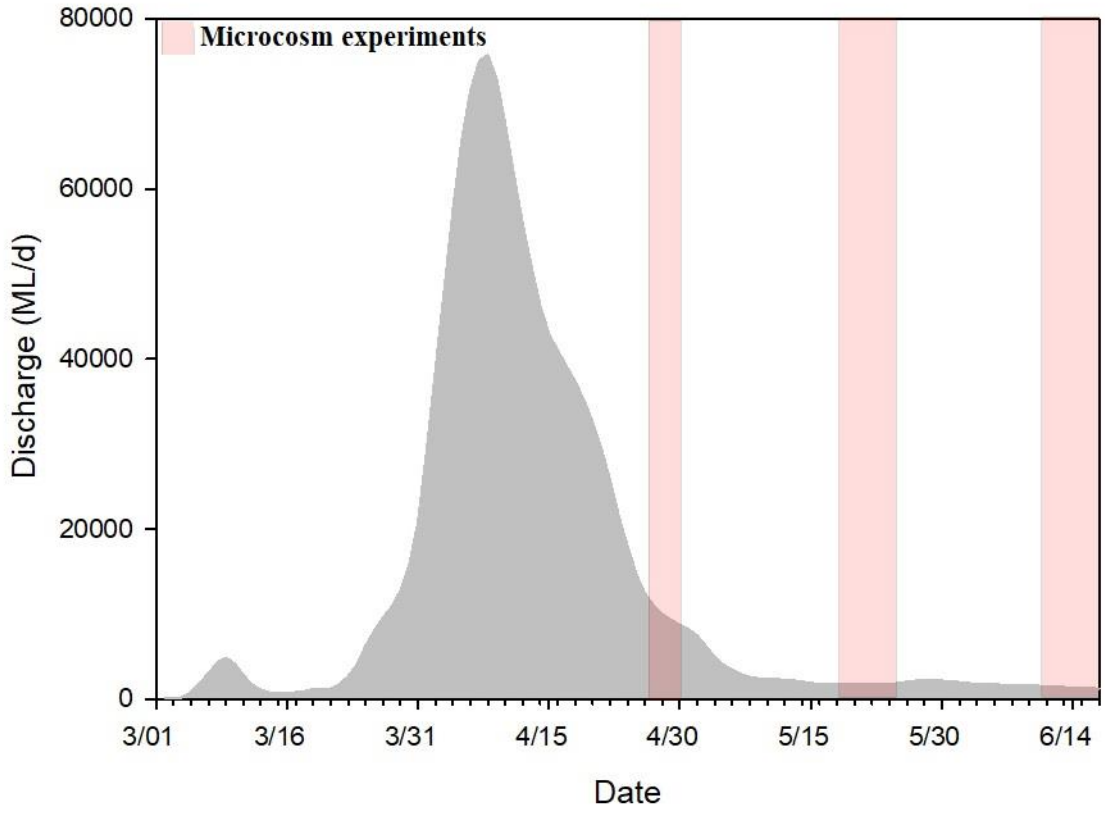

Figure 97. Hydrograph showing discharge at Dangar Bridge, red lines indicate when experiments were run. All experiments were conducted in 2021.

4.1.1 Physico-chemical parameters

Temperature, electrical conductivity, pH and dissolved oxygen (percentage and mg/L) were measured using a Hydrolab field hand-meter Surveyor and MS5 minisonde probe. Turbidity was measured using a Hach 2100P turbidimeter.

4.1.2 Nutrients and organic carbon

Dissolved organic carbon (DOC), filterable reactive phosphorus (FRP) and dissolved oxides of nitrogen (NOx) samples were collected in pre-washed and sample rinsed 50 mL PET bottles, filtered to 0.45µm using cellulose acetate syringe filters and then frozen. DOC samples were analysed using the High Temperature Combustion Method (APHA, 2005) and all N and P samples were analysed using a segmented flow analyser (OI Analytical Model FS3100) according to standard methods (APHA, 2005). Total nutrient (TN/TP) samples were collected unfiltered and analysed according to the appropriate method (APHA, 2005).

4.1.3 Bacterial abundance

Bacterial abundance was sampled and analysed according to the methods in Carney et al., (2016). Briefly, 1mL of river or microcosm water was sampled, preserved with 80µL (2% v/v) of glutaraldehyde and snap frozen with liquid nitrogen. Samples were analysed using a LSRII flow cytometer (Becton Dickinson, San Jose, CA, USA) and bacterial populations were discriminated

according to cell side scatter (SSC) and SYBR green fluorescence (Seymour et al., 2007). In preparation for flow cytometric analysis, samples were stained with SYBR Green I nucleic acid stain and fluorescent reference beads (1µm in diameter) were added to each sample immediately before analyses in a final concentration of 10^5 mL^{-1}.

4.1.4 Phytoplankton biomass and community composition

Chlorophyll-*a* was measured by filtering 100 mL of river water on site via vacuum filtration onto GFC glass fibre filters (Whatman). Filters were wrapped in aluminium foil and frozen until analysis following the methods of Mueller and Mitrovic (2014). Samples for phytoplankton community composition were preserved with Lugol's Iodine solution (~0.25% v/v). Samples were identified and enumerated at 200 times magnification using a light microscope (Olympus BX41) and Sedgwick-Rafter counting chamber. If required, samples were concentrated 5x prior to counting by settling in 50 mL measuring cylinders for 24 hours. The upper 40 mL was removed after checking all phytoplankton had settled and were no longer present in the upper layer. Phytoplankton taxa were identified to a genus level using identification literature by (Prescott 1978).

4.1.5 Zooplankton

Zooplankton samples (3 replicates each site) were collected from the pelagic zone. River water samples (50 L) were bucket poured through a 35 µm plankton net, concentrated into a sample bottle and preserved with >50% ethanol (v/v). All copepods (adults and late stage copepodites) and cladocerans were classified as mesozooplankton. Mesozooplankton were counted and identified to order level for copepods and family level for cladocerans using Bogorov counting chambers and a dissecting microscope at a magnification of ×8. Nauplii and rotifers were counted using a Sedgewick-Rafter counting cell on a compound microscope at a magnification of ×100. Rotifers were identified to family level. The taxonomic key of Shiel (1995) was used for identification of mesozooplankton and rotifers.

4.1.6 Leachate preparation

Leachates were prepared from three tributaries (Mehi, Namoi and Macquarie Rivers) and added at two concentrations (2 mg C/L and 6 mg C/L, based off their DOC concentrations) (Table 2). A control treatment was included which did not contain a leachate addition. All seven treatments were performed in triplicate. Leachate concentrations were selected to replicate different sized flow events, while remaining low enough to prevent severe hypoxia from occurring in the microcosms.

Table 2. Treatments used for microcosm experiments including source and concentration of leachate additions.

Leachate source	DOC addition
Control (C)	N/A
Macquarie (Q)	2mg/L (Q2) and 6mg/L (Q6)
Namoi (N)	2mg/L (N2) and 6mg/L (N6)
Mehi (M)	2mg/L (M2) and 6mg/L (M6)

To prepare the leachate, floodplain materials were collected from two randomly distributed 4 m^2 quadrats on the floodplains of the Macquarie (D/S Wellington, -32.466475, 148.823351), Namoi (U/S Walgett, -30.017930, 148.120697) and Mehi Rivers (U/S Collarenebri -29.513532, 148.723606). All loose materials within the quadrat were collected, most of the organic material collected was dry, comprising of decaying leaves and sticks and some fresh grasses. River redgums (*Eucalytpus camaldulensis*) and *Hakea* species were the most common vegetation on the floodplains sampled at the sites on the Namoi and Mehi Rivers whereas redgums, *Casuarina* (mainly *Casuarina cunninghamiana*) and various grasses were the most common on the Macquarie River. All human litter such as plastics and glass were removed before bagging. The leachate was made using a similar technique to that of Mitrovic et al. (2014). Floodplain materials from each site were placed in 70 L bins and soaked in 50 L of reverse osmosis water for 2 weeks at 4°C in the dark. The resulting leachates were then filtered through a series of filter sizes (10 µm, 1.3 µm, 0.5 µm and 0.2 µm) using a vacuum pump and glass fibre filter papers to exclude particulate matter including terrestrial bacteria. Once filtration was complete the leachate was homogenised and frozen at -20°C. All particulate organic matter from leachates was collected, dried and weighed to ascertain the basic composition of each leachate (Figure 18).

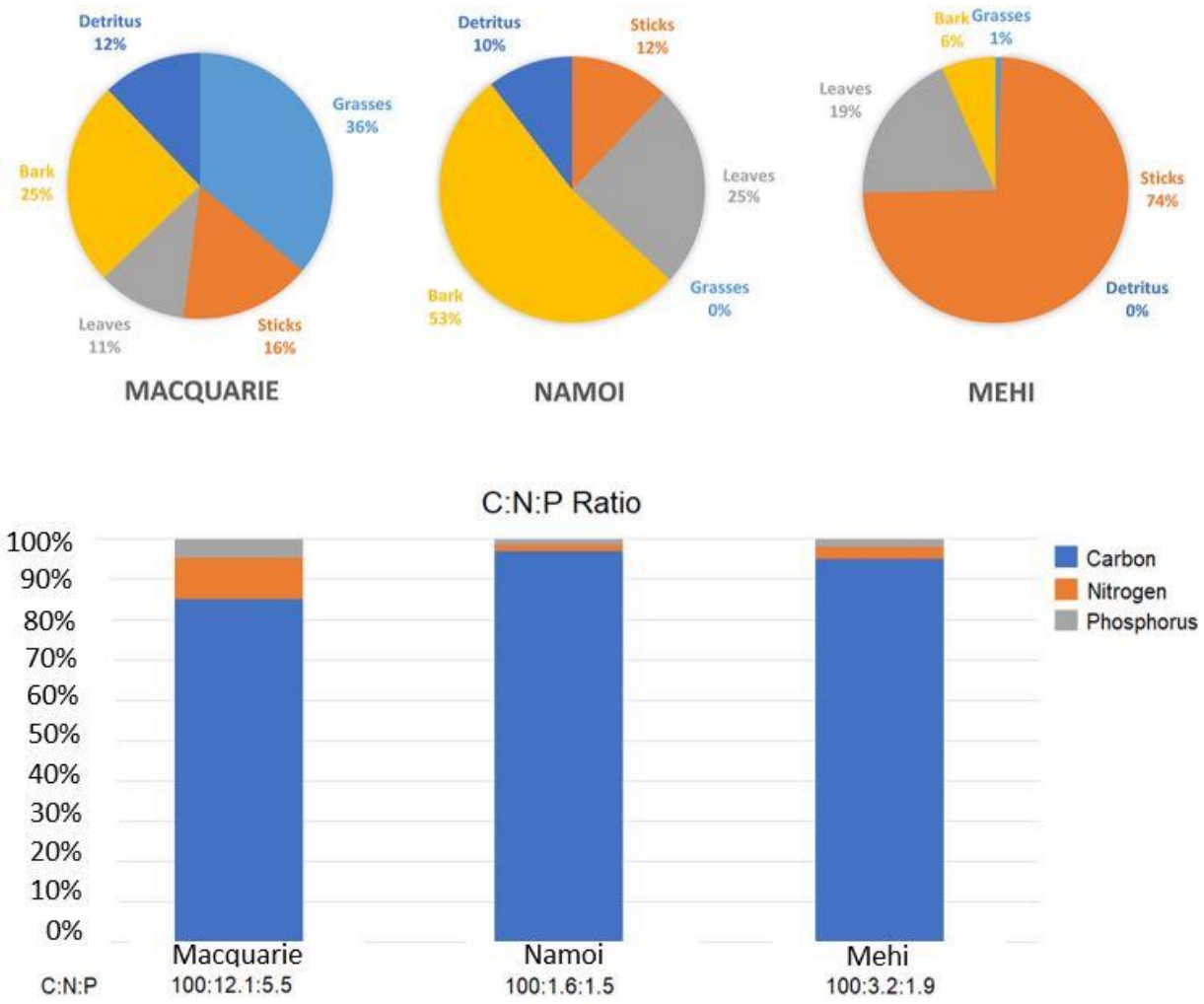

Figure 18. Constituents of leachates from each floodplain based on dry weight and Carbon:nitrogen:phosphorus ratio of each resulting leachate per 100mg C.

4.1.7 Statistical analysis

A multivariate PERMANOVA (Permutational analysis of variance) with pairwise comparisons was performed to analyse differences between treatments and sampling days for resource uptake (TN, TP and DOC) and food web responses (Chl-*a* and bacteria) in all microcosms. TN, TP and DOC were all analysed together as were Chl-*a* and bacteria. A separate analysis was also performed to compare differences in resource uptake and food web responses across experiments in case the timing of the experiments was significant. All data was normalised prior to analysis to account for large differences in concentrations and units.

4.2 Results

4.2.1 Food web responses

4.2.1.1 Bacteria

Of the three experiments, bacteria cell counts were lowest in Experiment 1, which occurred immediately post-flood. There were some differences between treatments after the two-day incubation, with M6, Q2 and Q6 (refer to Table 2 above) having significantly higher bacteria abundance compared to the control (Figure 19). Experiments 2 and 3 followed generally similar trends. In both experiments, Day 7 counts were typically higher than Day 2. N2 was notably lower than all other leachate treatments in both experiments, whereas M6 had the highest counts (Figure 19).

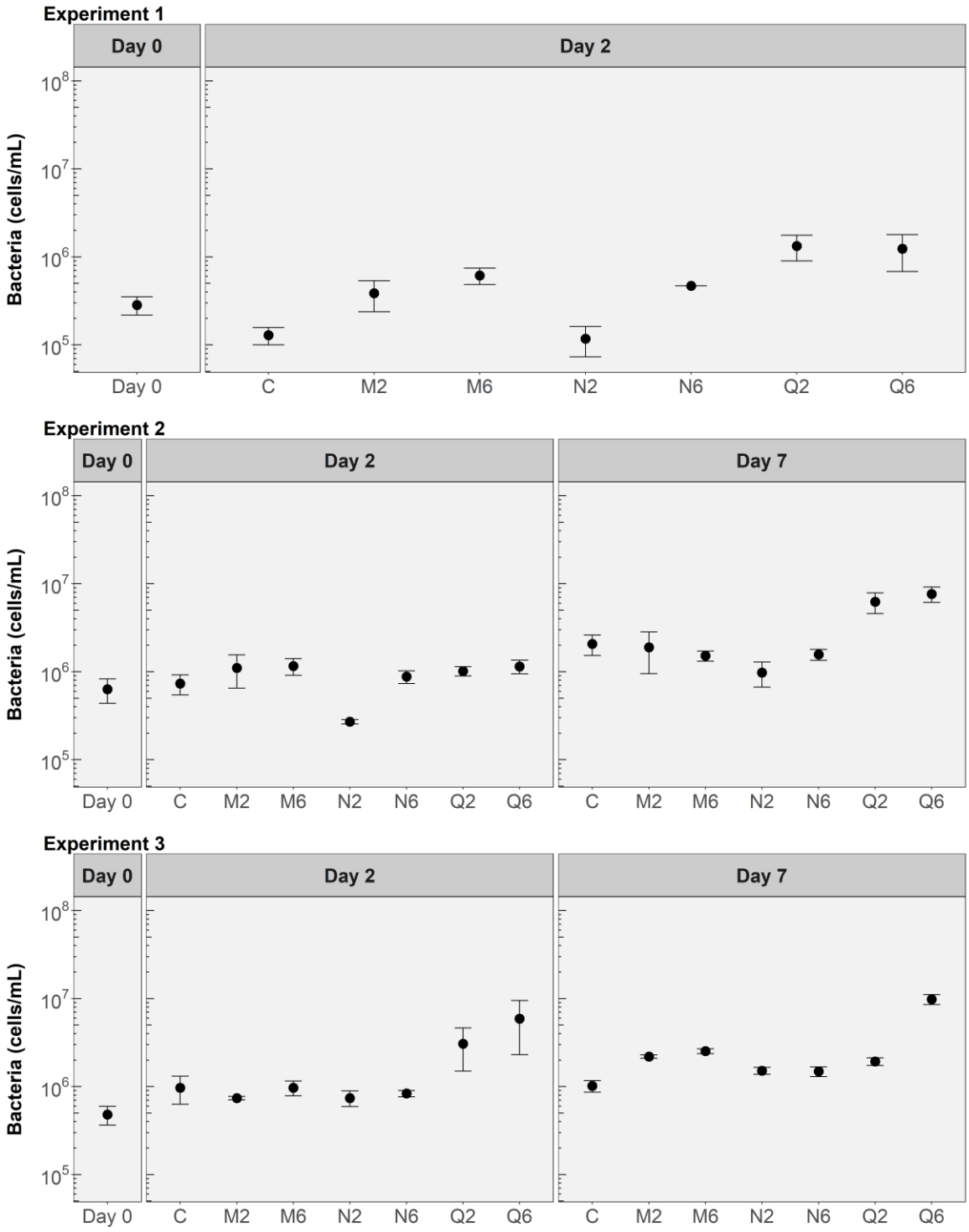

Figure 19. Average bacterial cell density (cells/mL) from different treatments in three microcosm experiments. Error bars are standard error of the mean. The y-axis is on a log10 scale.

4.2.1.2 Phytoplankton

In Experiment 1 there were no significant changes in Chlorophyll-*a* concentration in any treatments compared to the control after 2 days of incubation (Figure 20). Similarly, in Experiment 2 and 3, there was a limited response after 2 days. However, after 7 days of incubation Chl-*a* concentrations rose to extremely high concentrations. The largest response was observed in Experiment 2, where

Chl-*a* was >75 ug/L in all treatments after 7 days of incubation. There were also differences between treatments in Experiment 2, where Q2 and Q6 were higher than the control and all other treatments after 7 days. The M2 and M6 treatments also appeared marginally higher than the control. Similar trends were observed in Experiment 3, with Chl-*a* concentrations in the M2, M6, Q2 and Q6 treatments higher than the control, although to a lesser extent than those observed in Experiment 2 (Figure 20).

The initial phytoplankton community compositions were different in each of the experiments (Figure 21). In Experiment 1, the community was dominated by euglenoids and cryptomonads. After 2 days of incubation, euglenoids were less dominant in all treatments. Leachate additions generally resulted in a more diverse community composition, with a greater proportion of dinophyceae and green algae. This was particularly evident in the N6 and Q2 treatments. In Experiment 2, the initial community was strongly dominated by diatoms (Figure 21). After 2 days, there was a degree of variability in the phytoplankton communities between treatments, with Q2 having a greater amount of dinophyceae and euglenoids compared to the control, whereas most other treatments had a higher amount of diatoms compared to the control. After 7 days, all treatments reverted to one strongly dominated by diatoms. In Experiment 3 the initial community was composed of predominantly diatoms, green algae and cryptomonads. On day 2, there were some notable differences between treatments. Leachate additions appeared to promote a higher diversity community compared to the control. This is particularly evident in the N2 treatment which had a high proportion of cyanobacteria and green algae. By day 7 all treatments were similar to the control and were dominated by diatoms.

Food web response (Table 3, Chl-a and bacteria concentrations) were not significantly different from the control in any treatments in experiment 1. In experiment 2, only Q6 was significantly different from control (p=0.049) on day 2 whereas M2, Q2 and Q6 were significantly different from control (p<0.05) on day 7 and M6 was near significant (p=0.076). All treatments including the control were significantly different between day 0 and day 7 (p≤0.01). In experiment 3, no treatments were significantly different from the control on day 2 however M2, M6, Q2 and Q6 were all significantly different from the control on day 7 (p<0.05). All Mehi and Macquarie treatments were significantly different (p≤0.021) from day 0 on day 7 and N2 was near significant (p=0.063). Food web responses were significantly different between all experiments (Table 4, p<0.001, pseudo-f: 10.454).

Table 3 p-values from PERMANOVA comparisons of food web response (chlorophyll-a (ug/L) and bacteria (cells/mL)) in treatments vs control (days 2 and 7) and day 7 vs day 0 within treatments. Italicised numbers represent near significant values.

Foodweb response			Namoi (N)		Mehi (M)		Macquarie (Q)	
	Treatment vs	Control	2mg/L	6mg/L	2mg/L	6mg/L	2mg/L	6mg/L
Experiment 1	D2 vs Day 0	-	-	-	-	-	-	-
	C2	-	-	-	-	-	-	-
Experiment 2	C2	-	-	-	-	-	-	0.049
	C7	-	-	-	0.047	*0.076*	0.013	0.009
	D7 vs Day 0	0.001	0.006	0.01	0.004	0.004	0.002	0.005
Experiment 3	C2	-	-	-	-	-	-	-
	C7	-	-	-	0.08	0.047	0.025	0.002

	D7 vs Day 0	-	*0.063*	-	0.018	0.021	0.004	0.001

Table 4 Results from PERMANOVA main-test of resource uptake and food web growth between experiments.

Between experiments	p-value	Pseudo-f
Resource uptake	<0.001	12.938
Food web responses	<0.001	10.454

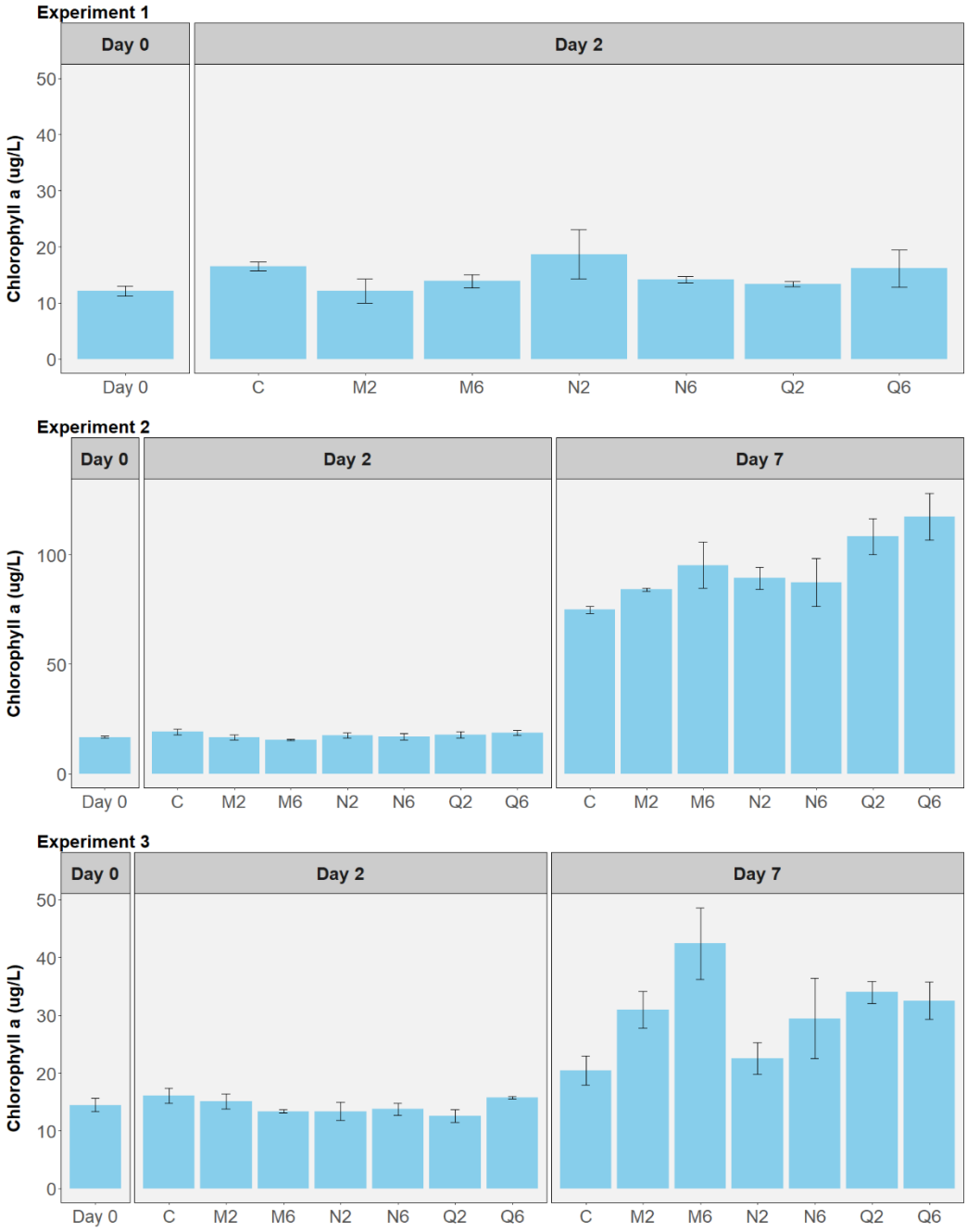

Figure 20. Average Chlorophyll-a concentrations (ug/L) from different treatments of three microcosm experiments. Error bars are standard error of the mean.

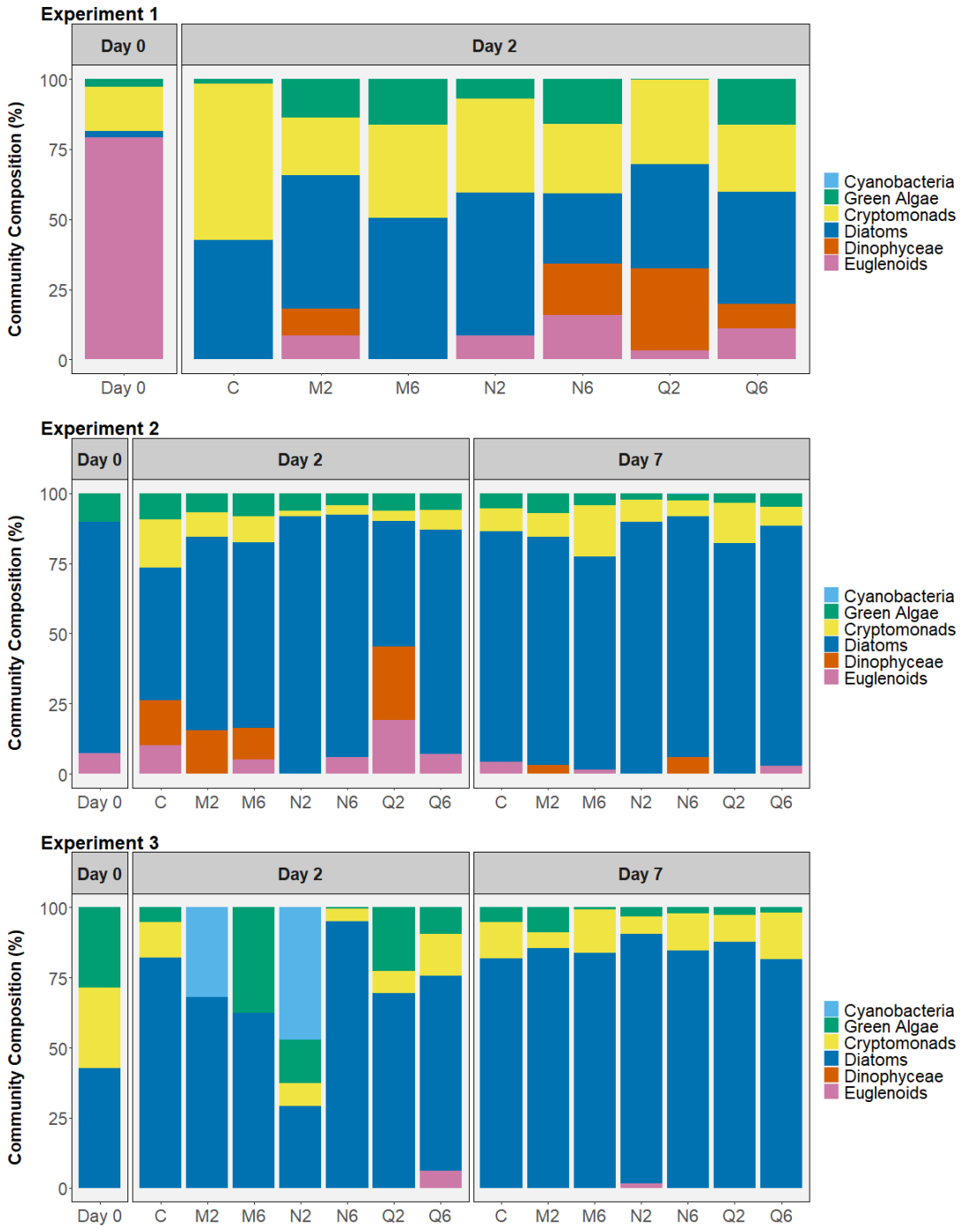

Figure 21. Phytoplankton community composition of different treatments in three microcosm experiments.

4.2.2 Resource uptake

Resource uptake was often different between treatments and through time. In Experiment 1 there were minimal changes between Day 0 and Day 2 in DOC (Figure 22), but there was evidence of nitrogen (Figure 23) and phosphorus (Figure 24) uptake across all treatments, however no treatments were significantly different from the control (M6 and Q6 were near significant (Table 5, p<0.06)). In Experiment 2, there was high uptake of nitrogen and phosphorus in all leachate treatments, whereas DOC uptake was less pronounced. Resource uptake was only significantly different from the control on day 7 in Q6 (p=0.015), though all treatments on day 7 were significantly different from day 0. In Experiment 3, DOC uptake was minimal in most treatments, with the exception of M2 and Q2 which was much higher. Nitrogen and phosphorus uptake was very high, particularly in the Mehi and Macquarie treatments. This led to several treatments (N6, M2, Q2, Q6) being significantly different (p<0.05) from the control on day 7. Resource uptake was significantly different across all experiments (Table 4, p<0.001, pseudo-f: 12.938).

Table 5 p-values from PERMANOVA comparisons on resource uptake (nutrient and DOC concentrations) in each treatment vs the control on the same day and day 7 vs day 0 within treatments. Italicised numbers represent near significant values.

Resource uptake			Namoi (N)		Mehi (M)		Macquarie (Q)	
	Treatment vs	Control	2mg/L	6mg/L	2mg/L	6mg/L	2mg/L	6mg/L
Experiment 1	D2 vs Day 0	*0.051*	-	*0.079*	*0.051*	0.041	-	*0.057*
	C2	-	-	-	-	*0.058*	-	*0.056*
Experiment 2	C2	-	-	-	-	-	-	-
	C7	-	-	-	-	-	-	0.015
	D7 vs Day 0	-	0.047	0.012	0.009	0.018	0.037	0.004
Experiment 3	C2	-	-	-	-	-	-	0.019
	C7	-	-	0.045	0.036	-	0.013	0.001
	D7 vs Day 0	-	-	-	*0.076*	-	-	0.009

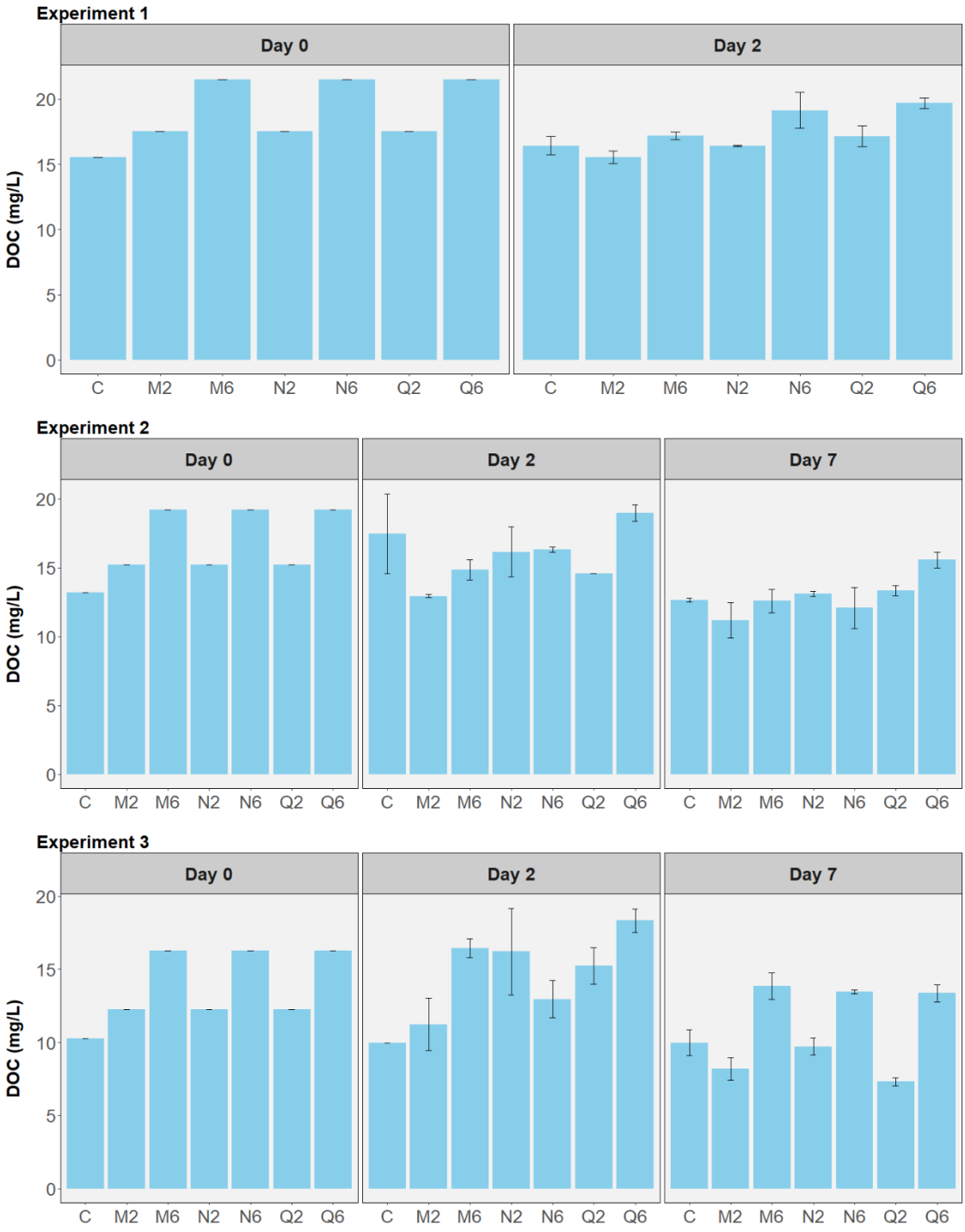

Figure 22 Average DOC concentrations with standard error of the mean for all treatments on days 0, 2 and 7

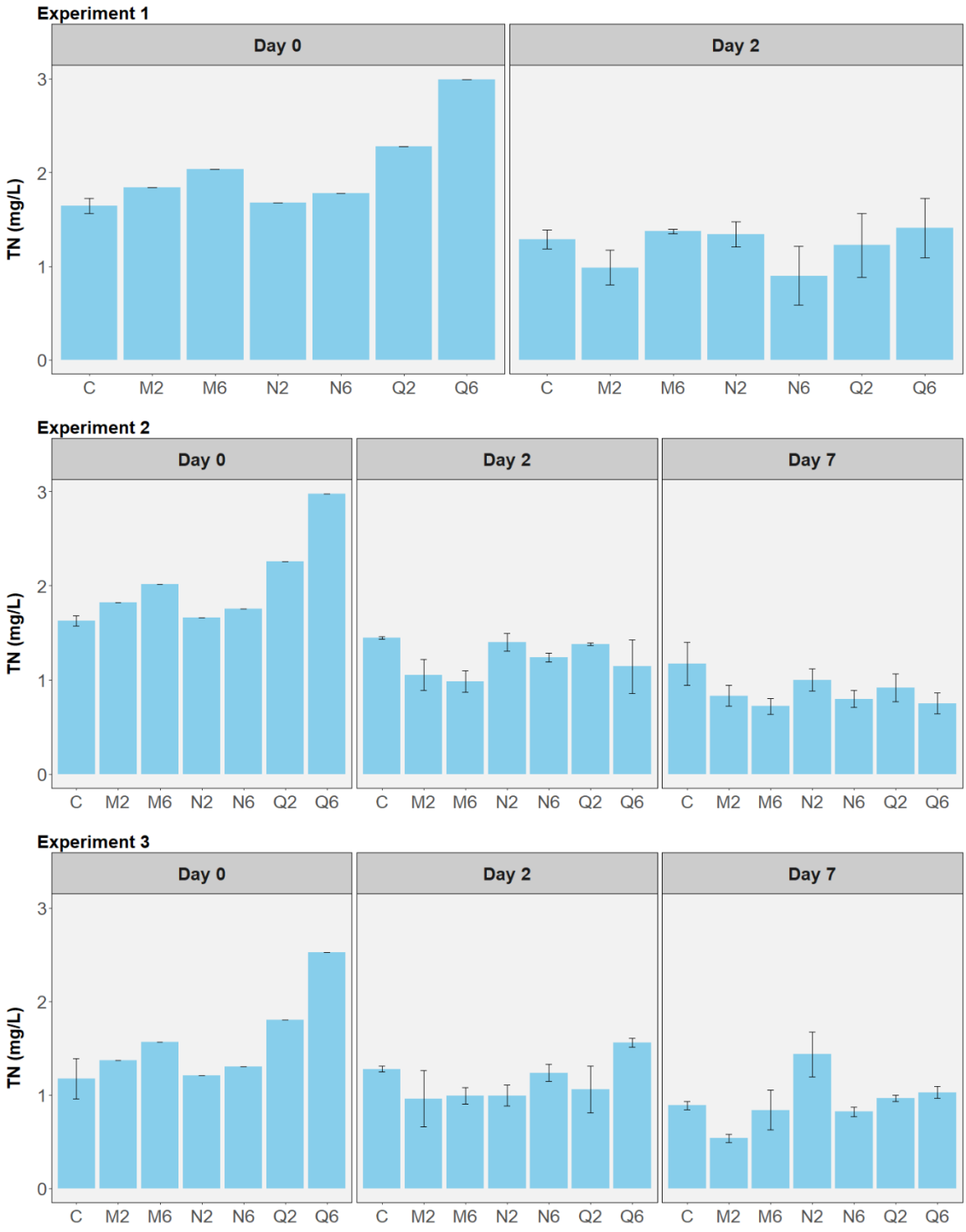

Figure 23 Average Total Nitrogen concentrations with standard error of the mean for all treatments on days 0, 2 and 7

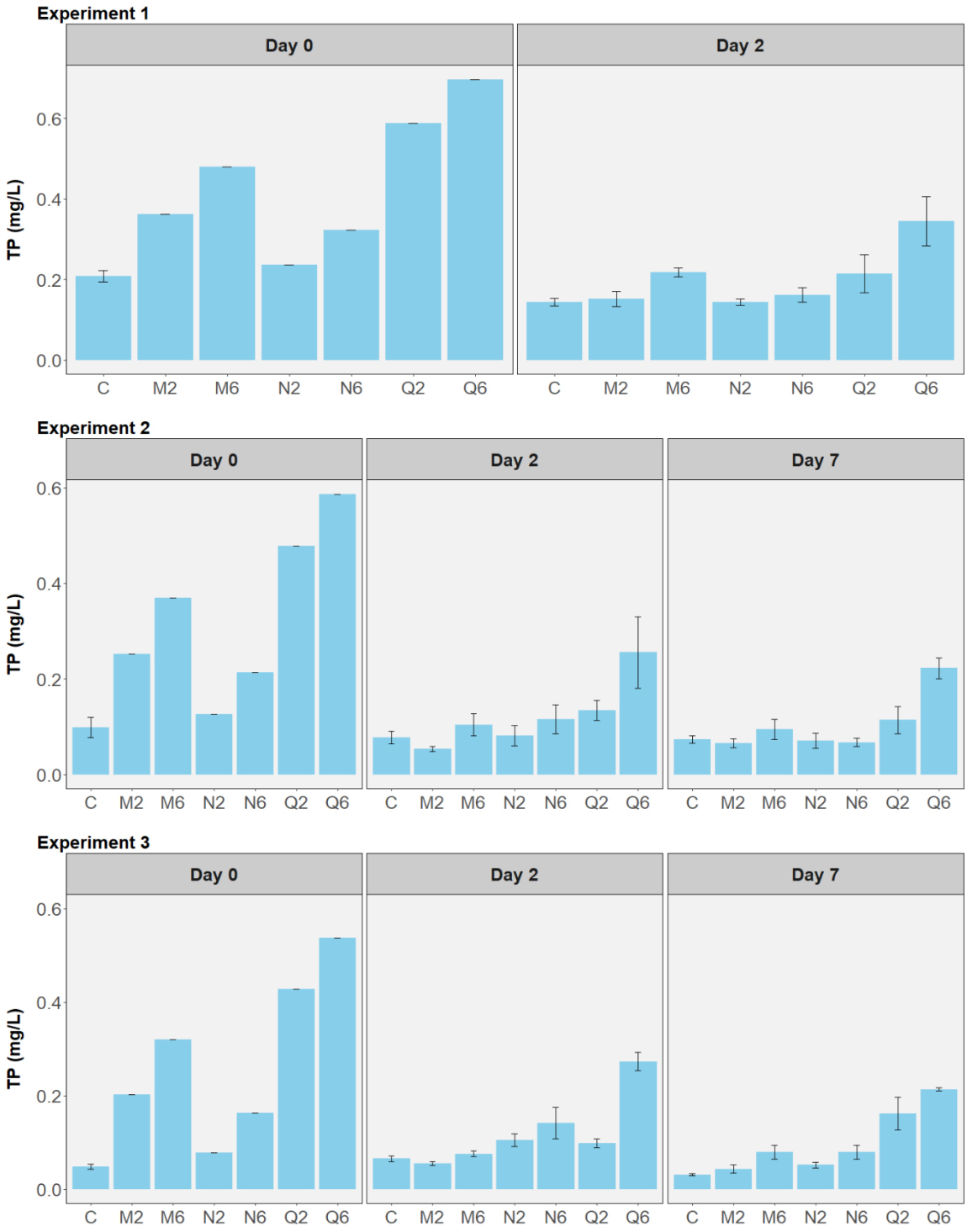

Figure 24 Average Total Phosphorus concentrations with standard error of the mean for all treatments on days 0, 2 and 7

5. Discussion

5.1.1 Relationship between discharge, resources and the lower food web

Discharge appeared to play an important role in mobilising some nutrients and DOC in the Barwon River and its tributaries. Linear regression analysis showed that total phosphorus (TP), phosphate, total nitrogen (TN) and ammonia were positively correlated to discharge at all sites, while nitrate was negatively correlated to discharge on the Barwon main stem and not significantly related to discharge on the Namoi and Mehi Rivers (see Appendix). DOC was not significantly related to discharge (see Appendix) however concentrations peaked during and immediately after the April flood event, suggesting discharge was important for regulating DOC concentrations. Similarly, most nutrients peaked during the April flood suggesting the large overbank flow mobilised high amounts of allochthonous nutrients, as suggested in the flood pulse concept (Junk et al., 1989) and seen previously during a flood on the Murray River (Nielsen et al., 2016). Positive relationships between discharge and nutrient and/or DOC concentrations have also been found previously on the Namoi (Westhorpe et al., 2008; Westhorpe and Mitrovic, 2012) and Gwydir Rivers (Woodward et al., 2015).

Nitrogen and phosphorus are crucial for supporting riverine productivity as they are important nutrients for the growth of phytoplankton and bacterial communities, which form the base of freshwater food webs (Hecky and Kilham 1988; Stahl et al., 2013). Dissolved oxygen concentrations were lowest during the April flood event (Figure 5), indicating low instream photosynthesis. This is supported by low Chlorophyll-*a* (Chl-*a*) concentrations and low phytoplankton counts during the April flood event. This is typical of flood events in Australian rivers as turbidity reduces light for photosynthesis and sharp increases in discharge lead to the dilution of phytoplankton (Townsend et al., 2017). Following the flood event, dissolved oxygen and phytoplankton biovolume increased steadily on the main stem (Figure 5, 12), whereas phytoplankton biovolume was extremely high in both tributaries following the flood, reaching 22 mm^3/L on the Namoi River. Phytoplankton were likely utilizing nutrients recently mobilised by the flood and could proliferate under reduced flow conditions. This is consistent with previous studies by Nielsen et al. (2016) and Townsend et al. (2017).

Changes in the phytoplankton community composition may have reflected changing growing conditions and resource availability. Mixotrophic algae dominated during and immediately after the April flood event, suggesting organic matter and bacteria may have been the dominant source of energy during this period, particularly as low light conditions would have greatly reduced photosynthesis. Once flood conditions had fully receded in May/June and light availability increased with declining turbidity (Figure 6), autotrophic diatoms dominated the phytoplankton community. The change from mixotrophs to obligate autotrophs suggests photosynthesis became the dominant source of energy for basal production (Figure 12). Interestingly, bacteria concentrations were generally low during the April flood event despite relatively high DOC concentrations, most likely due to poor growing conditions caused by dilution and in-stream turbulence. The Mehi River was an exception, where bacteria concentrations peaked during the flood event. As with phytoplankton, bacteria generally increased following the flood event, likely using nutrient and organic carbon mobilised by the flood. Westhorpe et al. (2010) found nitrogen and phosphorus additions to *in situ* microcosms in the Namoi River significantly increased Chl-*a* concentrations and when combined with DOC additions, significantly boosted bacterial production. This suggests that increases in river

discharge that raise DOC and nutrient concentrations may result in enhanced primary and/or heterotrophic production (Gawne et al., 2007; Cook et al., 2015). These findings have been observed in Australian semi-arid rivers, where high flow events and floodplain inundation have been shown to provide important pulses of energy for connected riverine food webs (Cook et al., 2015; Wallace and Furst, 2016).

The large response of zooplankton to the April flood observed in this study and subsequent decline in abundance once flood conditions subsided (Figure 13, 14) is consistent with the boom and bust conditions of lowland rivers in semi-arid Australia (Sterner et al., 2008). Previous studies have found both rotifer and mesozooplankton abundance increases considerably after flood events (Shiel et al., 2006; Ning et al., 2013; Furst et al., 2014), at times increasing orders of magnitude compared to base flow conditions (Nielsen et al., 2016; Rees et al., 2020). Generally, zooplankton concentrations were much higher at the downstream study sites (BUSN, BDSN, Namoi) during the April flood than upstream. Zooplankton hatching from newly inundated floodplain with egg banks and downstream transport of individuals (from upstream main stem or tributary inputs) during high flows may have greatly increased downstream zooplankton populations and account for the largest proportion of zooplankton observed downstream during this period (Jenkins and Boulton, 2003; James et al., 2008). As key food resources for zooplankton such as phytoplankton and bacteria were low during the flood event, in-situ growth may not have contributed a significant proportion of the observed zooplankton numbers on the lower reaches of the Barwon River. Despite increasing phytoplankton and bacteria on the Barwon main stem, zooplankton numbers remained low during May and June, possibly due to seasonality and winter dormancy in some copepod families (James et al., 2008).

5.1.2 Effect of tributaries

Tributary inflows can be an important source of organic matter and nutrients to the main stem of a river (Kiffney et al., 2006; Rohlfs et al. 2016). This study demonstrated that some nutrients (TP, phosphate and ammonia) on the Namoi and Mehi Rivers were often equal to or greater than concentrations on the Barwon main stem. This indicates that tributary inflows may be an important source of nutrients for main stem food webs. However, nitrate concentrations were always much lower on the tributaries than on the main stem (by a factor of 10) and this appeared to influence differences in total nitrogen across sites (Figure 8). Lower nitrate concentrations on both tributaries may have been due to different catchment sources, closer proximity to riparian vegetation (Woodward et al., 2009), biological uptake and potentially bacterial denitrification (Groffman, 1994). DOC was a similar concentration at all sites during the April flood however, once flood conditions had receded, DOC was generally lower on both tributaries than on the Barwon main stem. Low discharge on the tributaries in May and June may have contributed to the low DOC concentrations, as DOC is often more hydrologically-driven on tributaries compared to larger rivers (Agren et al., 2007).

Both tributaries generally contained higher concentrations of phytoplankton than the Barwon main stem, particularly in the weeks immediately following the April flood event (Figure 5). Differences in bacterial concentrations were more pronounced between the Barwon main stem and tributaries with bacterial concentrations often an order of magnitude higher in the tributaries than the Barwon. Previous studies have found tributary inflows to be an important source of migrant biota for rivers

(Kiffney et al., 2006) with inflows affecting planktonic community structure and food web production (Rice et al., 2008).

During booms in phytoplankton and bacteria on the tributaries, nutrient and DOC concentrations were generally similar between main stem and tributaries. It is therefore unlikely differences in nutrient concentrations were the key driver of change between them. For example, DOC was lower in the tributaries than the main stem while bacteria were an order of magnitude higher in the tributaries. As DOC is a limiting resource for heterotrophic bacteria it is possible the DOC present in the tributaries was of a much higher quality than that of the Barwon main stem and therefore more readily available for bacterial uptake. Differences in the bioavailability of DOC may be related to differences in catchment attributes such as terrestrial primary productivity (Wilson *et al.*, 2013), soils (Autio *et al.*, 2016) and land use (Petrone et al., 2009). Further, autochthonous DOC produced by phytoplankton and local macrophytes is considered more bioavailable than DOC derived from terrestrial origins (Bertilsson et al., 2003). Given the higher concentrations of phytoplankton and proximity to riparian vegetation in tributaries in this study, it is possible that autochthonous DOC played a role in the bioavailability of the DOC and consequent increased bacterial concentrations. However, as bacteria also increased during periods of low Chl-*a* concentrations, such as during the April flood on the Mehi and in May/June at both tributary sites, it is likely that bacteria were at least partially using highly bioavailable terrestrial DOC as an energy source as seen in previous studies (Moran et al., 2014). This difference in bacteria and DOC concentrations between tributaries and the main stem may broadly indicate the DOC mobilised on the Namoi and Mehi rivers is of a considerably higher quality than that of the Barwon.

The findings from the microcosm study support those of the monitoring study suggesting DOC and nutrients from the tributaries were more bioavailable than the Barwon main stem. The addition of leachates of floodplain material from tributary catchments resulted in increased primary production and bacterial production after 7 days of incubation. The greatest response in the phytoplankton community was observed in Experiment 2. This experiment coincided with the 19th May sampling trip, in which the concentrations of nutrients and DOC had rapidly declined since the post-flood peak. The leachate additions provided a pulse of nutrients and DOC which alleviated limitation, leading to higher chlorophyll-*a* concentrations in all leachate treatments compared to the control. The uptake of nitrogen and phosphorus was very high in this experiment, indicated by the rapid decrease in N and P concentrations after 7 days. Nutrient uptake was generally highest in the Mehi and Macquarie treatments. The minimal responses in the Namoi treatments is likely a result of the high carbon to nutrient ratios in the leachate (C:N:P ratio of 100:1.63:1.45) with nutrient concentrations in the Namoi leachate insufficient to increase phytoplankton growth which remained nutrient limited despite leachate additions. Low levels of nitrogen and phosphorus in the Namoi leachate was not supported by the monitoring study as N, P and C concentrations were similar in the Namoi and Mehi rivers. The difference in leachate concentrations are likely due to differences in floodplain material collected; and may not reflect the catchment as a whole.

The stimulation of the algal community was not observed to the same extent in either Experiment 1 or Experiment 3. In Experiment 1, the system was already loaded with high concentrations of resources from the recent flow event. The high availability of ambient resources would have minimised or obscured any response to leachate additions. Whereas in Experiment 3, phytoplankton growth occurred at a lesser degree than Experiment 2. This was likely due to colder temperatures limiting phytoplankton growth. However, bacteria did respond to leachate additions. Again, this was

particularly evident in the Mehi and Macquarie treatments, likely due to higher nitrogen and phosphorus concentrations in the leachate or due to more bioavailable carbon derived from their respective floodplains. The results of Experiment 2 and 3 reinforce the capacity for allochthonous inputs to provide highly bioavailable resources to the basal food web.

Higher concentrations of important food resources such as phytoplankton and bacteria appeared to play a role in supporting higher populations of zooplankton on the Mehi and Namoi Rivers once flood conditions had receded. Nauplii peaked at very high levels during the phytoplankton bloom on the Namoi immediately after the April flood event. Similarly, total rotifer concentrations on both tributaries peaked following the April flood and remained high until mid-May, whereas rotifer concentrations on the main stem continually declined following the flood event. Bacterivorous rotifer species such as *Keratella*, *Brachionu*s and *Filinia* (Arndt, 1993) dominated the rotifer community during the post flood boom in late April to mid-May, suggesting DOC and bacteria were playing an important role in supporting secondary production in both tributaries. This is consistent with previous research on the Namoi River (Mitrovic et al., 2014, Balzer, 2021) and broader experimental studies (Balzer, 2021).

Overall, it appeared the Mehi and Namoi Rivers may have been more productive per unit of discharge than the Barwon River. Bacteria, phytoplankton and most zooplankton were all in higher concentration on the tributaries once flood conditions had subsided, even when nutrients and DOC concentrations were similar or less than that of the Barwon River. It is therefore possible that the nutrients and organic carbon present in the Mehi and Namoi Rivers is of a higher quality to that of the Barwon River, leading to higher primary and secondary production on both Rivers. The relative influence of a tributary on main stem water quality and production is dependent on the ratio of tributary to main stem discharge (Benda et al., 2004). On the Mehi, total loads of phosphate, TN and ammonia contributed to main stem loads at disproportionately high levels compared to contribution of discharge during the April flood. Nutrient loads on the Namoi River were proportional to discharge throughout the study, suggesting the impact of the Namoi on Barwon main stem nutrient loads was closely related to flow magnitude. Total daily loads of phytoplankton and bacteria were also often disproportionately higher than discharge on both tributaries when compared to downstream sites on the Barwon, suggesting inflows from the tributaries would likely transport important basal producers into the Barwon River. These disproportionately large contributions of biota from both tributaries indicate flow events down the Namoi and Mehi Rivers may play an important role in increasing basal food web production in the Barwon River.

The influence of tributaries on main stem production may have been far greater during periods of low flow on the Barwon main stem. Bioavailable carbon and nutrients added to rivers under low flow conditions have been shown to lead to large increases in bacteria and zooplankton (Mitrovic et al. 2014; Balzer 2021). However, during and after the April flood event when tributary inflows were highest, carbon and nutrient concentrations were already high on the main stem, potentially reducing the impact of water quality changes through tributary inputs. This meant the main influence of tributaries was direct transport of biota into the main stem (bacteria, algae and zooplankton) rather than *in-situ* increases in production from higher nutrient loads leading to growth. Similarly, the effect of leachates during the microcosm experiments may have been greater during periods of low flow when nutrient and carbon concentrations may have been more limiting of basal production.

Seasonal variation in bioavailable carbon may also play a role with April (autumn) a time when large amounts of relatively fresh terrestrial OM are available. Redgums typically shed leaves during the warmer months and also with stress cues, such as extremely warm weather and dry conditions. The seasonal timing of flooding and drying events on floodplains is important as flow events in summer are expected to provide a higher yield of allochthonous organic carbon and nutrients as increased temperatures lead to greater heterotrophic production (Baldwin and Mitchell 2000). Therefore protection of events based on seasonality maybe another important factor to consider.

5.1.3 Management implications and recommendations

Discharge appeared important for mobilising nutrients and organic carbon on the Barwon River and its tributaries the Mehi and Namoi Rivers. However, concentrations of nutrients and DOC were often similar between the main stem and tributaries despite discharge on tributaries averaging <15% than discharge on the Barwon. This suggests relatively small flow events on the Namoi or Mehi Rivers may affect food web production in a similar way to that of larger flow events on the Barwon River. Further, phytoplankton and bacteria appeared to be supported in higher concentrations after flows on the tributaries than the Barwon River, with the tributaries often contributing disproportionately high loads of phytoplankton or bacteria to main stem loads relative to discharge (Figure 16). This data suggests comparatively small flow events down either tributary may result in high nutrient and DOC yields, leading to greater basal production in the tributaries and subsequently, the Barwon River. Tributary flows entering the Barwon-Darling River when the main stem is at relatively low flow, such as after tributary catchment localised flow events will likely have a much greater effect on the main stem, both in terms of the biota imported and the utilisation of resources delivered.

Zooplankton also appeared to be positively related to discharge at all sites however were only at high numbers on the main stem during and immediately after the April flood event. Conversely, nauplii and rotifer concentrations on the Namoi and Mehi appeared to be supported by higher phytoplankton and bacterial growth leading to the longer-term booms in rotifer populations in both tributaries. This increase in zooplankton growth suggests flow events down tributaries may increase secondary production for several weeks following a flow event, as seen in the Lake Eyre River basin (Shiel et al., 2006). As zooplankton form the bulk of fish diets during their larval and juvenile stages (Humphries, 1999), it is likely that flows down tributaries may contribute important food resources for fish and increase native fish recruitment if flows occur during or after the spawning season.

The outcomes of this project support two key objectives within the NSW Government River Flow Objectives framework; 2. Protect natural low flows, and 6. Maintain or mimic natural flow variability in all streams. This is also relevant during and following extended dry periods, and larger response in the food web may be seen after dry periods helping the system recover as flow conditions improve. Flows from tributaries into the Barwon Darling may provide for upstream system needs but if protected will achieve food web improvements downstream as well. Rules to protect these important flows (end-of-system) have been addressed in some WSPS. For example in the Namoi Water Sharing plan Clause 48 of the Plan specifies limits to total extractions by all Lower Namoi supplementary water access licence holders during periods when flows are above specified threshold flow levels. These rules contribute to a number of interim river flow objectives; protecting important rises in water levels; maintaining wetland and floodplain inundation; and maintaining natural flow variability.

Overall this study supports the importance of tributary flows to the main stem Barwon-Darling River. Flow rules that protect flows in tributaries entering the main stem should see ecological improvements in river productivity through increased resources and biota such as algae and zooplankton. These benefits may be greater when tributary flows enter the main stem of the Barwon-Darling River during periods of low flow.

5.1.3.1 Recommendations

This study provided some preliminary evidence of the importance of tributary inflows to supporting the main stem food web on the Barwon-Darling River. However, given that a large flood event occurred simultaneously on all tributaries and the Barwon River, it is difficult to determine and compare the individual roles of the tributaries, as their impact was obscured by the high availability of resources already present in the Barwon. A longer study period encompassing different flow periods and inflow volumes would offer far clearer results on the influence of tributaries on production within the Barwon River. This would help understand the impacts of different sized flow events, particularly flows that were isolated to a single tributary and did not coincide with large main stem flows. Capturing different flow sizes would help understand the influences of individual flows across a range of environmental conditions (such as antecedent conditions and seasonality) and how varying flows affect nutrients, organic matter, biota mobilisation and the ratio of nutrient to discharge across differing flow sizes. Further, understanding differences between tributaries such as bioavailability, nutrient loads and phytoplankton community composition would allow for better modelling of influence from specific tributaries. Future studies would also benefit from multiple sample sites downstream of the confluence to assess the extent of downstream transport of tributary resources and how long ecological benefits persist post-flow. The influence of weirs on longitudinal transport of tributary resources could also be examined. Monitoring of additional tributaries may also offer important information on the variable influences of tributaries with differing land-use, riparian vegetation and flow variability.

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