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MMCP Collaboration Final Report 2019 - Biofilm succession patterns and ecosystem dynamics in the Edward-Wakool River system

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The MMCP Collaboration Final Report 2019 – Biofilm succession patterns and ecosystem dynamics in the Edward-Wakool River system

Final Report prepared for the Murray–Darling Basin Authority.

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Cover Image: Biofilm growing on a River Red Gum block incubated in the Wakool River

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Contents

Background **1**
Overview **2**
Methods **4**
Sites 4
Water quality and nutrients 5
Biofilms 6
Production 7
Statistical analyses 8
Results **8**
Water quality and nutrients 8
Dissolved organic carbon changes within light/dark bottles 13
Dissolved organic carbon components relationship with DOC indices, chlorophyll-a and water quality variables 14
Biofilms 15
Biofilm dry weight, LOI and percent loss 15
Biofilm Chlorophyll-a 17
Biofilm C:N 19
Biofilm fatty acid composition 19
Correlations 21
Production 24
Key management implications **26**
References **28**

Tables

Table 1. Dissolved organic carbon (DOC) quality indices used in this study. 5
Table 2. Pearson correlation coefficients relating DOC components with BIX and chlorophyll a. *P* values ≤0.05 are highlighted in bold and suggest a significant relationship. 15
Table 3 Significant (<0.05) Pearson correlations between biofilm Chl-a concentration, total dry weight and percent loss and abiotic and biotic variables 24

Figures

Figure 1. Discharge in the Niemur and Wakool rivers and Yallakool Creek from June 2018–January 2019 (shaded area depicts study period, https://realtimedata.waternsw.com.au/). 1

Figure 2. Location of study sites. 4

Figure 3. River Red Gum blocks attached to floating platforms (a) deployed to sample biofilms in the (b) Niemur River, (c) Yallakool Creek and (d) Wakool River. 7

Figure 4. Sampling production in the Edward–Wakool River system; (a) dissolved oxygen logger to measure open water production tethered to floating biofilm platform, (b) and (c) light and dark bottle array to measure pelagic metabolism, and (d) deployment of the light and dark bottle array in Yallakool Creek. 7

Figure 5. Mean water quality values collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 9

Figure 6. Mean nutrient and chlor-*a* values collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).....10

Figure 7. Mean DOC values collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 11

Figure 8. Dissolved organic carbon (DOC) components derived using fluorescence excitation emission scans followed by PARAFAC analysis. 12

Figure 9. Percentage contribution of humic- and protein-like substances from river water DOC collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 12

Figure 10. Percentage contribution of humic- and protein-like substances from DOC from light and dark bottles incubated in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 13

Figure 11. Dissolved organic matter (DOM) indices for the Niemur, Wakool and Yallakool rivers from the 24 October 2018 to 3 January 2019; a) absorbance ratio at 254 nm to 365 nm (abs254/365) as an indicator of molecular weight; b) Biological Index (BIX) as an indicator of autochthonous production; c) Fluorescence Index (FI) as an indicator of source of DOM; and specific UV absorbance coefficient at 254nm ($SUVA_{254}$) as an indicator of aromaticity (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 14

Figure 12. Loss on ignition (A), total dry weight (B) and percent weight loss after ignition (C) of biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 16

Figure 13. River Red Gum blocks from the Wakool River (a) 3 weeks after deployment (half the block has been scrubbed of biofilm) and (b) 11 weeks after deployment. 16

Figure 14. Chlor-*a* concentration of biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 17

Figure 15. Collection of biofilm platforms in January 2019: (a) platform covered in cyanobacteria (likely *Nostoc* spp.), (b) recovery of dissolved oxygen logger from platform at the Wakool River site, and (c) close up of cyanobacteria (likely *Nostoc* spp.) growing on platforms after 11 weeks submergence in the Wakool River. 18

Figure 16. Sampling at the Niemur River site in January 2019; (a) 11 week old biofilm growing on River Red Gum block, (b) floating platform after retrieval with last block to be harvested, and (c) processing water quality and nutrient samples. 18

Figure 17. Total percent carbon (A), total percent nitrogen (B), and C:N (C) of biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 19

Figure 18 Bootstrapped means (black symbols) and 95% estimate regions derived from fatty acid profiles as percentage of total lipids for biofilms from Niemur River, Yallakool Creek and Wakool River from Trip 1 (3 week old biofilms) to Trip 9 (11 week old biofilms), red arrow indicating trajectory of change, MDS, multidimensional scaling. 20

Figure 19 Total mass (mg m^2, top panel) and proportion of total lipids (bottom panel) of essential PUFAs alpha-linolenic (ALA; 18:3ω3), arachidonic acid ARA (20:4ω6) and linoleic acid (LA; 18:2ω6) within biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles). 21

Figure 20 Significant ($p<0.05$) Pearson coefficient correlations for biofilm dry weight and abiotic and biotic variables 22

Figure 21 Significant ($p<0.05$) Pearson coefficient correlations for biofilm percent loss and abiotic and biotic variables 23

Figure 22. Stream metabolism dynamics GPP (A), NPP (B), CR (C), and littoral zone NPP (D) estimated as O_2 mg/L per hour calculated from open water estimates (crosses) and light and dark bottles (circles) from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (error bars ±1 SE, shaded area around lines represent 95% confidence intervals). ..25

Background

Initially, the focus of this project was to assess changes in biofilm quality in response to managed flows. We chose to investigate biofilm ecology in rivers within the Edward–Wakool River system, since there were environmental flows planned for 2018–2019. However, due to extremely dry conditions throughout the Murray–Darling Basin (MDB) in 2018, all planned environmental flows were suspended within the Edward–Wakool River system in order for the MDBA to transfer water to Lake Victoria prior to commencement of sampling for this project (October 2018). In contrast to planned variability flows, each of the rivers in our study catchment were maintained at high levels for the duration of our study (Figure 1). Discharge in all three rivers dropped marginally over the course of the study, but flow variation was minimal.

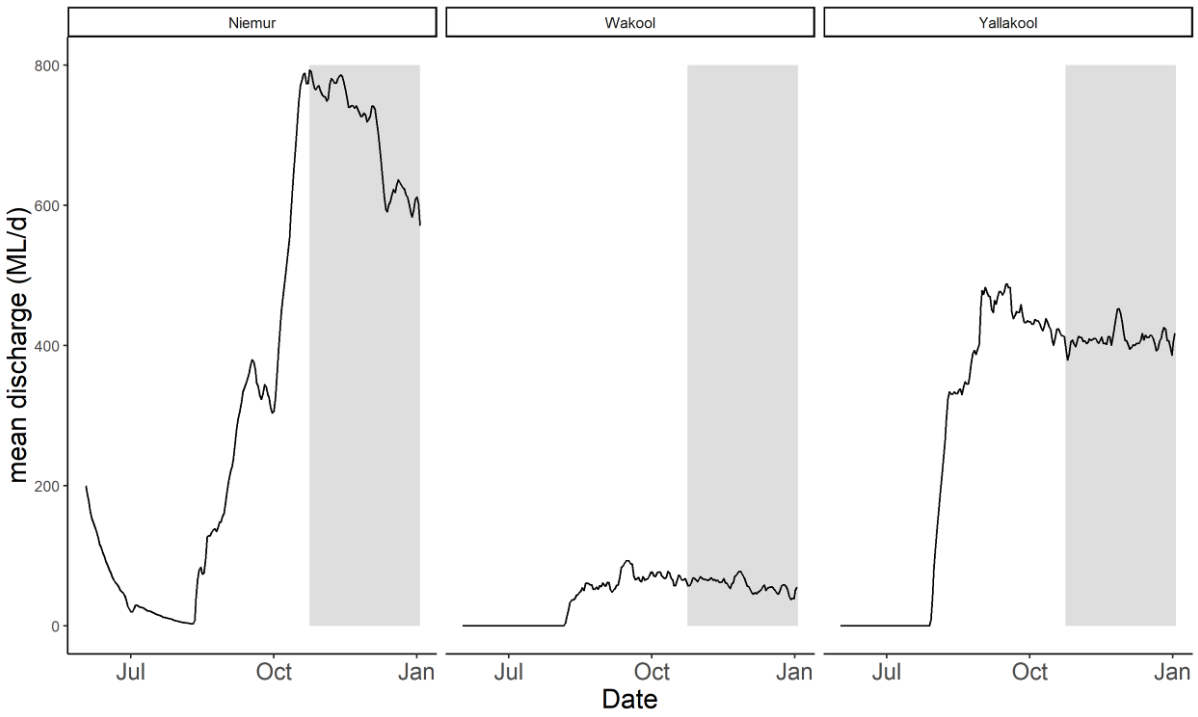

Figure 1. Discharge in the Niemur and Wakool rivers and Yallakool Creek from June 2018–January 2019 (shaded area depicts study period, https://realtimedata.waternsw.com.au/).

While changes to the planned environmental flow regime were disappointing, it provided us with a unique opportunity to investigate successional patterns of biofilms through time, replicated in three adjacent streams, under relatively constant flow conditions. Information surrounding basal food quality under such scenarios is of particular applied interest, since a better understanding of how biofilm quality changes through time could help inform decision making by water managers for optimisation of flow duration. For example, if the quality of biofilms growing on snags reaches a maximum after one week of inundation, it might make good sense to vary water height by small amounts relatively frequently; alternatively, if biofilm quality peaks 10 weeks after wetting, it may be more sensible to maintain water levels for longer periods to facilitate growth of high quality food resources for consumers.

Overview

Water level variations from managed flow releases are known to influence both rates of biofilm productivity (Ryder 2004) and structural attributes (Burns and Walker 2000; Ryder et al. 2006) of biofilms that inhabit hard surfaces within rivers. However, little attention has been directed at understanding how managed flows may alter the potential food quality of river biofilms and the subsequent implications for higher consumers (e.g. invertebrates, fish and water birds). Flow directly influences biofilm communities that inhabit hard surfaces within rivers via mobilization of carbon, nutrients (nitrogen and phosphorus), and sloughing. Hard surfaces within streams are hot spots for production, and support diverse macroinvertebrate communities; in effect, the biofilm communities are the first responders to changed flow. Biofilms are also important food sources, providing the basal resource for higher order consumers. As such, responses in biofilms can have a significant impact on community compositions at higher trophic levels. Improved biodiversity is a key target for environmental water management activities, but assessment tools and time restraints often fail to demonstrate desired outcomes. A key limitation is understanding the basal level ecology and its influence on higher trophic levels.

Environmental (e) deoxyribonucleic acid (DNA) detection is an effective method to determine temporal change of biofilm communities. eDNA is founded on next-generation sequencing (NGS) techniques that offer new and innovative approaches to examine a diverse arrangement of aquatic taxa (Furlan *et al.* 2016; McInerney & Rees 2017). eDNA detection (also referred to as metabarcoding), is potentially more sensitive than classical capture-based methods and offers a qualitative measure of both prokaryotes and eukaryotes and has been used to characterise biotic communities within streams (McInerney *et al.* 2016) and floodplain soil (Baldwin *et al.* 2013). Next generation sequencing of biofilms can assess a broad range of organisms present within aquatic systems using sequence-based methods that rely on cheap high-throughput sequencing of DNA and ribonucleic acid (RNA).

While eDNA can tell us 'what is there', determining the quality of biofilms requires further analyses. Food quality, in its coarsest form, may be assessed by ecological stoichiometry (for example Carbon:Nitrogen (C:N) Elser *et al.* 2000); however, consumers can be limited by availability of complex organic compounds such as amino acids (Dwyer *et al.* 2018), sterols and fatty acids (Twining *et al.* 2016). Within freshwater ecosystems, green algae and diatoms are considered high-quality food resources for herbivorous taxa due to their high concentration of nutrients and long-chain poly-unsaturated fatty acids (LC–PUFA) (Brett & Müller-Navarra 1997; Guo *et al.* 2015). Some omega–3 (ω3) and omega–6 (ω6) PUFAs, such as eicosapentaenoic EPA (20:5ω3), docosahexaenoic DHA (22:6ω3) and arachidonic acid ARA (20:4ω6) are essential for physiological functions of consumers (e.g. growth and reproduction, Sargent *et al.* 1999). These compounds, often referred to as essential fatty acids, are not able to be synthesized in sufficient quantities by animals themselves, and must be either sourced from their diet (Parrish 2009) or synthesized from shorter-chain PUFAs, such as alpha-linolenic (ALA; 18:3ω3) and linoleic acid (LA; 18:2ω6), which are plentiful in terrestrial plants (Guo *et al.* 2016). An improved understanding of what flow regimes best support biofilm synthesis of high quality essential nutrients to support higher order consumers is critical for effective water management.

Stream metabolism, measured as rates of aquatic primary production and whole ecosystem respiration, are functional indicators of aquatic ecosystem condition (Grace *et al.* 2015; Mulholland *et al.* 2001). Gross primary production (GPP) and community respiration (CR) rates are controlled by exchanges between hydrology, vegetation (terrestrial riparian and aquatic), geomorphology,

climate, water chemistry and biota of the stream environment, along with overall catchment condition (Grace & Imberger 2006; Mulholland *et al.* 2001).

In aquatic environments, metabolic activity can be defined as the production of oxygen during daylight hours by benthic plants and phytoplankton (autotrophs), countered by the consumption of oxygen by benthic and planktonic organisms such as bacteria, fungi and animals (heterotrophs) (Odum 1956). Oxygen production by algae can occur in the water column or within biofilms on hard surfaces, but is constrained by exposure to light. Oxygen consumption (respiration) by heterotrophic organisms such as bacteria and fungi is not limited by light, and may occur on surface biofilms, within the tissue matrix of organic structures, such as leaves and wood and within benthic sediments.

Dissolved organic carbon (DOC) is an important food source to heterotrophs within river systems (Holland *et al.* 2012; Meyer 1994). The bioavailability of DOC to heterotrophic organisms depends on the source of DOC; whether it's allochthonous (terrestrially derived) or autocthonous (microbially derived within the river), the amount of protein-like to humic-like material present, its aromaticity and its molecular weight. Thus, understanding the amount and type of DOC present will give us an understanding on the bioavailability of the DOC present and whether production within the system is reliant on allochthonous and/or autochthonous DOC.

The project objectives are to:

1. Provide an improved understanding of basal level ecology and processes of hard surface biofilms following inundation.

2. Demonstrate the use of new tools and the development of protocols to better manage environmental water allocations.

3. Generate information that can be directly used by water managers to report on outcomes of changed flow regimes.

4. Provide managers with the capacity to act and refine future management options.

Information generated form this work provides managers with detailed information on the succession of biofilm quality following inundation. Managed environmental flows that are not of sufficient magnitude to inundate adjacent floodplains are routinely employed in regulated rivers, and are generally seen as beneficial to ecosystems. However, empirical data describing fine scale–benefits are scarce, and managers currently lack information surrounding the influence of flow magnitude and timing on basal resource food quality. This information is key for prioritization of water resources for maximum ecological benefit, since basal resources sit at the foundation of all aquatic food webs.

In the absence of environmental flows, this project employed biochemical and DNA techniques to measure structural and functional changes of biofilms over an eight week period, commencing three weeks after initial inundation. Biochemical tools are used to provide information of food nutrition, while DNA tools are used to provide rapid, detailed analyses of structural aspects of microbial communities (DNA work is not included in this report due to laboratory processing constraints, but will be included during manuscript development). Applying both tools simultaneously represents a novel approach to demonstrating ecological responses to hydrology. Application of molecular DNA techniques demonstrate how biofilm communities growing on hard surfaces in rivers (from prokaryotes e.g. bacteria, through to higher eukaryotes e.g. algae, fungi and insects) respond to inundation following complete desiccation. In order to help explain patterns of biofilm succession,

we also measured water quality, nutrients and dissolved organic carbon (DOC) during the 8 week sample period. In addition, we investigated riverine productivity and which types of DOC are being used within the river ecosystems, and tracked river metabolism in our three streams during the study period. We also separated pelagic metabolism by estimating metabolism in the photic zone using the light/dark bottle method as an assay in order to estimate metabolic dynamics occurring on hard surfaces.

Methods

Sites

Sites were selected at locations on three rivers within the Edward–Wakool River system; Wakool River (-35.522576, 144.519656), Yallakool Creek (-35.506229, 144.484940) and Niemur River (-35.267449; 144.237214). Initially, this river system was selected based on the likelihood of managed flows, the former two sites being monitored by the Commonwealth Environmental Water Office's (CEWO) Long-Term Intervention Monitoring (LTIM) project, and their capacity to value add to existing programs. The latter site on the Niemur River was selected because it is downstream of Werai Forest, and provided potential contrasting nutrient dynamics to the other sites if flooding was to occur. All three sites are typical of lowland rivers in the mid-Murray; the channels are relatively incised with clay banks, and the riparian vegetation is dominated by *Eucalyptus camaldulensis* (River Red Gum) and *Acacia stenaphylla* (River Cooba) at the margins, with *E. largiflorens* (Black Box) growing on higher elevations. Understories are dominated by introduced grasses and native chenopods, with a range of submerged and emergent macrophytes growing in slackwater areas.

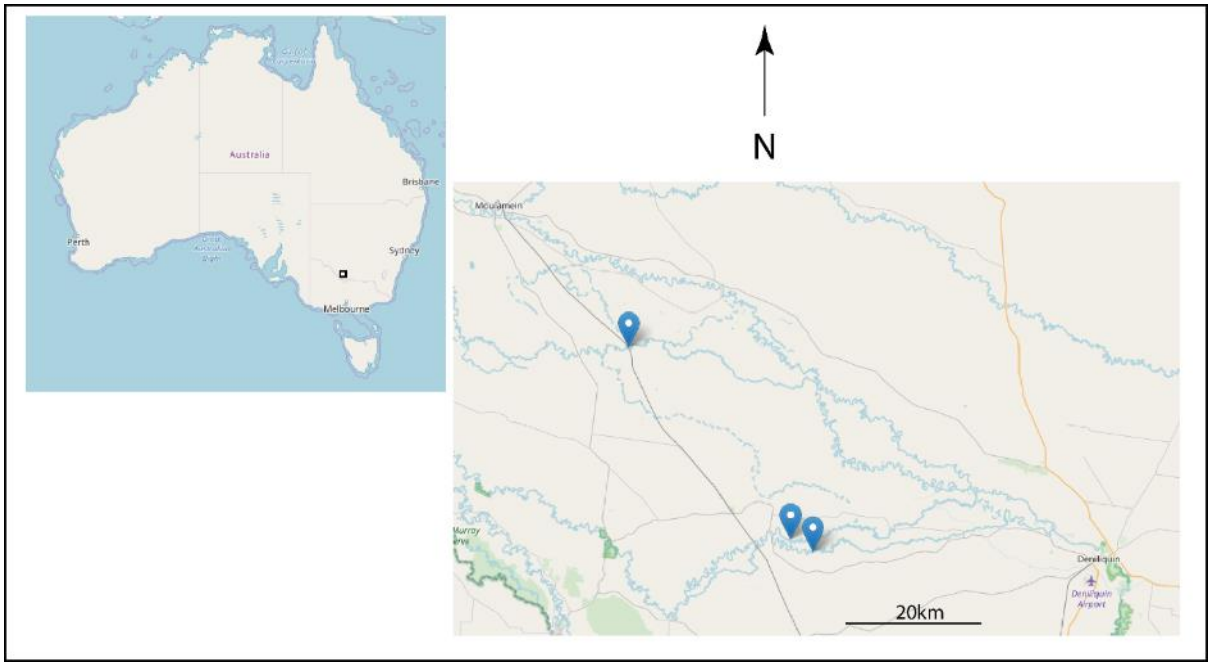

Figure 2. Location of study sites.

Water quality and nutrients

We measured water quality on ten occasions at our three sites, corresponding to the initial placement of field equipment and subsequent biofilm and production sampling. Water-quality samples were collected between 1200 and 1500 h to minimize temporal confounding of spatial patterns. We recorded temperature, conductivity, pH, dissolved O_2 (DO), and turbidity at each location with an YSI multiprobe (YSI incorporated, Yellow Springs, USA). Water samples were also collected from each location to measure Ammonium (NH_4), oxides of nitrogen (NO_x), total nitrogen (TN), total phosphorus (TP), filterable reactive phosphorus (FRP), dissolved organic C (DOC), and chlorophyll-*a* (Chl *a*) see McInerney *et al.* (2017) for full details on methods for analysis of water samples).

Samples were also collected for the characterisation of DOC within the water column and from light and dark bottles (see below) after six hours of exposure. Fluorescence excitation emission (FEEM) scans along with absorbance scans were performed using a quartz fluorimeter cuvette in a HORIBA Aqualog® (HORIBA Scientific) to determine the quality of DOC and its key components within river water and light dark bottles. FEEM scans along with simultaneous absorbance measurements were conducted on all samples, with excitation wavelengths in 2-nm steps between 250–450 nm, and emission wavelengths of 250–620 nm (Holland *et al.* 2018). The FEEMs were modelled using parallel factor analysis (PARAFAC) (PLS-toolbox in MATLAB: Eigenvectors Research Inc, WA, USA) to determine presence of key DOC components. Relative DOC aromaticity, molecular weight, and source were determined using a range of absorbance and fluorescence indices as described in Table 1.

Table 1. Dissolved organic carbon (DOC) quality indices used in this study.

Measurement	Equation	Indicator	Reference
Specific UV absorbance @ 254 nm ($SUVA_{254}$ (L mgC^{-1} m^{-1})	Abs_{254}/DOC	Relative aromaticity: Higher value indicates DOC with greater aromaticity	Weishaar *et al.* (2003)
absorbance ratio at 254 nm to 365 nm ($Abs_{254/365}$)	Abs254/365	Relative molecular weight: Lower the value the higher the molecular weight	Dahlén *et al.* (1996)
Biological Index (BIX)	Emission 380 nm/ 430 nm @ excitation 310 nm	Indicator of autotrophic productivity. Values >1 correspond to recently produced DOC.	Huguet *et al.* (2009)
Fluorescence Index 9FI)	Emission 470 nm/ 520 nm @ excitation 370 nm	Indicates DOC source: Allochthonous (FI ~ 1.2) vs. autochthonous (FI ~ 1.8) DOM	Cory and McKnight (2005)

Biofilms

Floating platforms supporting nine River red gum blocks (length 180 mm, width 105 mm) were constructed (Figure 3). Three platforms were deployed at each site on 24 October 2018. River Red Gum blocks were leached in boiling water prior to platform construction, and were arranged such that the surface of the blocks were ~ 180 mm below the surface of the water when suspended. Commencing three weeks after deployment, biofilms were sampled on nine occasions up until 3 January 2019. On each sampling occasion, a single River Red Gum block was randomly selected and removed from each of the platforms. The biofilm was removed from the upper most surface of the blocks using a stiff tooth brush and rinsed into a 200 mL PET jar using deionised water, before being frozen. In the laboratory, biofilms were sub-sampled for determination of loss on ignition (LOI), percentage carbon and nitrogen, chlorophyll-*a* and fatty acids.

For LOI, wet samples were weighed before drying at 500–550 °C for two hours and re-weighing. For determination of percentage of total carbon and nitrogen, biofilms were freeze dried and sent to an external laboratory and analysed with a LECO TruMac CNS Analyser (LECO Corporation, Saint Joseph, USA), following standard methods from Rayment and Lyons (2011). For chlorophyll-*a*, pigments were extracted in 90% filtered Ethanol (AR100) and placed in a water bath for five minutes at 75 °C. Chlorophyll-*a* was measured by spectrophotometric absorption and concentrations calculated as µg/L. For fatty acid determination, frozen samples were sent to an external laboratory where methods followed those used by Conlan et al. (2017). Briefly, for all samples, lipid was extracted from dry samples soaked in dichloromethane:methanol (CH2Cl2:CH3OH) and quantified gravimetrically on a four-figure balance. Lipid class analysis used an Iatroscan MK 6 s thin layer chromatography-flame ionisation detector. Fatty acids were then extracted and esterified into methyl esters using the acid catalysed methylation method (Christie 2003). Gas chromatography was then used to identify the fatty acid methyl esters relative to known external standards.

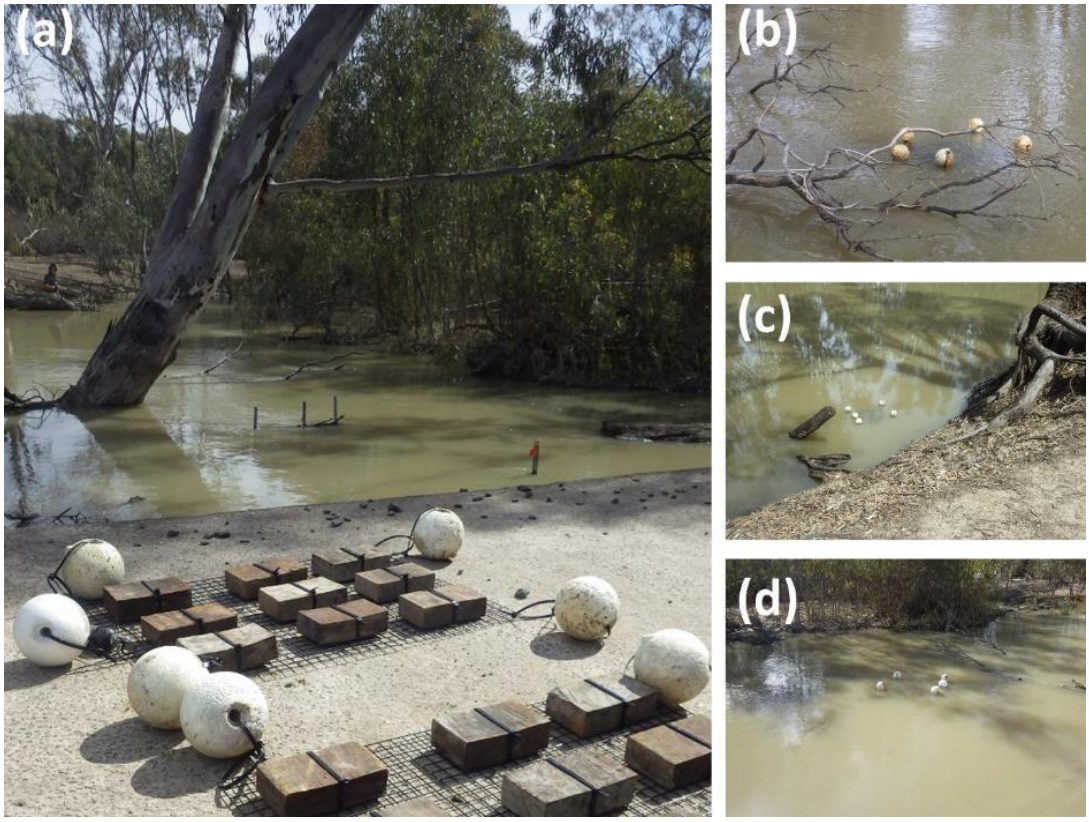

Figure 3. River Red Gum blocks attached to floating platforms (a) deployed to sample biofilms in the (b) Niemur River, (c) Yallakool Creek and (d) Wakool River.

Production

We used two approaches to estimate production; (1) single station open water methods were used to estimate whole-stream production and (2) light and dark bottles were used to estimate pelagic production. For single station whole-stream estimates, two D-Opto dissolved oxygen loggers (Zebra-Tech LTD, Nelson, New Zealand) were attached to biofilm platforms at each site, and set to log dissolved oxygen and temperature every 10 mins for the project duration (Figure 4 (c)). Odyssey® Waterproof Photosynthetic Active Radiation Loggers (Odyssey, Christchurch, New Zealand) measuring photosynthetically active radiation (PAR), were deployed on the bank adjacent to floating platforms, and logged light for the project duration. Gross Primary Production (GPP), Community Respiration (CR) and Net Production (NP) were calculated using the BAyesian Single-station Estimation method (Grace *et al.* 2015).

To estimate pelagic production at each site, we constructed floating rigs to support three replicate light and dark bottles (Figure 4). Dissolved oxygen and temperature within bottles were measured prior to incubation, which occurred over 6 daylight hours, and were then measured again on retrieval. Light and dark bottles were deployed on nine occasions throughout the study, corresponding to biofilm collection. Methods outlined in Grace and Imberger (2006) were used to calculate assays of GPP, CR and NP during the 6 hour incubation period.

Figure 4. Sampling production in the Edward–Wakool River system; (a) dissolved oxygen logger to measure open water production tethered to floating biofilm platform, (b) and (c) light and dark bottle array to measure pelagic metabolism, and (d) deployment of the light and dark bottle array in Yallakool Creek.

Statistical analyses

Scatter and boxplots were used to visualise changes in variables over time and, in most cases, LOWESS smoothed lines were incorporated into plots to visualise trends in the data. Pearson correlations were generated to explore possible correlations between biofilm characteristics (i.e. dry weight, percent loss, and biofilm Chl-a) and water quality variables. For those variables with significant correlations ($p < 0.05$) scatterplots were generated with linear trend lines indicating the direction of the correlations. PERMANOVA (Anderson et al. 2008) was used to test for differences in fatty acid profiles between sites and through time using a two factor design with site (3 levels) and time (9 levels) as fixed factors. Whole stream metabolism was estimated using the R package BASEmetab (Grace et al. 2015) and retrieving instantaneous rates. Two hundred thousand MCMC iterations were performed on the data with the first 50000 discarded as a burnin. For each sample day, the instantaneous rates occurring between the times light/dark bottles were set to when they were retrieved were summed to calculate the total production of oxygen within the river over the sample period. Production per hour was calculated by dividing the total production by the number of hours.

Results

Water quality and nutrients

Water quality was similar among our three rivers. Temperature followed a seasonal trend among all sites, with mean values remaining between 18–22 °C from October to December before increasing in summer to peak at 27.9 ±0.0 SE °C in the Niemur River in January (Figure 5). Conductivity was similar in the Niemur River and Yallakool Creek, with mean values increasing gradually from 42 ±0.0 µS cm^{-1} in October to 55 ±0.0 µS cm^{-1} in January as river levels slowly decreased. Mean conductivity in the Wakool was slightly higher, ranging from 54 ±0.0 SE µS cm^{-1} in October to 82 ±0.0 SE µS cm^{-1} in January. Mean values for pH ranged from 6.45 in the Wakool River on 18 December 2018 to 7.32 in the Niemur River on 3 January 2019. Mean turbidity was generally highest within the Wakool River, ranging from 80.1 ±3.2 NTU on 15 November 2018 to 36.4 ±1.0 NTU on 28 November (Figure 5). Turbidity followed the same pattern among all three rivers, with maximum mean values occurring between 13–15 November 2018 and minimum mean values occurring on 28 November 2018. Both measures of dissolved oxygen (% saturation and mg/L), decreased during the study period.

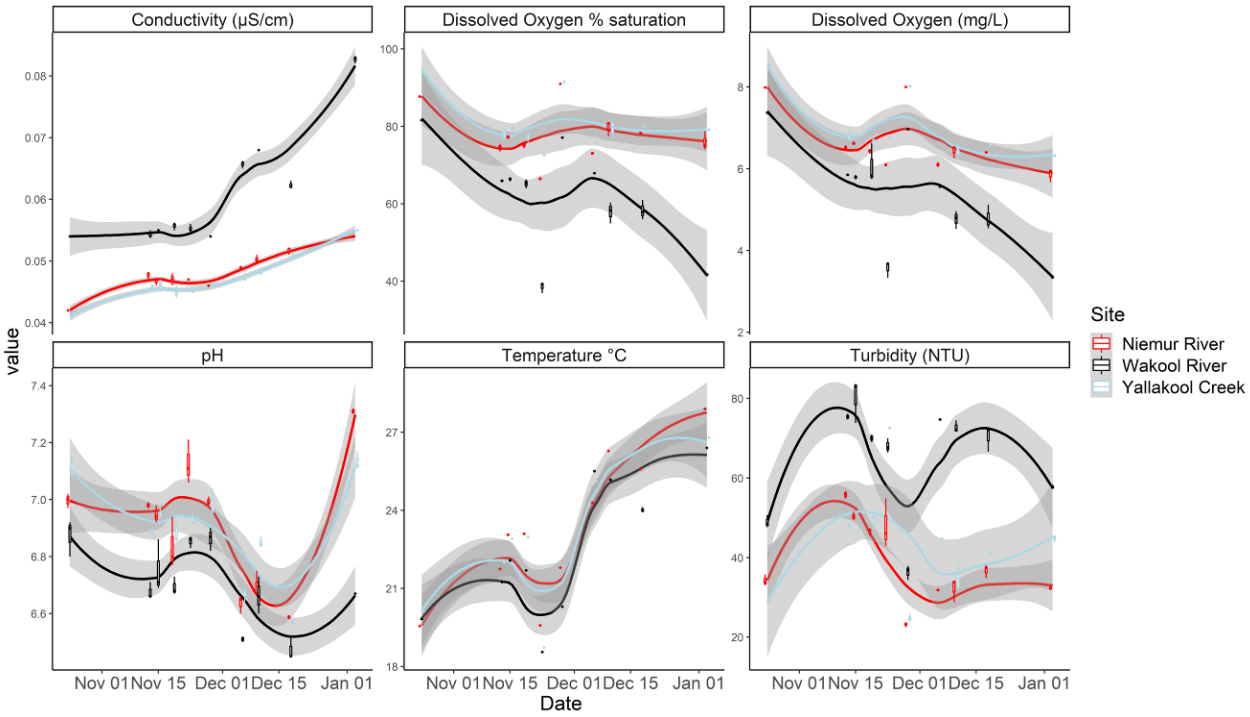

Figure 5. Mean water quality values collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

All measures of nitrogen (NH_4, NO_x and TN) and TP followed similar patterns across our three rivers; values were generally higher at the beginning of the study and remained relatively constant from 13 November–3 January (Figure 6). FRP did not vary appreciably throughout the study. Chl-*a* was also relatively constant, although concentration at all sites dipped below 10 µg L^{-1} between 15 November and 6 December (Figure 6).

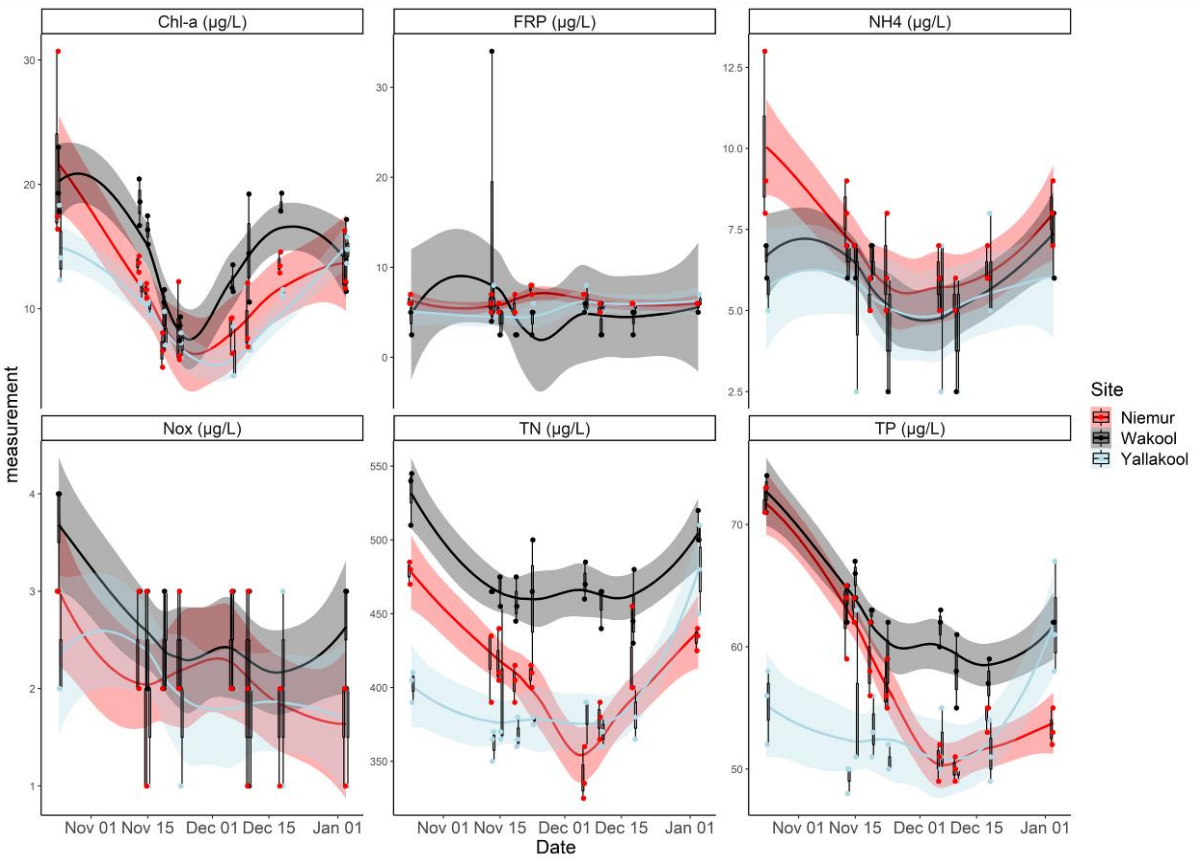

Figure 6. Mean nutrient and chlor-*a* values collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Mean DOC values remained relatively stable among our three rivers during the study period, with mean values ranging from 1.87 ±0.03 SE mg L^{-1} in the Wakool River on 15 November 2018 to 3.2 ±0.06 SE mg L^{-1} in the Niemur River on the 24 October 2018 and in Yallakool Creek, 3 January 2019 (Figure 7). Patterns were similar among rivers, with highest values recorded at the time of biofilm platform deployment and lowest values recorded during the early part of the study. DOC increased gradually towards the end of the study period, but values were generally low, reflective of stable river conditions.

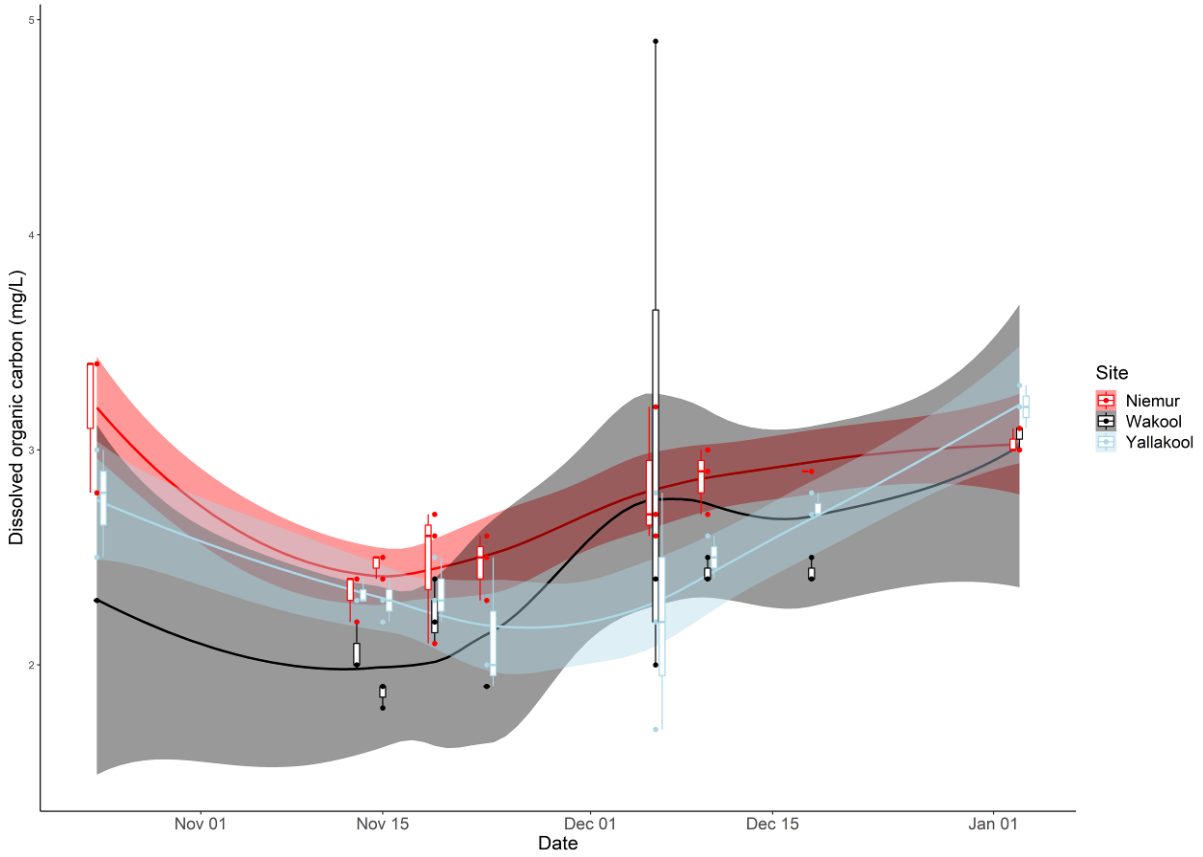

Figure 7. Mean DOC values collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Fluorescence excitation emission scans of dissolved organic matter in the Niemur River, Wakool River and Yallakool Creek water followed by PARAFAC analysis revealed the presence of three main DOC components (Figure 8). Component 1 was characterised by excitation of 240 and 290; emission 340 and is described in the literature as freshly produced autochthonous proteinous material (Brym *et al.* 2014; Murphy *et al.* 2011; Schittich *et al.* 2018; Stedmon *et al.* 2007; Yamashita *et al.* 2013; Yamashita *et al.* 2010) and will be hereby referred to as protein-like1. Component 2 was characterised by an excitation of 255 and 340; emission 444 and is described as terrestrial humic-like DOC (Chen *et al.* 2017; D'Andrilli *et al.* 2019; Derrien *et al.* 2018; Derrien *et al.* 2019; Jørgensen *et al.* 2011) and will be referred to as humic-like. Component 3 is also described as proteinous in nature, similar to tyrosine and will be referred to as protein-like 2 (Murphy *et al.* 2011; Retelletti Brogi *et al.* 2019).

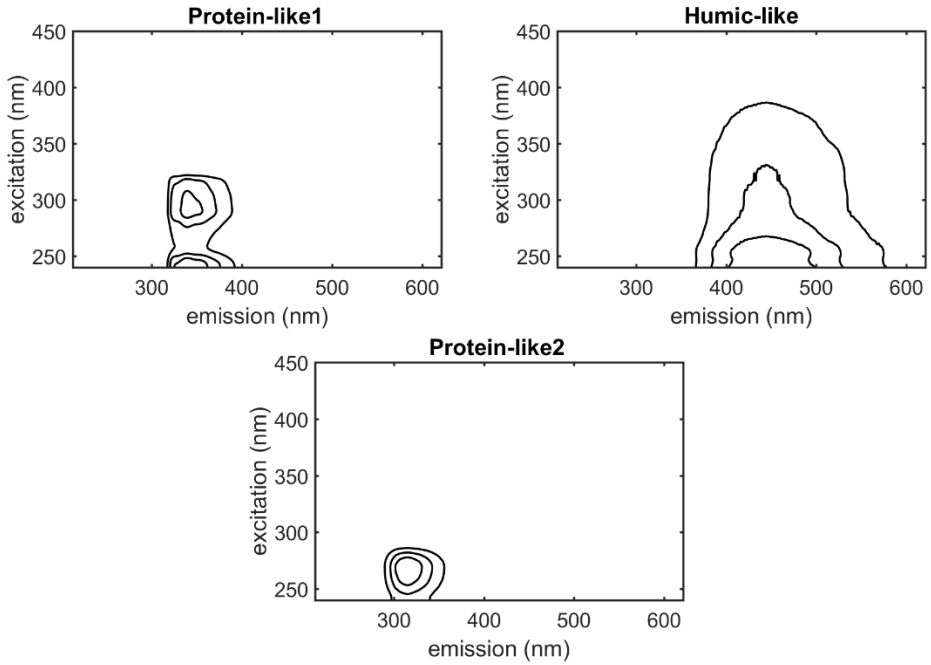

Figure 8. Dissolved organic carbon (DOC) components derived using fluorescence excitation emission scans followed by PARAFAC analysis.

Temporal variation in the relative abundance of DOC components was shown for all three rivers (Figure 9). The humic-like component dominated all sites, except in the Wakool (23 November 2018) and the Niemur (4 of December) where the protein-like 1 component became the most dominant (Figure 9). The Yallakool also experienced an increase in protein-like DOC during this period, however, the DOC more closely resembled that of the protein-like 2 component (Figure 9). The high abundance of the humic-like component found within these systems is indicative of the important role allochthonous DOC plays in these systems. The high abundance of protein-like components shown over the period between 23 November to the 4 December represents freshly produced autochthonous DOC and is likely to reflect an increase in microbial (algal and/or bacterial) abundance during this period.

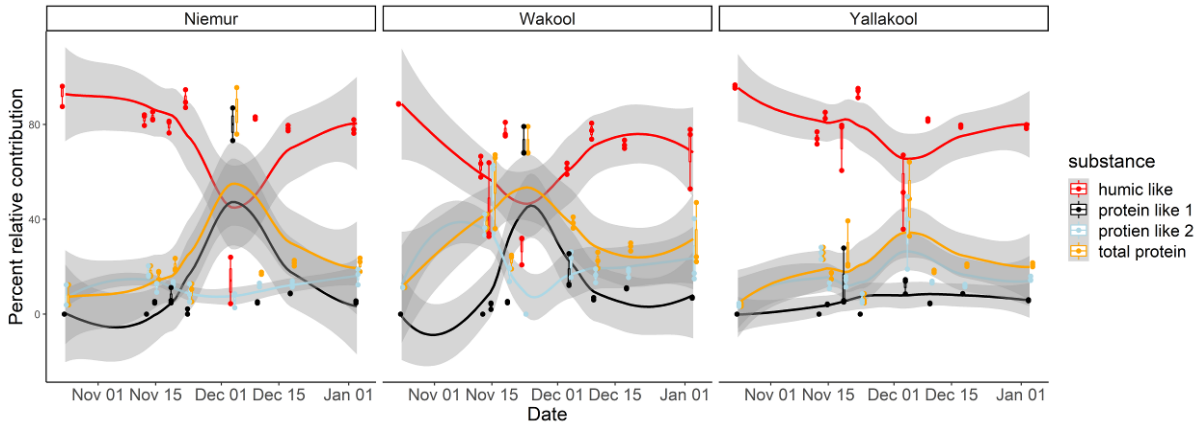

Figure 9. Percentage contribution of humic- and protein-like substances from river water DOC collected from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Dissolved organic carbon changes within light/dark bottles

Humic-like DOC was shown to decrease after six hours within both the light and dark bottles while both protein-like components increased (Figure 10). The increase in protein-like DOC and decrease in the humic-like component within the bottles represents microbial degradation of the humic-like component and the production of protein-like material. Protein-like components in this study are likely to reflect the release of extracellular polymeric substances (EPS) produced by algae and bacteria (Li *et al.* 2015; Villacorte *et al.* 2015; Xu *et al.* 2013).

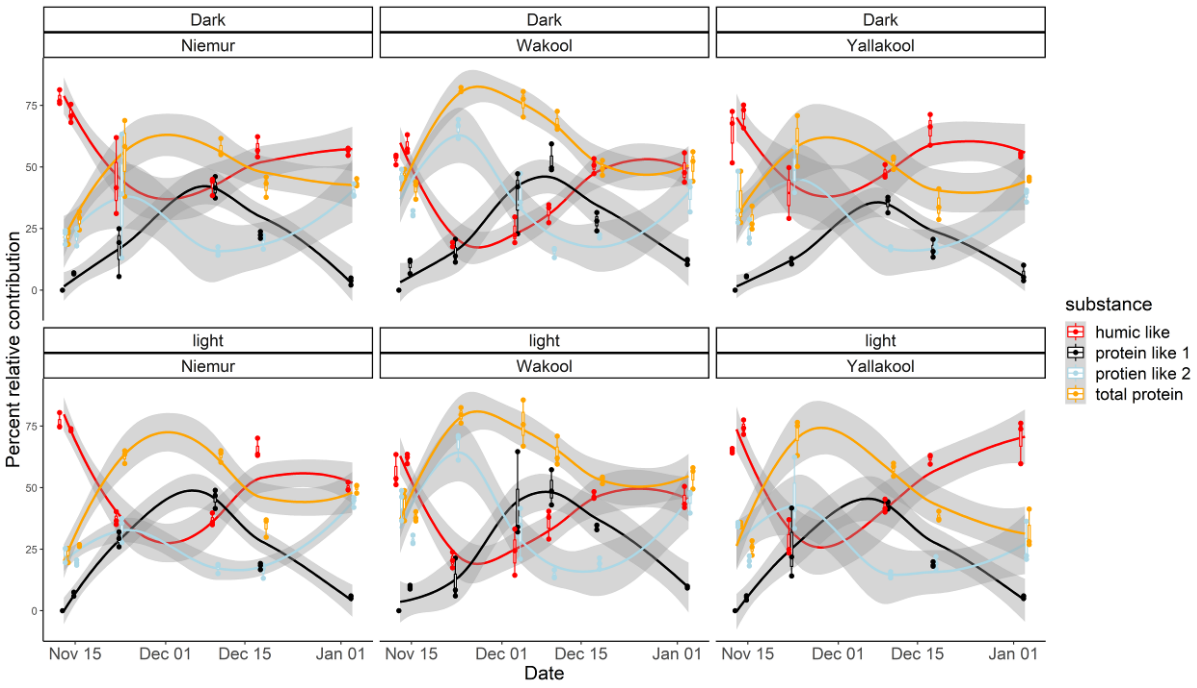

Figure 10. Percentage contribution of humic- and protein-like substances from DOC from light and dark bottles incubated in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

DOC aromaticity as indicated by $SUVA_{254}$ values was shown to decrease in all three rivers over the sampling period (Figure 11). $SUVA_{254}$ values in freshwater generally range from 1–6 L mgC^{-1} m^{-1}, with higher values reported from systems with a high terrestrial inputs and lower values indicating higher microbial inputs (Jaffé *et al.* 2008). All three river systems during the first two sampling periods reported $SUVA_{254}$ values greater than six, suggesting a strong presence of terrestrial DOC during this period. The decline in $SUVA_{254}$ over the sampling period within all three systems suggests the DOC is decreasing in aromaticity indicating an increase in microbial DOC. FI used to indicate the source of DOM, ranged from 1.43–1.69, suggesting that DOM pool consisted of a mixture of allochthonous and autochthonous DOM (Cory & McKnight 2005). Relative molecular weight was also shown to vary temporally with DOC in the Niemur and Yallkool rivers with the lowest molecular weight (i.e. highest $abs_{254/365}$) recorded in early December 2018. In contrast, the DOC within the Wakool River was of the lowest molecular weight during the first sampling trip (Figure 11). $Abs_{254/365}$ values generally range between 2.5–15 in freshwaters globally (Holland *et al.* 2018). Temporal variation in autochthonous production as indicated by BIX was shown in all three rivers. Increased autochthonous production (higher BIX) was shown to occur in late November in the Wakool River and early December in the

Niemur and Yallakool rivers (Figure). BIX values > 1.0 indicate the presence of freshly produced autochthonous DOM, whereas values < 0.6 indicate that the presence of autochthonous DOM is low (Dalmagro *et al.* 2019; Hansen *et al.* 2016).

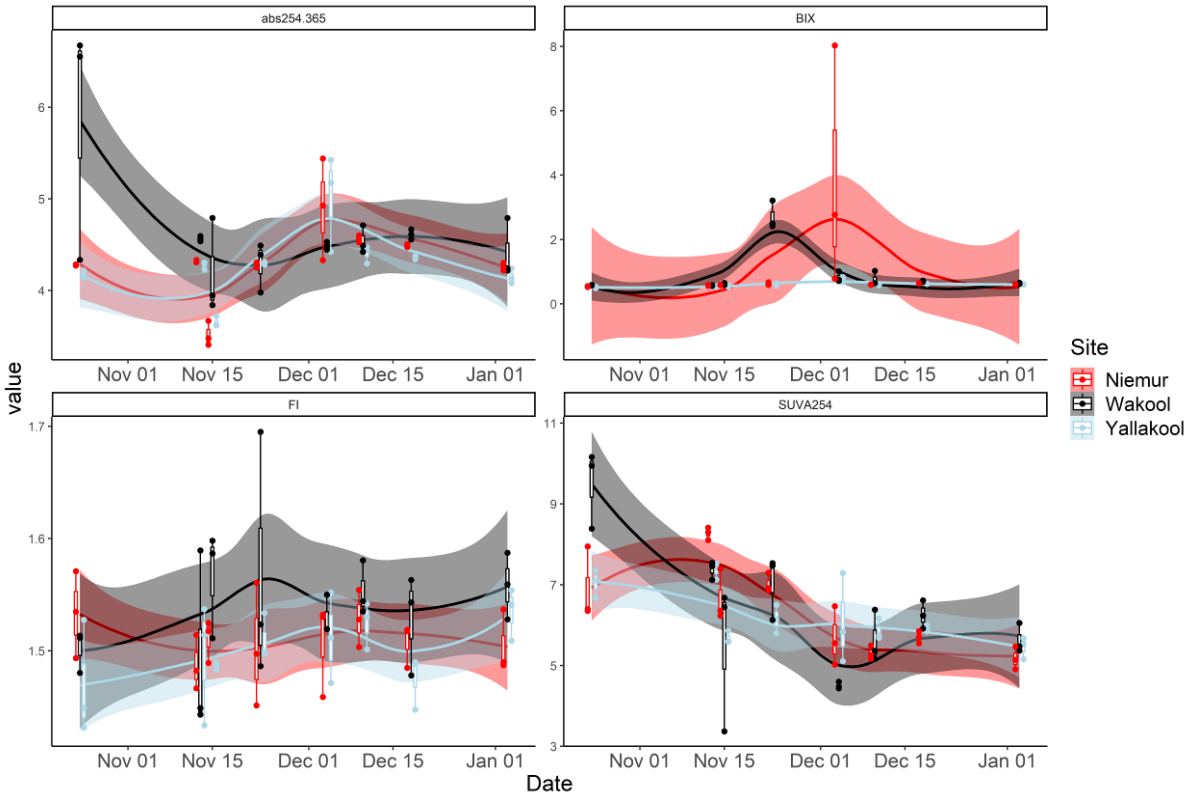

Figure 11. Dissolved organic matter (DOM) indices for the Niemur, Wakool and Yallakool rivers from the 24 October 2018 to 3 January 2019; a) absorbance ratio at 254 nm to 365 nm (abs254/365) as an indicator of molecular weight; b) Biological Index (BIX) as an indicator of autochthonous production; c) Fluorescence Index (FI) as an indicator of source of DOM; and specific UV absorbance coefficient at 254nm ($SUVA_{254}$) as an indicator of aromaticity (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Dissolved organic carbon components relationship with DOC indices, chlorophyll-a and water quality variables

An increase in the abundance of protein-like1 DOC and a decrease in humic-like DOC was related to an increase in autochthonous production as indicated by the BIX index in all three rivers (Table 2). This is no surprise as protein-like1 DOC is commonly associated with increased microbial production in rivers (Brym *et al.* 2014; Murphy *et al.* 2011; Schittich *et al.* 2018; Stedmon *et al.* 2007; Yamashita *et al.* 2013; Yamashita *et al.* 2010). Surprisingly no relationship was shown between protein-like1 DOC and chlorophyll-*a* in the Niemur and Yallakool rivers with a negative relationship shown in the Wakool (Table 2). The lack of a negative relationship between chlorophyll and the protein-like component indicates the presence of a more heterotrophic microbial community within the water column. The lack of a significant difference between the light and dark bottles and the production of protein-like DOC within all three rivers also suggests that protein-like DOC in the water column is linked to bacterial production rather than having an algal origin.

Table 2. Pearson correlation coefficients relating DOC components with BIX and chlorophyll a. *P* values ≤0.05 are highlighted in bold and suggest a significant relationship.

	River	Protein-like1		Humic-like	
		correlation coefficient	P Value	correlation coefficient	P Value
BIX	Niemur	0.903	**<0.001**	-0.899	**<0.001**
	Wakool	0.98	**<0.001**	-0.685	**<0.001**
	Yallakool	0.825	**<0.001**	-0.732	**<0.001**
Chl-*a*	Yallakool	-0.350	0.093	0.357	0.087
	Wakool	-0.724	**<0.001**	0.499	**0.015**
	Niemur	-0.326	0.139	0.362	0.098

Biofilms

Biofilm dry weight, LOI and percent loss

The lowest mean total dry weight of biofilms (161.1 ± 15.05 SE gm^2) was recorded in the Niemur River on the 19 November 2018 and highest mean dry weight (780. 8 ± 193.24 SE gm^2) was recorded in Yallakool Creek on 3 January 2019 (Figure 12 B). Total dry weight reflects all material residing on blocks and includes organic (living and dead plant and animal material) and inorganic material (sediment, sand, Figure 13) and not surprisingly given stable river levels, this generally increased with time across all three rivers. Loss on ignition (LOI) represents the organic only component and is more reflective of living biofilms. Lowest mean LOI (6.87 ± 1.47 SE gm^2) was recorded from blocks placed in Yallakool Creek three weeks after deployment (first sample collection) and highest mean LOI (55.17 ± 13.79 SE gm^2) was recorded from blocks collected from the Niemur River on 3 January, 11 weeks after deployment (Figure 12 A). Percent loss reflects the proportion of the total biofilm that is made up of organic material, and biofilms in the Niemur River were consistently higher in organic matter than in the other two streams (Figure 12 C).

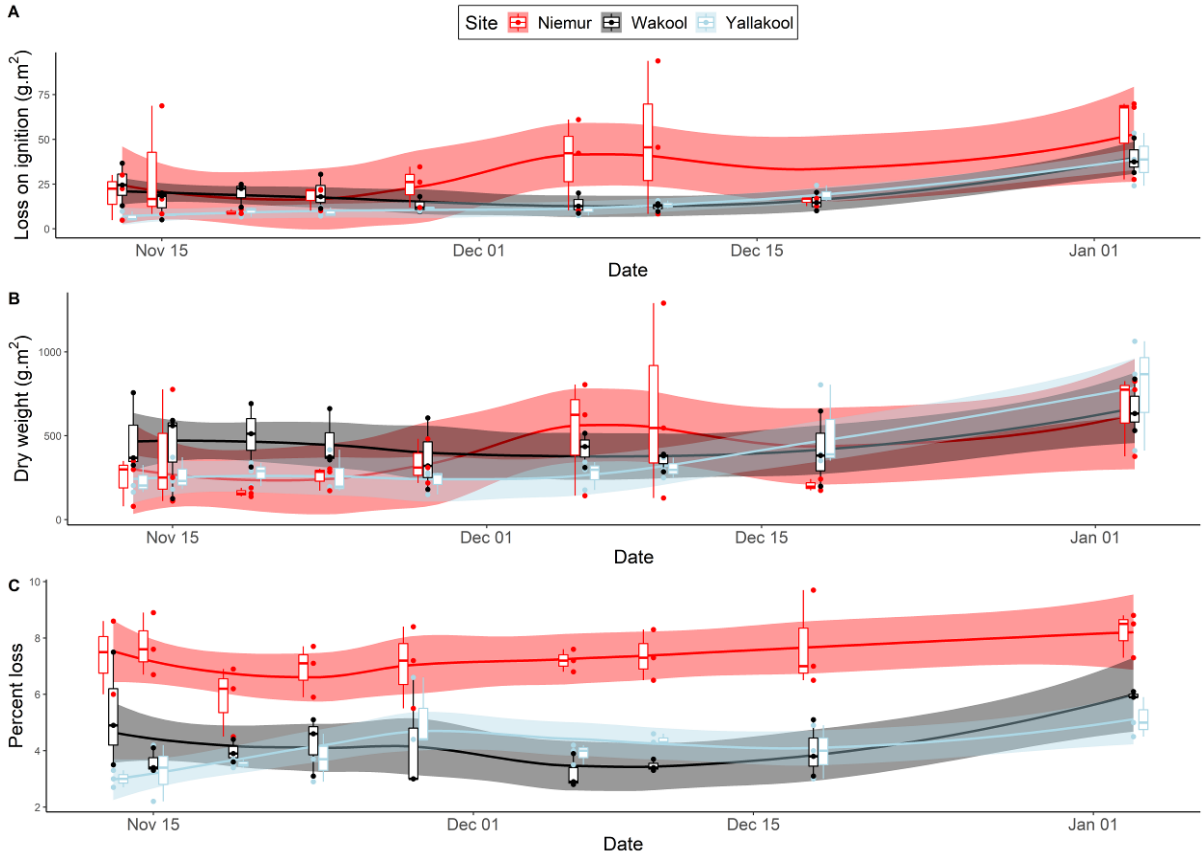

Figure 12. Loss on ignition (A), total dry weight (B) and percent weight loss after ignition (C) of biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

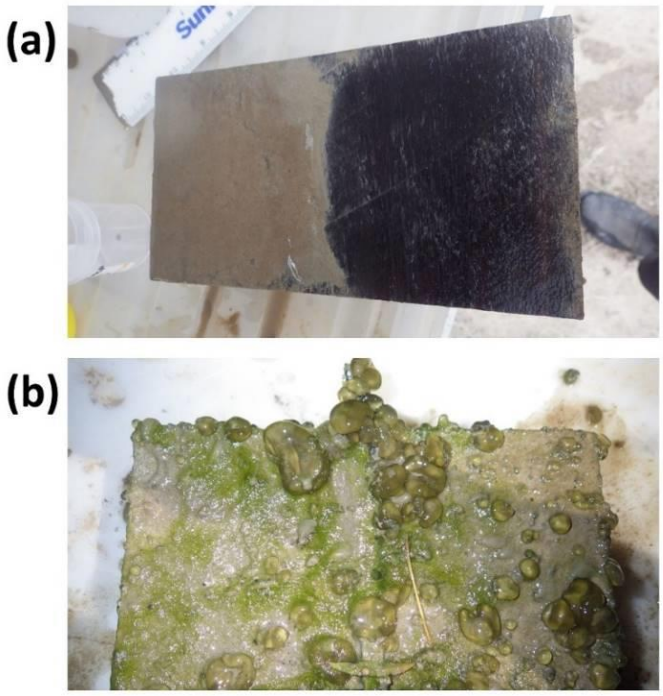

Figure 13. River Red Gum blocks from the Wakool River (a) 3 weeks after deployment (half the block has been scrubbed of biofilm) and (b) 11 weeks after deployment.

Biofilm Chlorophyll-a

Mean chlorophyll-*a* concentration of biofilms remained low (mean values < 6 µg L^{-1}) for the first five weeks following deployment of the blocks (up to 28 November) before increasing to 20 µg L^{-1} at Wakool in January 2019 (Figure 14). Chlorophyll-*a* concentration within biofilms is reflective of the amount of photosynthetically active material, and can represent a proxy for both algal and cyanobacterial components of biofilms.

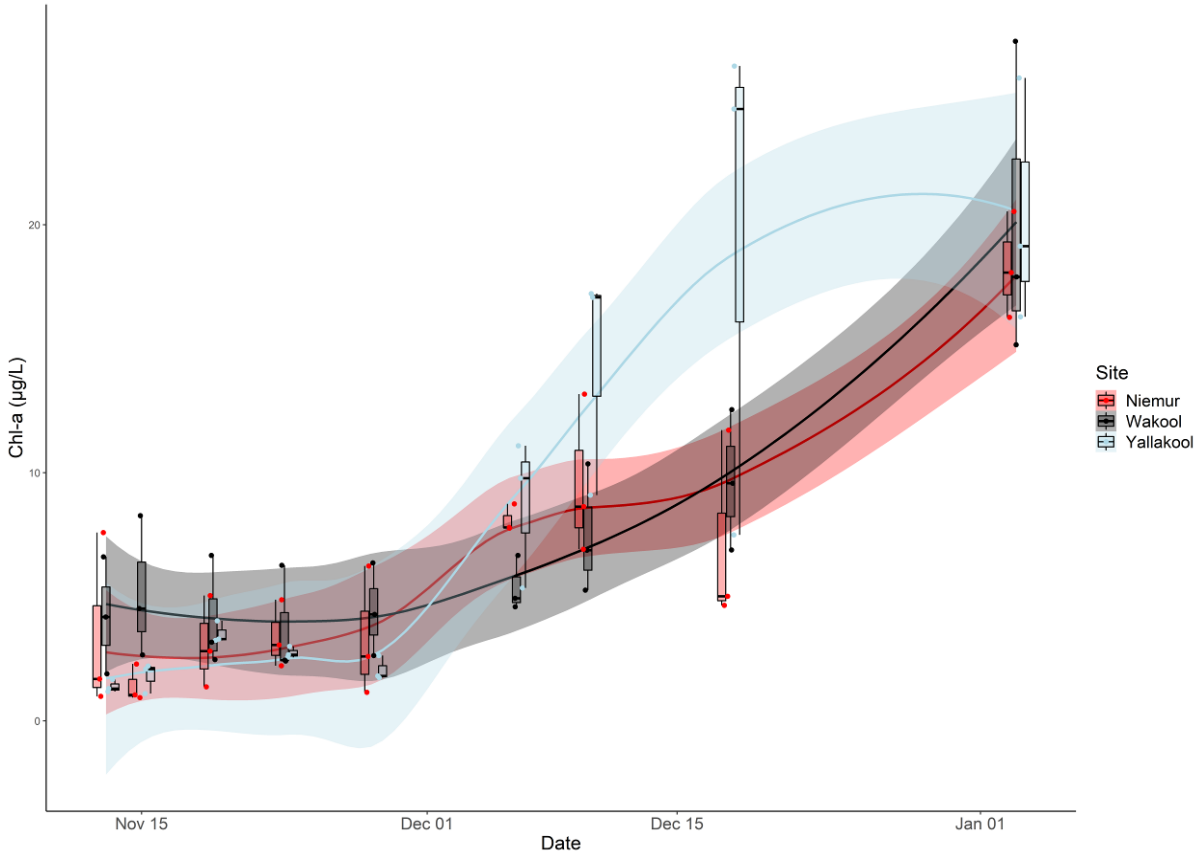

Figure 14. Chlor-*a* concentration of biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Figure 15. Collection of biofilm platforms in January 2019: (a) platform covered in cyanobacteria (likely *Nostoc* spp.), (b) recovery of dissolved oxygen logger from platform at the Wakool River site, and (c) close up of cyanobacteria (likely *Nostoc* spp.) growing on platforms after 11 weeks submergence in the Wakool River.

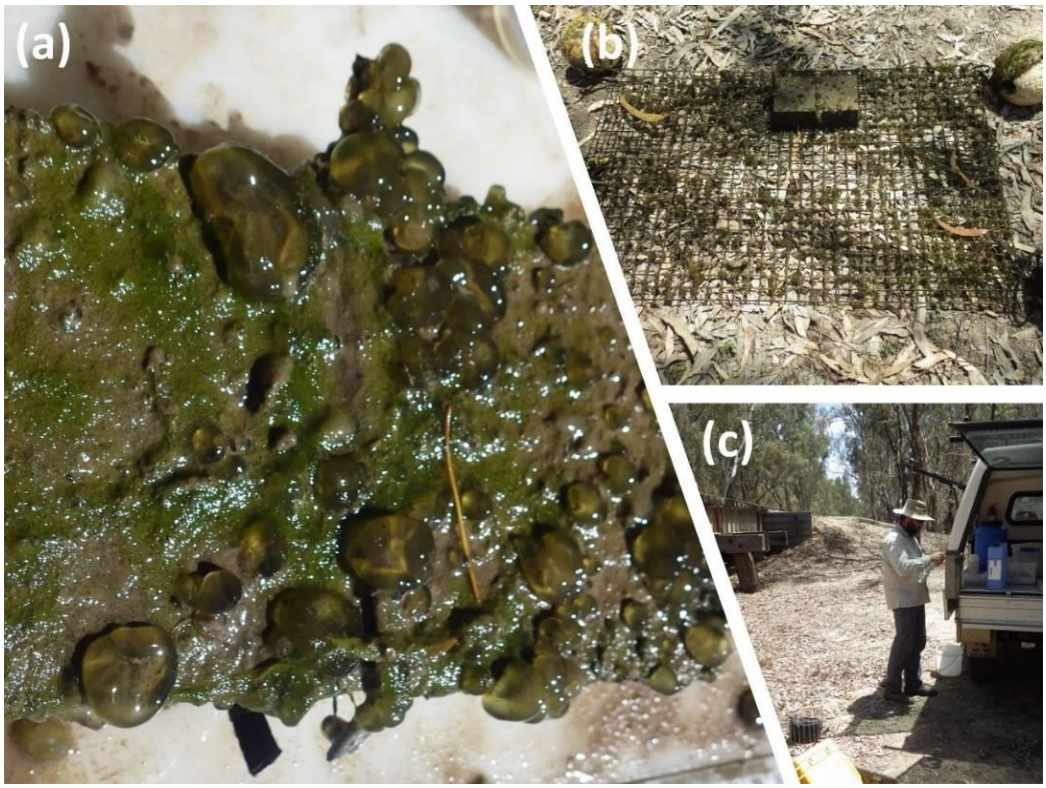

Figure 16. Sampling at the Niemur River site in January 2019; (a) 11 week old biofilm growing on River Red Gum block, (b) floating platform after retrieval with last block to be harvested, and (c) processing water quality and nutrient samples.

Biofilm C:N

The ratio of carbon to nitrogen (C:N) within biofilms can be used as a course indicator of food quality. Mean percentage total carbon was lowest in Yallakool Creek biofilms on 13 November 2018 (0.73 ± 0.04 SE) and highest in the Niemur River on 18 December 2018 (2.64 ± 0.53 SE, Figure 17 (A)). Mean percentage total nitrogen was lowest in Yallakool Creek biofilms on 23 November 2018 (0.03 ± 0.00 SE) and highest in the Niemur River on 3 January 2019 (0.25 ± 0.02 SE, Figure 17 (B)). Mean C:N ratio among biofilms at all sites was variable during the early part of the study, but in Yallakool Creek, ranged from 6.9 ± 0.75 SE on 28 November to 35.8 ± 0.00 SE on 23 November (Figure 17 C). During the latter part of the study (weeks 6–11 after deployment of blocks), C:N of biofilms within all rivers remained relatively low (~10–20), and did not vary substantially.

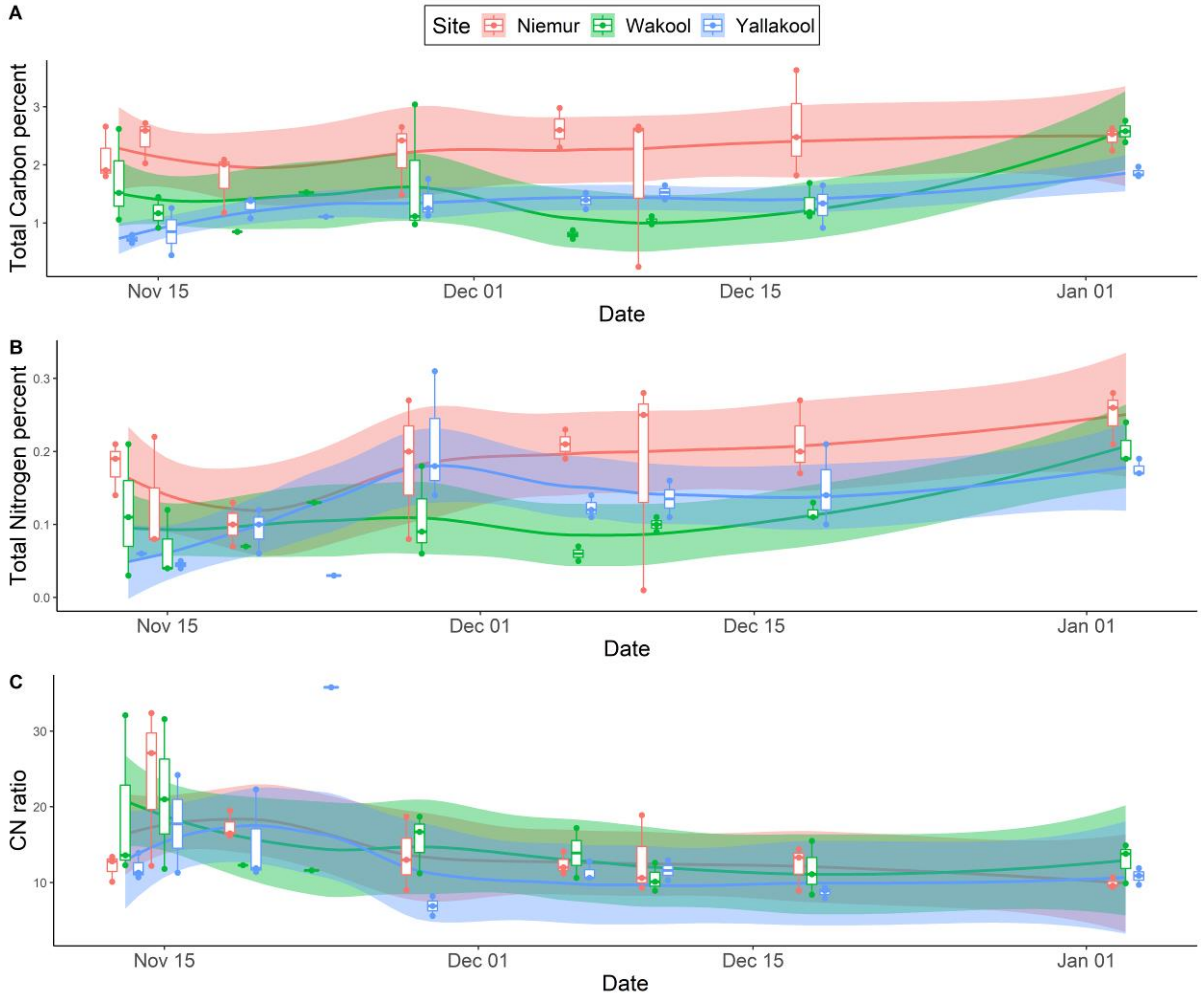

Figure 17. Total percent carbon (A), total percent nitrogen (B), and C:N (C) of biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

Biofilm fatty acid composition

A total of 52 individual fatty acids were detected from biofilms during the study. Biofilm fatty acid profiles were similar between rivers (*Pseudo–F* = 0.95, *P*=0.52) but varied significantly with time (*Pseudo–F* = 1.60, *P* < 0.001, PERMANOVA). Pairwise comparisons indicated that fatty acid profiles of

3 week old biofilms differed significantly from the profiles of 9 and 11 week old biofilms (t = 1.50, *P* = 0.019 and t = 2.01, *P* = 0.003, respectively, Figure 18).

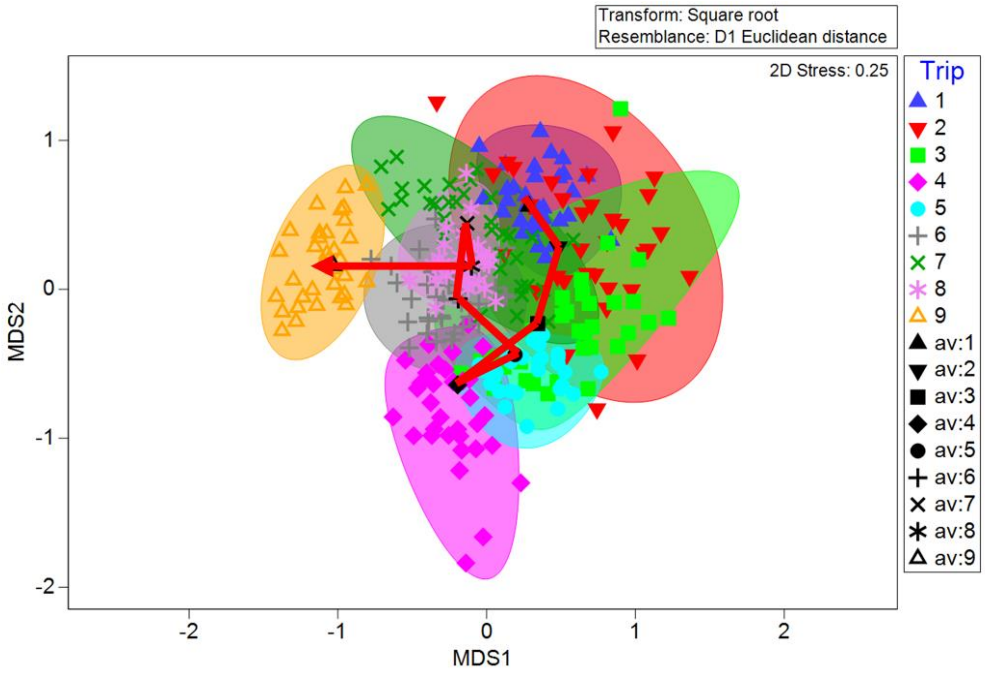

Figure 18 Bootstrapped means (black symbols) and 95% estimate regions derived from fatty acid profiles as percentage of total lipids for biofilms from Niemur River, Yallakool Creek and Wakool River from Trip 1 (3 week old biofilms) to Trip 9 (11 week old biofilms), red arrow indicating trajectory of change, MDS, multidimensional scaling.

Essential PUFAs, eicosapentaenoic EPA (20:5ω3) and docosahexaenoic DHA (22:6ω3) were not detected from biofilms in any of our three rivers. Essential PUFAs alpha-linolenic (ALA; 18:3ω3), linoleic acid (LA; 18:2ω6) and arachidonic acid ARA (20:4ω6) were detected and displayed similar patterns during the study (Figure 19). Mean values for ALA across all rivers generally ranged between 100–200 mg m^2, while LA was slightly lower, with mean values generally ranging between 50–100 mg m^2. ARA was detected in lower quantities, never exceeding 10 mg m^2. As a proportion of total lipids, ALA and LA remained relatively stable throughout the study, with mean percentages ranging 8-20% for the former 4-10% for the latter.

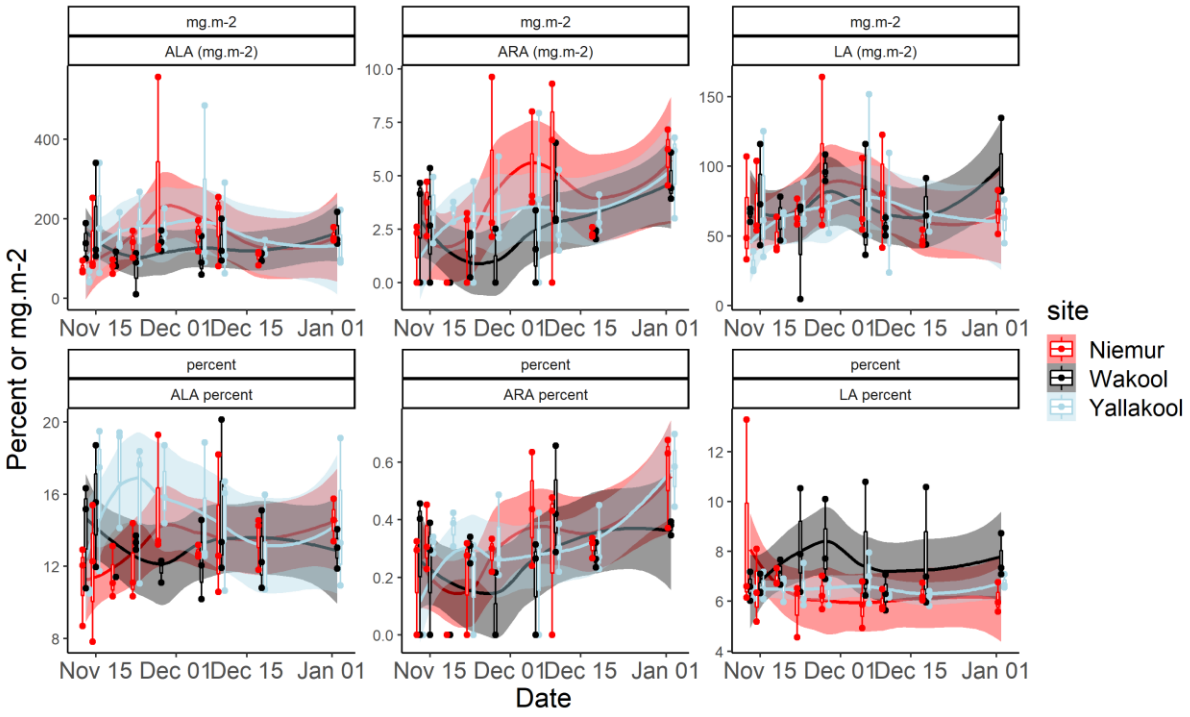

Figure 19 Total mass (mg m^2, top panel) and proportion of total lipids (bottom panel) of essential PUFAs alpha-linolenic (ALA; 18:3ω3), arachidonic acid ARA (20:4ω6) and linoleic acid (LA; 18:2ω6) within biofilms grown in the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (shaded area around lines represent 95% confidence intervals, boxplots representing upper and lower quartiles, whiskers 95th percentiles).

As a proportion of total lipids, ARA within biofilms among all sites was low in the first samples taken 3 weeks after deployment of the platforms (~0.2%) and was highest (~0.3-5 %) on blocks retrieved after 11 weeks deployment. Both total mass and proportion of ALA, ARA, and LA displayed a similar pattern across all three rivers, with values beginning relatively low and increasing until about 5 weeks after deployment, before dropping and then increasing again up to completion of the study at 11 weeks (Figure 19).

Correlations

Significant positive correlations were observed between total dry weight of biofilms and Chlorophyll-*a* concentration, conductivity the number of growth days, temperature and TN (Figure 20, Table 3). There was a significant negative correlation between dissolved oxygen total dry weight of biofilms.

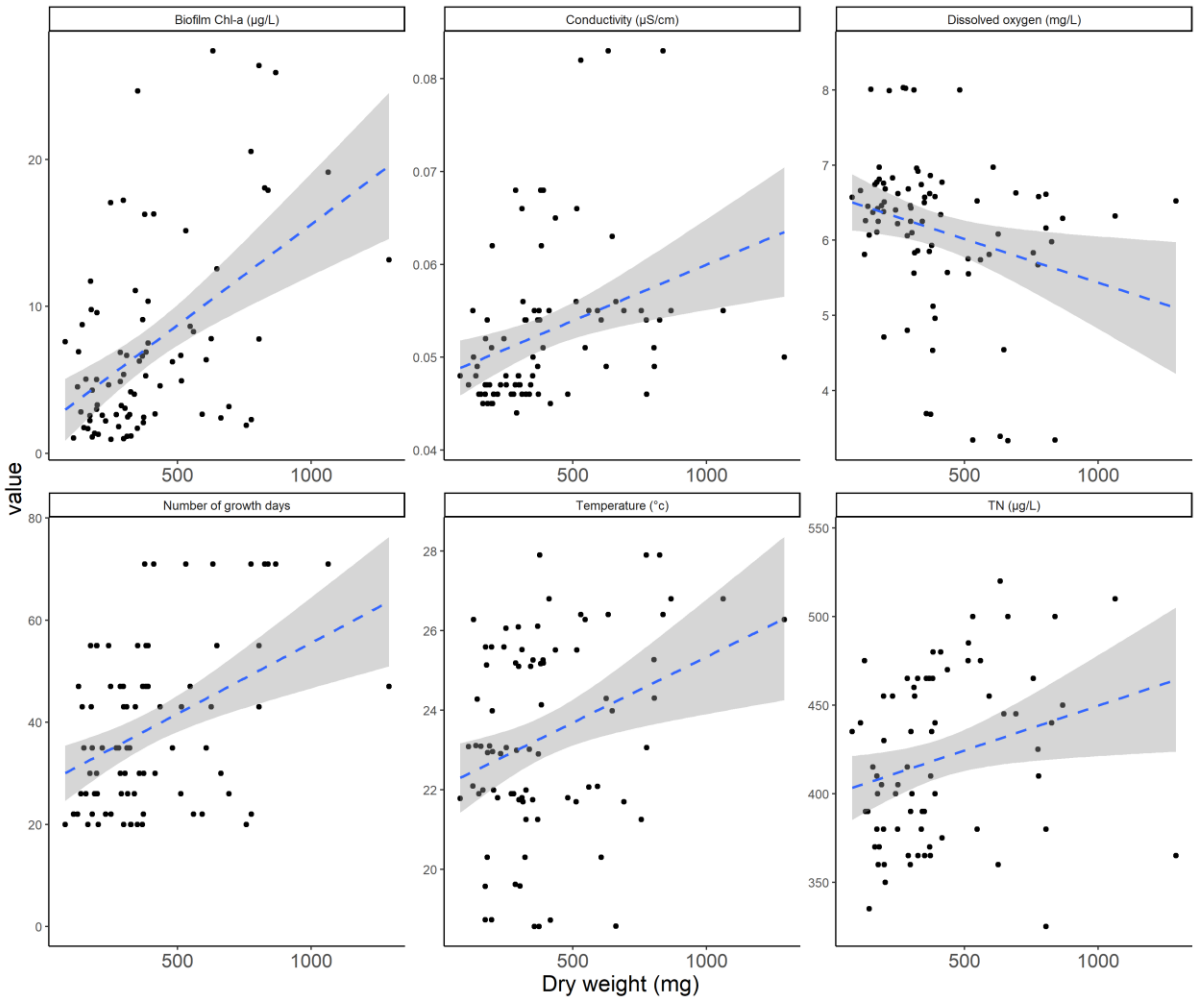

Figure 20 Significant (p<0.05) Pearson coefficient correlations for biofilm dry weight and abiotic and biotic variables

Percent weight loss following ignition (% of total biofilm made up of organic matter) showed significant positive correlations with discharge, water height, FRP, pH and NH_4, while correlating negatively with flow velocity at biofilm platforms and turbidity (Figure 21, Table 3).

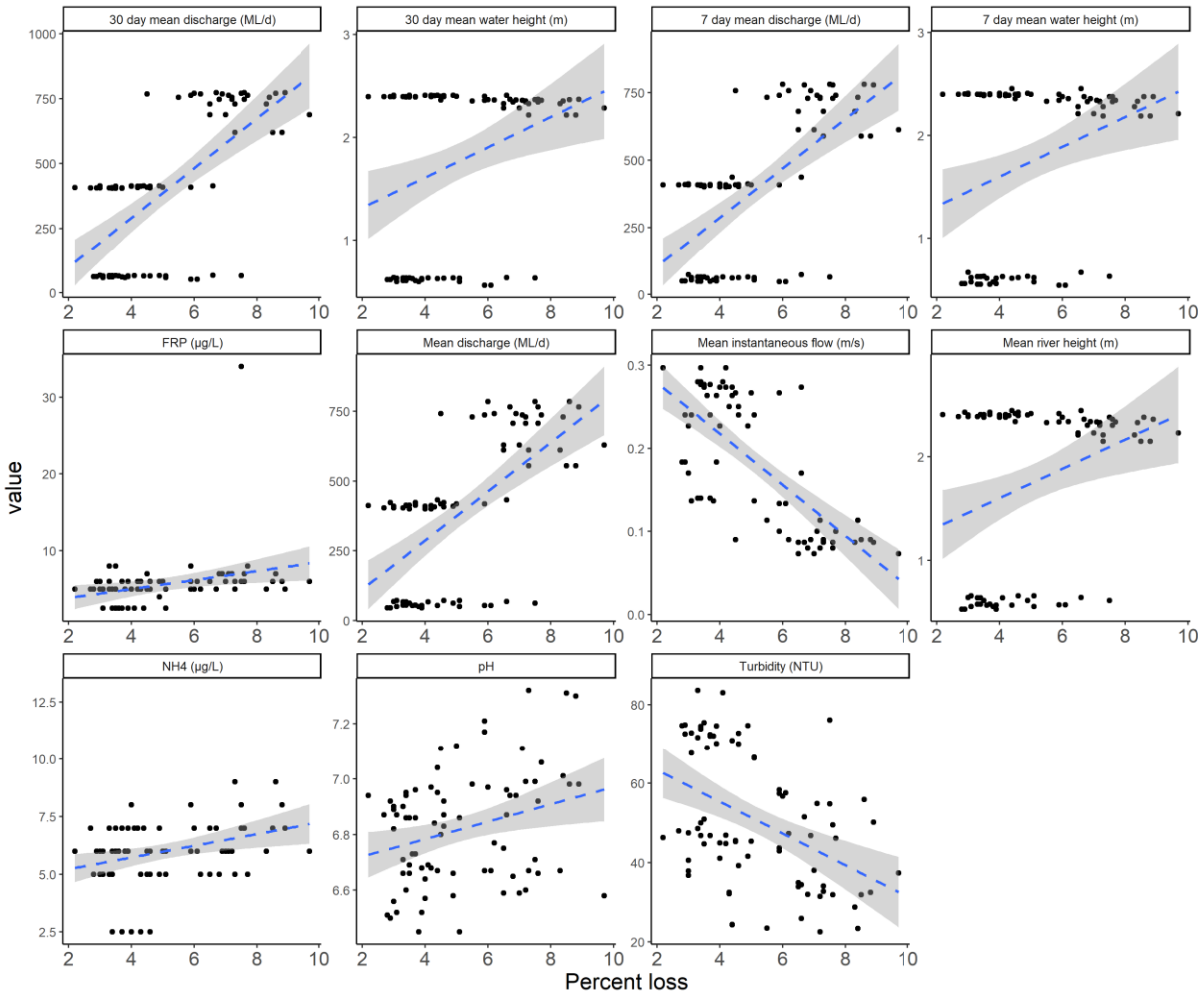

Figure 21 Significant (p<0.05) Pearson coefficient correlations for biofilm percent loss and abiotic and biotic variables

Table 3 Significant (<0.05) Pearson correlations between biofilm Chl-a concentration, total dry weight and percent loss and abiotic and biotic variables.

Variables	Biofilm Chl-a (µg/L)		Dry weight (g/m^2)		Percent loss	
	correlation coefficient	*P*-value	correlation coefficient	*P*-value	correlation coefficient	*P*-value
Mean instant flow (m/s)					-0.71	<0.001
Biofilm Chl-a (µg/L)			0.50	<0.001		
Conductivity (µS/cm)	0.42	<0.001	0.34	0.002		
Mean discharge (ML/d)					0.62	<0.001
7 day mean discharge (ML/d)					0.63	<0.001
30 day mean discharge (ML/d)					0.65	<0.001
Dissolved oxygen (mg/L)	-0.31	0.005	-0.27	0.017		
FRP (µg/L)					0.30	0.011
Mean river height (m)					0.32	0.004
NH4 (µg/L)					0.33	0.005
Number of growth days	0.81	<0.001	0.41	<0.001		
pH					0.29	0.009
Specific UV absorbance @ 254nm(L mg C/m)	-0.38	0.002				
Temperature (°c)	0.68	<0.001	0.32	0.004		
TN (µg/L)			0.26	0.026		
Turbidity (NTU)					-0.44	<0.001
7 day mean water height (m)					0.32	0.003
30 day mean water height (m)					0.33	0.003

Production

Whole stream GPP calculated from single station open water estimates was generally low across all three sites, ranging from 0.01 mg O_2 L^{-1} h^{-1} in the Niemur River on 23 November 2018 to 0.47 mg O_2 L^{-1} h^{-1} on 10 December 2018 (Figure 22 A). Community respiration ranged from 0.13 mg O_2 L^{-1} h^{-1} in Yallakool Creek on 23 November to 5.67 mg O_2 L^{-1} h^{-1} in Yallakool Creek on 13 November 2018 (Figure 22 C). Whole stream NPP was therefore largely negative, reflective of heterotrophic ecosystems, ranging from -0.09 mg $O_2L^{-1}h^{-1}$ in Yallakool Creek on 13 November to -5.66 mg O_2 L^{-1} h^{-1} in Yallakool Creek on 13 November 2018 (Figure 22 B). In contrast, mean GPP estimates calculated from the pelagic photic zone were generally higher, ranging from 0.13 ±0.02 mg O_2 L^{-1} h^{-1} in the Niemur River on 13 November 2018 to 0.42 ±0.01 mg O_2 L^{-1} h^{-1} in the Wakool River on 3 January 2019 (Figure 22 A). Mean CR in the photic zone was lower than whole stream estimates, ranging between 0 and -0.06 ±0.02 mg O_2 L^{-1} h^{-1} (Figure 22 C). Subsequently, mean NPP estimates from the pelagic photic zone were higher than whole stream estimates, ranging from 0.09 ±0.01 mg O_2 L^{-1} h^{-1} to 0.42 ±0.01 mg O_2 L^{-1} h^{-1}.

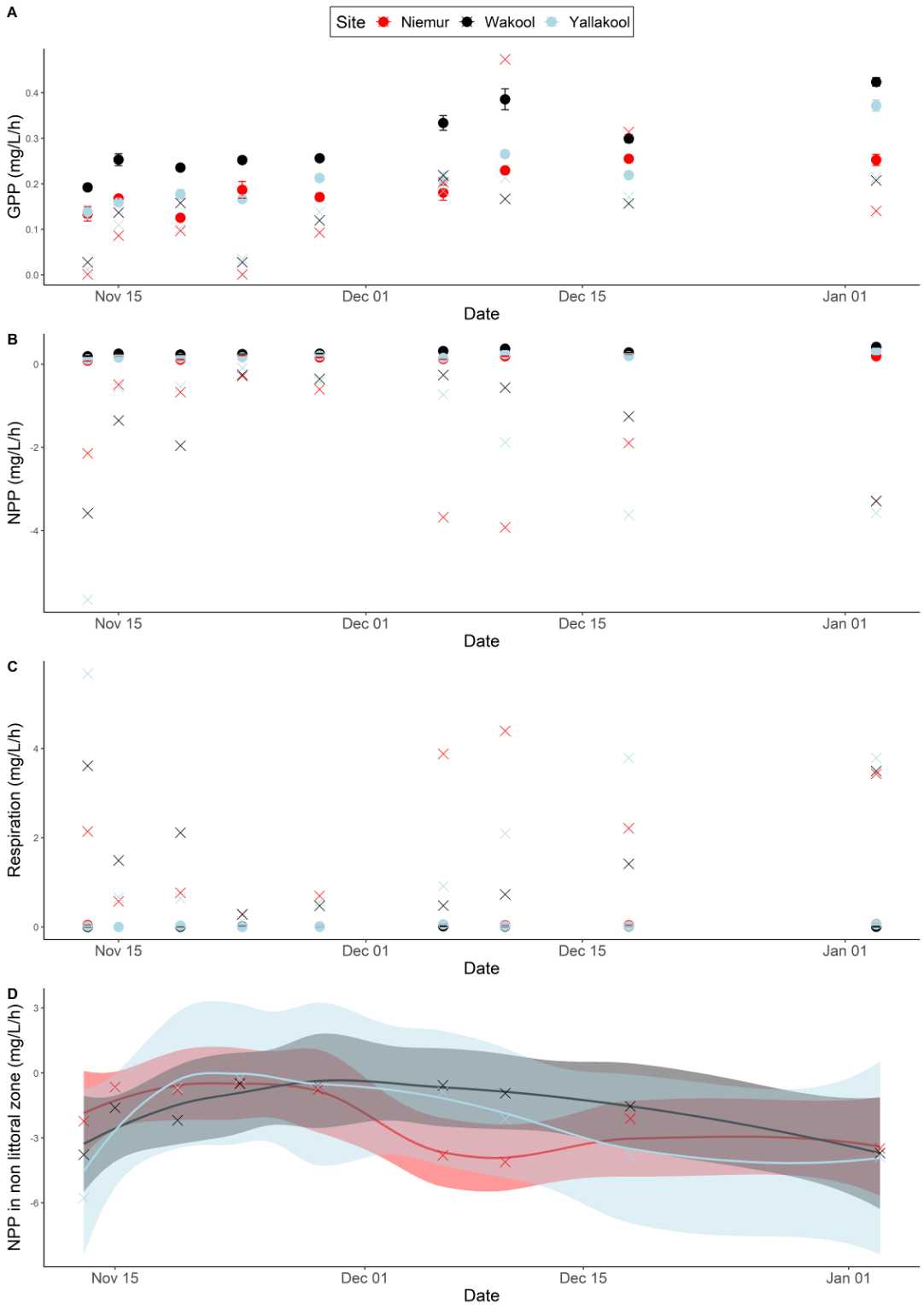

Figure 22. Stream metabolism dynamics GPP (A), NPP (B), CR (C), and littoral zone NPP (D) estimated as O_2 mg/L per hour calculated from open water estimates (crosses) and light and dark bottles (circles) from the Niemur River, Yallakool Creek and Wakool River from 24 October 2018–3 January 2019 (error bars ±1 SE, shaded area around lines represent 95% confidence intervals).

Both light/dark bottle assays and whole stream estimates showed that GPP in all three rivers was low (<0.5 mg $L^{-1}h^{-1}$) and the similarity between whole stream and light/dark bottle values suggest that in our study systems, most GPP is derived from pelagic production by phytoplankton. If surface

biofilms were contributing significantly to overall GPP, we would expect higher whole stream values compared to our pelagic assay. CR estimates from the light dark/bottle assays were also low, however in contrast, whole stream estimates of CR were higher (Figure 22 C), indicating that in all three streams metabolism is dominated by respiration occurring on stream surfaces and within the benthos. Despite evidence for elevated heterotrophic microbial community activity in the water column (as discussed previously in the DOC section), pelagic GPP (measured from light/dark bottle assay) exceeded pelagic CR values, resulting in positive NPP in the photic zone of the water column (Figure 22 B). Unlike photosynthetic production which requires sunlight, respiration can occur in both light and dark environments, and in turbid lowland systems such as the Edward Wakool, most of the pelagic production is likely only occurring in the top 30-50cm of the water column. Microbial decomposition of organic matter and oxygen consumption by heterotrophs within our streams is greater than the production of oxygen from autotrophic activity, ultimately resulting in negative NPP values for the whole stream. Subsequently the NPP in the photic zone (Whole stream NPP minus pelagic NPP) was predominantly negative (Figure 22 D), reflective of respiration occurring on surfaces and in sediments. Photic zone NPP decreased slightly during the study, perhaps as a product increased microbial activity with increased water temperature with season.

Key management implications

Initially this project set out to measure biofilm responses to managed flows, but due to unforeseen circumstances, this was not possible. We did however compile an enormous amount of useful information that extends our knowledge of biofilm succession patterns in response to a steady-state ecosystem condition in lowland rivers.

1. **Importance of allochthonous DOC in the Edward Wakool**

   Fluorescence excitation emission scans of dissolved organic matter in the Niemur River, Wakool River and Yallakool Creek water followed by PARAFAC analysis revealed the presence of three main DOC components. Our work showed the presence of a more heterotrophic microbial community within the water column. The lack of a significant difference between the light and dark bottles and the production of protein-like DOC within all three rivers also suggests that protein-like DOC in the water column is linked to bacterial production in place of algal activity. The high abundance of the humic-like component found within these systems is indicative of the important role allochthonous DOC plays in these systems. Protection of allochthonous sources of DOC (e.g. riparian vegetation, inundation of floodplains) is an important management consideration.

2. **Biofilm succession**

   It took approximately 5 weeks for chlorophyll- *a* in biofilm to increase markedly, indicating algal growth. We show a range of key metrics to which biofilms are responding (e.g. discharge, nutrients, turbidity). This response coincided with increases in essential fatty acids critical for animal development. Green algae is known to be an excellent source of PUFA, and here we show that some essential fatty acids peaked in concentration after 11 weeks deployment. This was supported by C:N data, which was lowest (lower is reflective of higher food quality) after 11 weeks. This is important information, since there is evidence in the literature that biofilms become a poorer quality food source through time as they become dominated by filamentous algae and cyanobacteria. Here we show that biofilm quality was retained for up to 11 weeks following inundation, and based on some key metrics (e.g. C:N, fatty acids profiles), peak quality was reached at the end of the study. More work is required to extend our study past 11 weeks,

but the information generated from this research has important implications for directing inundation duration of managed flows.

3. Stream metabolism

Our work indicates that during steady high flows over spring and summer that streams within the Edward-Wakool River system are strongly heterotrophic. Importantly, our technique showed that GPP in these ecosystems is predominantly generated within the water column by phytoplankton. Surfaces (combining hard surface biofilms on snags and soft benthos) are predominantly heterotrophic and consume more oxygen than they produce via microbial processing of organic material. Our data provide important baseline information of ecosystem dynamics within these systems that will be useful for comparison with metabolism responses to managed flows.

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