

# River bacterioplankton community responses to a high inflow event

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**ABSTRACT:** Microbes drive chemical cycling and productivity within river ecosystems, but their influence may shift when intense allochthonous inputs accompany high freshwater inflow (flood) events. Investigating how floods influence microbial processes is fundamentally important for our understanding of river ecology, but is generally overlooked. We analysed bacterioplankton community composition (BCC) and abundance over 4 mo following an enormous flood event in the Hunter River, Australia, that resulted in a major fish kill. Concentrations of dissolved organic carbon (DOC) and inorganic nutrients (N and P) were up to 3 times higher during the flood event compared to prior and subsequent months. Bacterial cell abundances were up to 10 times higher at impacted sites during the flood event. Using Automated Ribosomal Intergenic Spacer Analysis we found significant shifts in BCC between the flood impacted month and subsequent months ( $p < 0.05$ ). Distance linear modelling indicated that DOC and dissolved N and P correlated most strongly with BCC patterns during the high inflow, whereas community dynamics correlated most strongly with nitrogen oxides and ammonium during the river's recovery phase. 16S rRNA amplicon pyrosequencing revealed that common soil-associated and facultative anaerobic genera of *Proteobacteria* were most dominant during the flood period, suggesting that a proportion of the bacterial community observed during this event were potentially inactive soil microbes transported into the river via terrestrial runoff. During the recovery period, *Cyanobacteria* and freshwater-associated genera of *Actinobacteria* and *Proteobacteria* became dominant in 16S rRNA pyrosequencing profiles. These observations indicate that allochthonous nutrients delivered via floods can significantly stimulate bacterial growth, underpinning substrate-controlled succession of bacterial communities and ultimately shaping the ecology within river ecosystems.

**KEY WORDS:** Bacterioplankton · ARISA · Lotic ecology · Network analyses

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## INTRODUCTION

Riverine ecosystems comprise a variety of ecologically important habitats and support high levels of organism diversity (Arthington et al. 2006, Dudgeon et al. 2006). Underpinning the function, stability and productivity of flowing fresh water (or lotic) ecosystems are the microorganisms that form the base

of the food web (Stahl et al. 2006, Blum & Mills 2012). Microbes including bacteria, phytoplankton and microzooplankton are important drivers of lotic chemical and energy cycles and are abundant and dynamic members of the lotic biota (Havens et al. 2000)

Lotic bacterial communities are biogeographically diverse and are typically characterised by local habi-

tat-specific endemism, yet are more phylogenetically similar to other freshwater habitats than marine and soil groups (Zwart et al. 2002). Longitudinal gradients in microbial community composition and functional groups are common in rivers and estuaries, as a consequence of changing hydrology and salinity concentrations (Crump et al. 2004, Maranger et al. 2005). Similarly, lotic bacterial community assemblages can shift seasonally, with patterns in bacterial composition typically driven by algal (autochthonous) carbon during low precipitation seasons and by allochthonous carbon during wetter seasons (Almeida et al. 2005, Pinhassi et al. 2006, Hitchcock & Mitrovic 2013). Compared to marine environments, bacterial abundance, productivity and respiration rates can be significantly higher in lotic systems (Del Giorgio & Cole 1998, Del Giorgio & Williams 2005) due to higher nutrient inputs from terrestrial inputs (Cole & Caraco 2001, Pollard & Ducklow 2011), and as a consequence riverine ecosystems are often net heterotrophic (Hadwen et al. 2010). Our knowledge of the ecology of lotic microbes is much less developed than our understanding of marine and lake microbial communities. In particular, there is currently limited knowledge of how microbial communities respond to the constantly shifting environmental conditions that are characteristic of river ecosystems (Curtis & Sloan 2004, Schultz et al. 2013).

Physical and chemical conditions within lotic systems can shift slowly, such as between seasons, or very abruptly, particularly following precipitation events that lead to high inflows and flooding (Westhorpe & Mitrovic 2012). As the foundation of lotic food webs, microbes are among the first organisms to experience and respond to changes in the dynamic physicochemical environment of rivers and their estuaries (Kirchman et al. 2004, Wear et al. 2013). Understanding how the abundance, composition and function of microbial assemblages change over different temporal scales is fundamental to our understanding of riverine ecology (Blum & Mills 2012).

Medium to large river inflows, where precipitation events lead to a subsequent increase in flow volume and velocity, are a pivotal regulator of physical habitat and the primary transporter of nutrients in lotic systems (Arthington & Pusey 2003). They couple abiotic components of terrestrial environments (such as flood plains) with biotic processes in aquatic systems (Junk et al. 1989, Tockner et al. 2000, Siczko & Peduzzi 2014). The frequency, variability, volume and quality of river inflows ultimately determine the ecological character of rivers and their associated estuaries (Poff & Zimmerman 2010). Notably, in

many parts of the world, major rivers and estuaries are subject to flow regulation, and receive reduced flow volumes that differ greatly from historical patterns (Vörösmarty et al. 2010). In determining the ecological significance of fresh water inflows, an understanding of natural biological responses to physical changes at the base of lotic food webs is imperative, but currently lacking.

In many aquatic and marine environments, heterotrophic bacterioplankton abundance and activity is tightly regulated by the availability of DOC obtained primarily from autochthonous sources including phytoplankton extracellular exudates and metazoan wastes (Azam et al. 1983, Wilson & Devlin 2013). However, rivers and estuaries periodically receive large inputs of particulate organic carbon (POC) and DOC from terrestrial (allochthonous) sources during seasonal inflows and floods (Farjalla et al. 2009), and this may temporarily uncouple the reliance of bacterioplankton on autochthonously derived C (Almeida et al. 2005, Ameryk et al. 2005). Such a shift may significantly alter chemical cycling and trophic dynamics within lotic ecosystems over a variety of temporal scales, as has been observed in lake environments (Kritzberg et al. 2004).

When large seasonal floods (that are induced by heavy precipitation events) breach river embankments, floodwaters inundate catchment and floodplains. Organic material from flood-impacted land washes into the river as the water levels recede. Carbon and nutrients then leach from the inundated area, often turning the water a dark tea colour, termed 'black water'. In river systems where considerable land modification has taken place (e.g. cleared pastures), organic carbon and inorganic nutrients accumulate in high concentrations in the flood waters, which contributes strongly to microbial activity and decomposition processes (Carvalho et al. 2003, Almeida et al. 2005, Farjalla et al. 2009). This, in combination with increased turbidity levels that often accompany inflows, can shift the base of the lotic system to a more heterotrophic state (Westhorpe et al. 2010), and in some cases leads to the formation of hypoxic or anoxic zones (Paerl et al. 1998, Zhang et al. 2010). In extreme cases, these low O<sub>2</sub> black waters can cause mass mortality in the food web and can detrimentally impact fishery stocks and river ecological health (Salles et al. 2006). The extent of these events will be influenced by changes in composition and activity of microbial populations that follow inflow events.

In marine and lake ecosystems, pulses of organic and inorganic nutrients, derived from a variety of

biotic (e.g. algal blooms) and abiotic (e.g. nutrient upwelling) events can lead to substrate-controlled succession of bacterial populations (Fawcett & Ward 2011, Teeling et al. 2012). It is probable that allochthonous resource pulses related to inflow events within rivers will have a similar effect on lotic microbial communities (Wear et al. 2013). Resource variability could then select for copiotrophic microbes which normally persist in low abundances but are capable of very efficient resource assimilation during nutrient pulses. However, to date very few studies have observed these processes in lotic systems.

Previous studies have performed nutrient manipulation experiments using microcosms to investigate heterotrophic bacterial growth response to simulated inflows, floods and their associated nutrient inputs (Hitchcock et al. 2010, Hitchcock & Mitrovic 2013, Mitrovic et al. 2014). These studies have indicated that heterotrophic bacterial growth in rivers and estuaries is often limited or co-limited by available DOC, N and P during periods of low river discharge (Jansson et al. 2006, Hadwen et al. 2010, Hitchcock et al. 2010, Hitchcock & Mitrovic 2013), but very little is known about how these events influence the composition and diversity of bacterial assemblages, or the implications for lotic chemical cycling and trophic dynamics. This knowledge is of growing importance because there is a global pattern of high flow events being returned to many major coastal rivers and estuaries as a result of flow management (Dudgeon 2010). Here we examined the compositional dynamics of a lotic microbial community during and after a major black water event, with the objective of determining to what extent ephemeral inputs of allochthonous organic material alter the microbial ecology of river ecosystems.

## MATERIALS AND METHODS

### Study site

Sampling was conducted in the Hunter River estuary system, located on the mid-eastern coastline of New South Wales (NSW), Australia (63.34° S, 37.26° E to 63.76° S, 37.91° E). Hunter River discharge to its estuary is regulated by dams and weirs, and 2 of the Hunter River system's main tributaries — the Williams and Paterson rivers — are also regulated. The Hunter River estuary was chosen as a suitable sample site for this study as it is represen-

tative of many regulated estuaries of the temperate east Australian coast, and has recently had new flow regulation rules assigned to it (NSWDPI 2003).

### Sampling regime

Sampling was conducted at high tide at 7 sites, across the tidal zone of the Hunter River estuary, as well as within its 2 main tributaries (Paterson River and Williams River). Sites 1, 3, 4, 6 and 7 were located in the Hunter River, Site 2 was located within the Paterson River and Site 5 was located in the Williams River (Fig. 1, Table 1). Biological sampling began in March 2013, in conjunction with a monthly water quality sampling regime which had begun in 2012. The first bacterial sampling occasion conducted in March 2013 coincided with an intense inflow where depletion in dissolved oxygen (DO) resulted in a mass fish kill. Following this black water event, sampling continued on a monthly basis until June 2013.

### Water sampling protocols

Hydrographic data were obtained from gauging stations operated by the NSW Office of Water throughout the Hunter River estuary and its catchment. Flow discharge, expressed as mean  $\text{m}^3 \text{d}^{-1}$ , was recorded every 15 min over each 24 h period and converted to mean daily flow by averaging flow discharge across 24 h intervals. *In situ* water quality measurements including temperature, pH, conductivity and DO were taken at all sites from 20 cm below the water surface, using a calibrated Hydrolab

Table 1. Site distance from estuary mouth and salinity at each site and sampling occasion in 2013. Data given in practical salinity units (PSU) converted from conductivity in  $\text{mS cm}^{-1}$

Location	Site	Distance from estuary mouth (km)	Salinity			
			Mar	Apr	May	Jun
Hunter River	1	49.1	<2	<2	<2	<2
	3	45.9	<2	<2	<2	<2
	4	35.4	<2	<2	<2	<2
	6	28.1	<2	<2	8.8	5.8
	7	16.9	<2	9.7	25.6	22.1
		Distance from Hunter River junction (km)				
Paterson River	2	55.0	<2	<2	<2	<2
Williams River	5	46.0	<2	<2	<2	<2

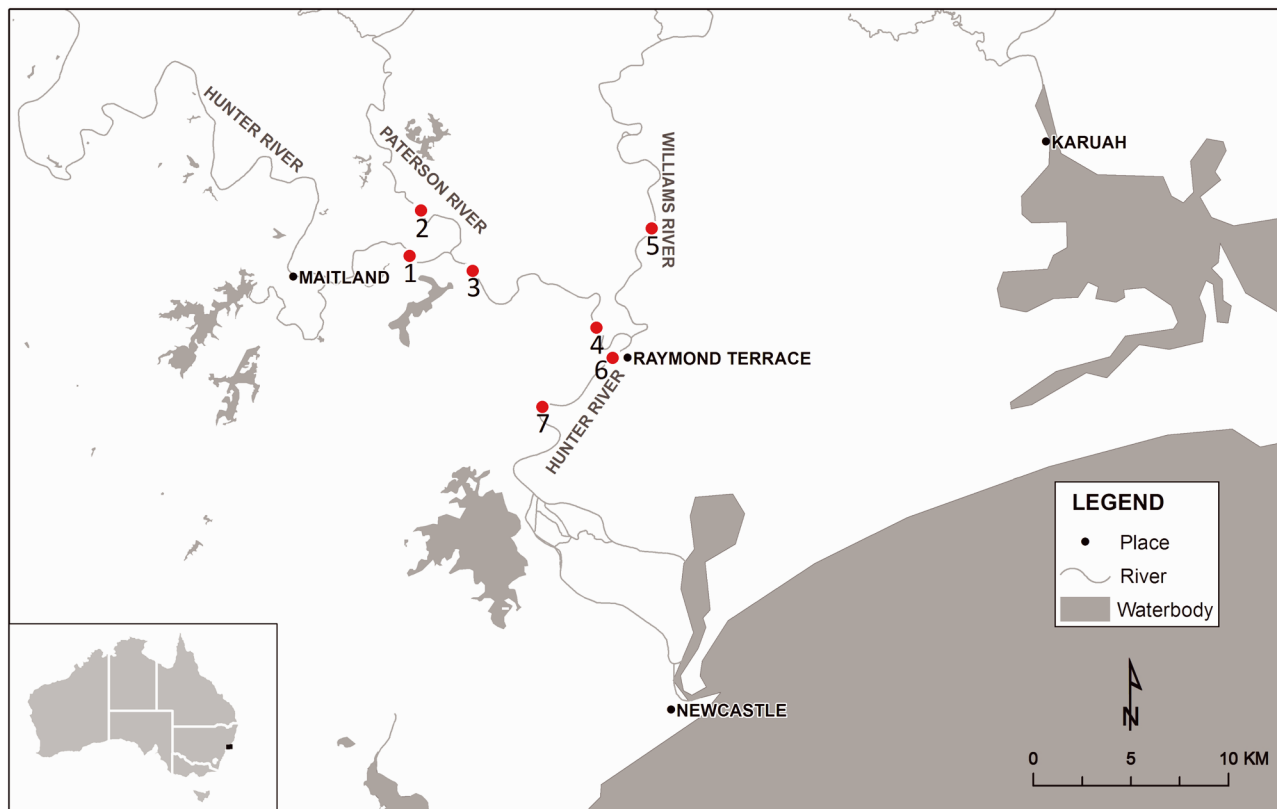


Fig. 1. Sampling sites within the tidal pool of the Hunter River and its 2 main tributaries, the Paterson River and the Williams River, New South Wales, Australia

Surveyor (Hydrolab) and MS5 Sonde probe (Hydrolab). Turbidity measurements were taken using a Hach turbidity Meter (Hach Company).

Surface samples for dissolved organic carbon (DOC) were collected in triplicate and immediately filtered through 0.45  $\mu\text{m}$  cellulose acetate filters (Sartorius Stedim Biotech) into pre-combusted 200 ml glass bottles, refrigerated and then acidified with 2N hydrochloric acid before analysis on a Shimadzu TOC-VCSH analyser using the High Temperature Combustion Method (Eaton et al. 1995, Westhorpe & Mitrovic 2012). Nutrient samples (50 ml) were separately analysed for total nitrogen (TN), dissolved organic nitrogen (DON), oxidised nitrogen ( $\text{NO}_x$ ), ammoniacal nitrogen ( $\text{NH}_4$ ), total phosphorus (TP) and filtered reactive phosphorus (FRP). Nutrient samples were filtered through 0.45  $\mu\text{m}$  filters, and stored in triple-rinsed 50 ml PET bottles and refrigerated at 4°C. Analysis was conducted using a segmented flow analyser (OI Analytical Model FS3100) in accordance with standard methods (Eaton et al. 1995).

Chlorophyll *a* (chl *a*) concentrations were determined by filtering 200 ml of surface water onto

0.75  $\mu\text{m}$  pore-size glass microfiber filters and were analysed using the methods of Eaton & Franson (2005).

### Bacterial cell enumeration

Triplicate 1 ml surface samples were fixed with glutaraldehyde (2% final concentration) and snap frozen in liquid nitrogen. Samples were stored at  $-80^\circ\text{C}$  prior to flow cytometric (FCM) analysis. In preparation for FCM, samples were quickly thawed in hot water and stained with SYBR Green I nucleic acid stain (1:10 000 final dilution; Molecular Probes) (Marie et al. 1997, Gasol & Del Giorgio 2000, Seymour et al. 2005). Fluorescent reference beads (1  $\mu\text{m}$  diameter, yellow/green; Molecular Probes) were added to each sample immediately prior to analyses at a final concentration of  $10^5 \text{ ml}^{-1}$ . Samples were analysed using an LSRII flow cytometer (Becton Dickinson) and bacterial populations were discriminated according to cell side scatter (SSC) and SYBR Green fluorescence. Data were analysed using WinMDI v. 2.9 software (WinMDI; Joseph Trotter, Salk

Institute for Biological Studies). Cell concentrations are reported in bacterial cells ml<sup>-1</sup>.

### DNA extraction

Triplicate 1 l surface water samples were collected in rinsed plastic bottles, and filtered using a peristaltic pump onto 5 µm and 0.2 µm membrane filters arranged in an in-line filtration set up. The 5 µm pore size membrane filter was employed to capture bacteria attached to suspended particles (Nair & Simidu 1987, Gram et al. 2010, Tang et al. 2011, Rösel & Grossart 2012), allowing discrimination from the free-living bacterial community captured on 0.2 µm membrane filters. In the absence of a universally agreed filter size standard appropriate for discriminating attached and free-living bacterial communities, we conservatively selected a 5 µm membrane pore size which will allow for the capture of medium to large particles colonised by bacteria (Azam & Hodson 1977, Acinas et al. 1999, Riemann & Winding 2001). Filters were immediately snap-frozen in liquid nitrogen, and stored at -80°C until DNA extraction was performed. Extraction of microbial genomic DNA was performed using a bead beating and chemical lysis kit (MoBio PowerWater) according to the manufacturer's directions. Extracted DNA was quantified using a Qubit Fluorometer (Life Technologies).

### Bacterial community fingerprinting

To investigate bacterial community diversity we used Automated Ribosomal Intergenic Spacer Analyses (ARISA) (Brown et al. 2005). PCR was performed using 16S and 23S Internal Transcribed Spacer (ITS) specific primers. These included the 6-carboxyfluorescein (FAM)-labelled forward primer 1392f (5'-GYA CAC ACC GCC CGT-3') and the reverse primer 23Sr (5'-GGG TTB CCC CAT TCR G-3') (Sigma-Aldrich). Each 20 µl PCR reaction mixture consisted of 2 µl of extracted DNA, 10 µM of each primer, 10 µl of GoTaq<sup>®</sup> Colorless Master Mix, (2×: GoTaq<sup>®</sup> DNA Polymerase supplied in 2× Colorless GoTaq<sup>®</sup> Reaction Buffer at pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl<sub>2</sub>. This reaction mixture was subjected to a PCR cycle of 94°C for 5 min, and then 35 cycles (94°C for 40 s, 56°C for 40 s, 72°C for 90 s), followed by 72°C for 5 min (Brown et al. 2005). Fragments were sized using a 3730xl DNA Analyzer (Applied Biosystems)

at the Australian Genome Research Facility (AGRF), using the LIZ1200 internal size.

ARISA profiles were analysed using PeakScanner software (v. 1, Applied Biosystems). Fragments with a relative fluorescence intensity (RFI) <0.09% of total amplified DNA were discarded as they are indistinguishable from background noise (Hewson & Fuhrman 2004). Fragments with <200 or >1200 base pairs (bp) were also discarded as they fall outside of the internal size standard range. Binning scripts in Custom R statistical software were used to discriminate fragments, whereby fragments differing by less than 2 bp were considered the same operational taxonomic unit (OTU) (Ramette 2009).

### 16S RNA amplicon pyrosequencing

16S rRNA amplicon pyrosequencing was used to identify and contrast the composition of bacterial communities from the attached (>5 µm) and free-living (>0.2 µm, <5 µm) bacterial populations during the March and April sampling expeditions, allowing us to compare the bacterial community during a black water event (March) to the subsequent recovery phase.

DNA samples were amplified with the 16S rRNA universal Eubacterial primers 27F (5-AGA GTT TGA TCC TGG CTC AG-3') and 519R (5-GTN TTA CNG CGG CKG CTG-3') (Kim et al. 2011, Kumar et al. 2011) and a PCR reaction using the HotStarTaq Plus Master Mix Kit (Qiagen) using the following cycling conditions: 94°C for 3 min, followed by 28 cycles of 94°C for 30 s; 53°C for 40 s and 72°C for 1 min; after which a final elongation step at 72°C for 5 min was performed. Post-PCR purification was performed using Agencourt Ampure beads (Agencourt Bioscience). Samples were sequenced using the Roche 454 FLX titanium platform at the Molecular Research Labs (Shallowater, Texas, USA).

DNA sequences were processed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso et al. 2010) as previously described for 454 pyrosequencing data (Gibbons et al. 2013). Briefly, DNA sequences were de-multiplexed and reads shorter than 200 bp, with a quality score <25 or containing homopolymers exceeding 6 bp were discarded. OTUs were defined at 97% sequence identity using UCLUST (Edgar et al. 2011) and assigned taxonomy against the Greengenes database (v. 13.5) (McDonald et al. 2011) using BLAST (Altschul et al. 1990, DeSantis et al. 2006). Chimeric sequences were detected using ChimeraSlayer (Haas et al. 2011) and filtered from the dataset.

## Statistical methods

Linear correlations between environmental variables and bacterial cell counts were measured by applying Pearson's Correlation Coefficient to data. Determination of variability between flood affected and post-flood months, sites and bacterial filter size classes was achieved by applying a suite of permutational multivariate analysis of variance (PERMANOVA) statistical methods in PRIMER (Primer-E) to environmental and biological (ARISA and pyrosequencing) data. To determine whether environmental conditions and bacterial community composition (BCC) at river sites varied significantly between 'during flood' and 'post flood' periods, PERMANOVA was used to compare the variance in the means of environmental and biological data from the flood impact month of March 2013, to the post flood months of April, May and June 2013 (Anderson 2001). An assumption of PERMANOVA is that data are equally dispersed (Anderson et al. 2007). Unequal dispersion in environmental variable data was tested using draftsman plots. Where unequal dispersion of data was detected, it was resolved by LOG+1 transformation, therefore reducing the skewness of the data while maintaining proportionality and satisfying the assumptions of PERMANOVA.

PERMANOVA with PRIMER + PERMANOVA software v. 6 (Anderson et al. 2007) was used to determine significant dissimilarity within biological (ARISA) and environmental data, between the flood impacted month and subsequent months. Data were then graphically represented using non-parametric multi-dimensional scaling (nMDS) plots. Distance based linear modelling (DistLM), using a stepwise procedure for adjusted  $R^2$ , was then used to select the environmental variables most likely to explain patterns in the biological data. This was graphically represented by a distance-based redundancy analysis (dbRDA) plot.

A detailed analysis of relationships between environmental variables and specific bacterial taxonomic units was also made using network analysis (Steele et al. 2011). Statistical associations between variables were determined using the maximal information coefficient (Reshef et al. 2011) by selecting the 50 most dominant bacterial genera identified from 28 samples across March and April, using the 16S rRNA amplicon pyrosequencing data and visualized in Cytoscape (Fuhrman & Steele 2008). All environmental variable and bacterial taxa interactions that were insignificant ( $p > 0.05$ ) or of correlative strength below 0.8 were excluded. The resultant network was

created using an edge-weighted force-directed layout which allowed visualisation of the correlation strength between individual bacterial genera and the measured environmental variables (Zhou et al. 2010, Steele et al. 2011). Relationships between variables have been represented by edges, in our network positive associations are represented by black lines and red lines indicate negative associations. The length of the edge relates the strength of correlation between variables, (e.g. shorter edges relate to stronger associations between variables). Variables (including bacterial groups and water chemistry measurements) have been represented by nodes, which have been colour-coded according to the bacterial phyla or environmental variable they belong to, and the size of nodes relates to their relative abundance.

## RESULTS

### Physico-chemical dynamics

We used environmental variable data (including all physical and nutrient measurements) collected during sampling occasions from January 30 until June 12, 2013, which encompassed the physicochemical conditions within the Hunter River estuary under a low flow state (January), during a major inflow event (March), and during the 3 mo recovery phase thereafter (April to June).

During the high flow period in March, flow rates at some sites increased massively from baseline levels of below  $300 \text{ ml d}^{-1}$  to in excess of  $90000 \text{ ml d}^{-1}$ . Since flow rate recordings began in this region in 1968, on average there have only been 1 to 2 events of this magnitude occurring each decade (<http://realtimedata.water.nsw.gov.au/water>). Strong linear correlations were identified between mean river discharge rates and water turbidity ( $r = 0.66$ ) and DOC ( $r = 0.84$ ), with DOC concentrations peaking in March during the high inflow event (Fig. 2). Flow discharge also correlated strongly with FRP ( $r = 0.79$ ) and DON ( $r = 0.77$ ), which were both also elevated in March (Fig. 2). Conversely,  $\text{NO}_x$  concentrations were lowest in March at Hunter River Sites 3, 4, 6 and 7 and Site 2 on the Paterson River (Fig. 2). Dissolved oxygen (DO) concentrations fell to anoxic levels during the March inflow event with the exception of Site 5, on the Williams River, which was relatively unaffected by the flood. During this period DO dropped to  $<0.2 \text{ mg l}^{-1}$  at Sites 2, 3, 4, 6 and 7, but remained above  $4 \text{ mg l}^{-1}$  at the upper Hunter River (Site 1). DO concentrations subsequently returned to pre-March

inflow levels of between 5 and 10 mg l<sup>-1</sup> across all sites by the next sampling effort in April. NH<sub>4</sub> concentrations were highest at all sites except on the Williams River (Site 5), in April.

Using the entire suite of water chemistry and nutrient parameters measured, significant variation was observed in physical conditions, between months (df<sub>3,27</sub>, perm<sub>998</sub>; pseudo-*F* = 10.98, *p* < 0.001) and sites (df<sub>6,27</sub>, perm<sub>996</sub>; pseudo-*F* = 2.05, *p* < 0.005), indicative of a highly dynamic aquatic environment. Although some parameters were dispersed homogeneously, (PERMDISP *p* > 0.05), the strength of significance (*p* < 0.01) in variation between months and sites (Fig. 2) suggests that the major inflow event in March was a strong driver of spatiotemporal heterogeneity in the physical condition of the Hunter River ecosystem.

### Chl *a* concentrations

Chl *a* varied considerably between months and sites, typically ranging between 2 and 40 µg l<sup>-1</sup>, with 2 exceptions where phytoplankton blooms were evident: Site 5 in March (83.3 µg l<sup>-1</sup>), and Site 4 in June (94.3 ± 0.88 µg l<sup>-1</sup>) (see Appendix 1). Chl *a* concentrations were generally lower at impacted sites during the flood event in March and higher during the recovery phase in April through to June. Chl *a* concentration exhibited weak, negative linear correlations with discharge (*r* = -0.26) and FRP (*r* = -0.31), and was positively correlated with DO (*r* = 0.47) and NO<sub>x</sub> (*r* = 0.20).

### Bacterial abundance

Bacterial cell concentrations were highest at all sites in March during the inflow event, with the exception of Site 1 (Hunter River) and Site 5 (Williams River). Across the entire time-series, bacterial cell abundance was highest at Site 2 on the Paterson River in March (2.79 × 10<sup>8</sup> ± 1.12 × 10<sup>7</sup> cells ml<sup>-1</sup>) and lowest at Site 7 on the Hunter River in June (7.57 × 10<sup>6</sup> ± 6.26 × 10<sup>4</sup> cells ml<sup>-1</sup>). Comparison of bacterial cell abundances during the flood period in March

to subsequent months using PERMANOVA revealed significant (*p* = 0.001) and strong (df<sub>1,27</sub>, perm<sub>997</sub>; pseudo-*F* = 31.504) temporal dissimilarity between the flood impacted month and subsequent months, which is consistent with bacterial cell concentrations being up to an order of magnitude higher in March compared to the following months.

### Bacterial community fingerprinting

BCC, determined using ARISA, was distinctly different in March during the flood event compared to the recovery period over the 3 subsequent months.

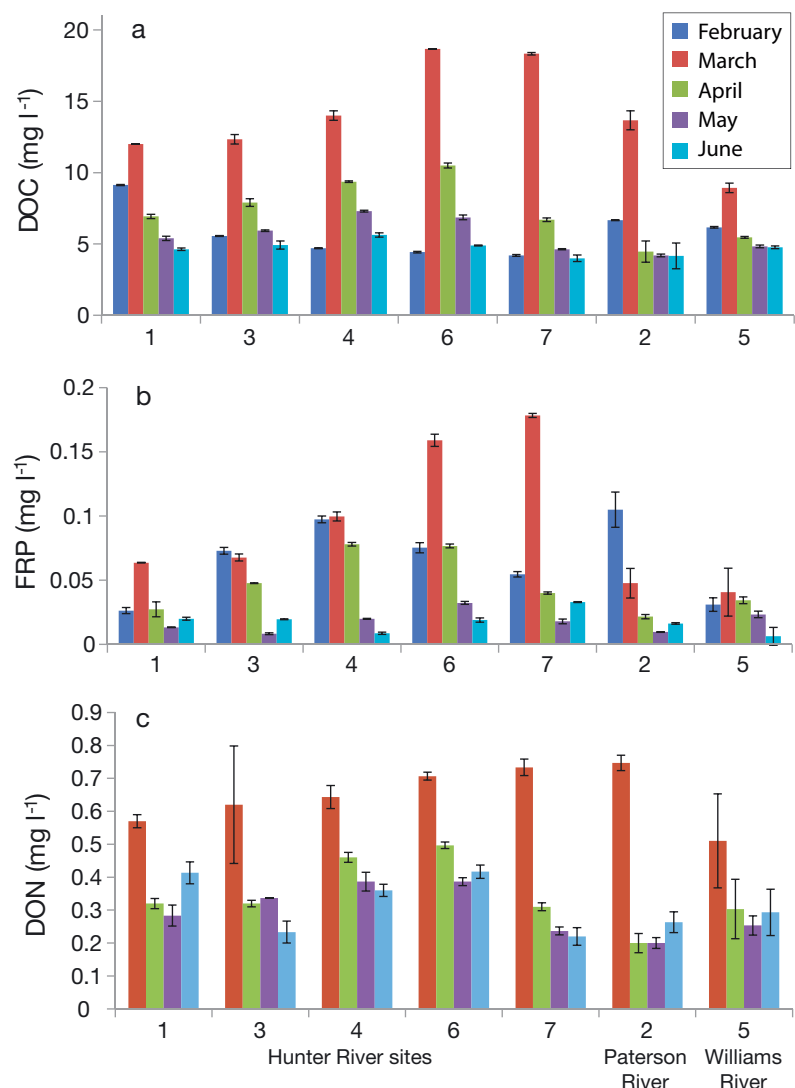


Fig. 2. Nutrient concentrations sampled at the Hunter River (in upstream to downstream sequence, Sites 1, 3, 4, 6 and 7, left to right) and tributary sites (Paterson River, Site 2; Williams River, Site 5) from February to June 2013. (a) Dissolved organic carbon (DOC), (b) filtered reactive phosphorus (FRP), and (c) dissolved organic nitrogen (DON). Error bars represent SE

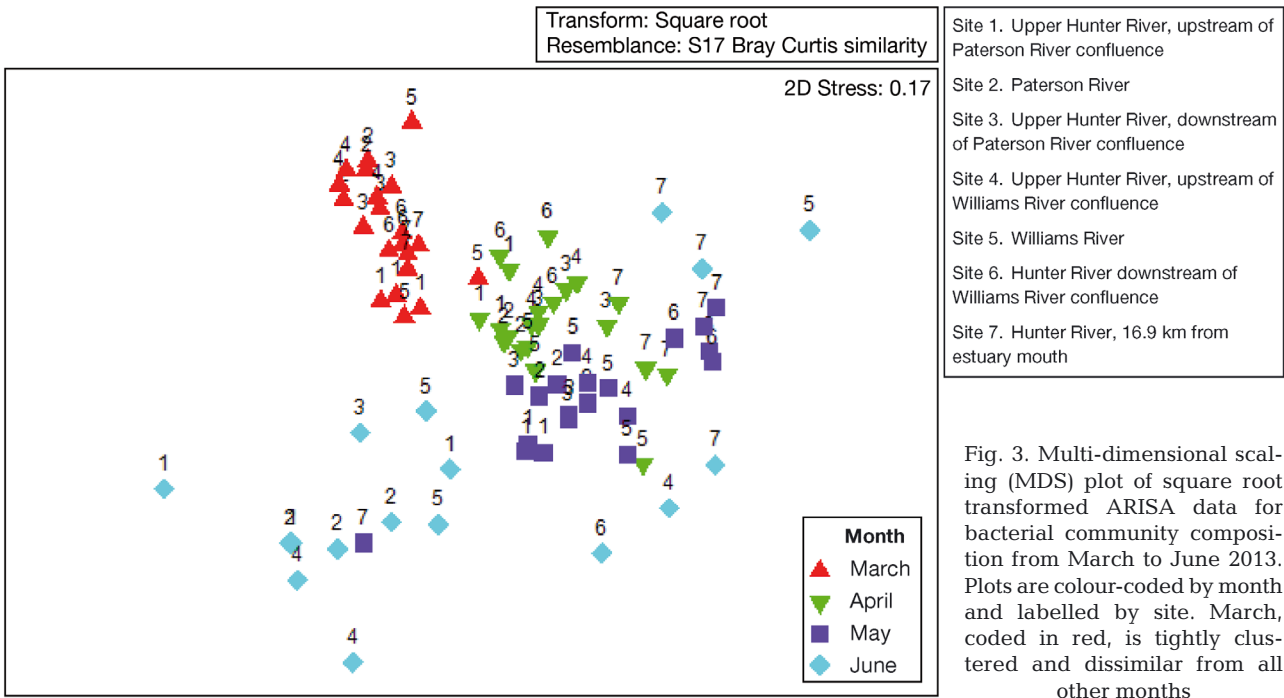


Fig. 3. Multi-dimensional scaling (MDS) plot of square root transformed ARISA data for bacterial community composition from March to June 2013. Plots are colour-coded by month and labelled by site. March, coded in red, is tightly clustered and dissimilar from all other months

PERMANOVA assumptions of normality were met (PERMDISP  $p < 0.05$ ) and the patterns in ARISA data revealed a significant ( $p = 0.001$ ) month and site interaction ( $df_{18,78}$ ,  $perm_{999}$ ,  $pseudo-F = 2.76$ ). BCC shifted very strongly between the flood-impacted month (March) and subsequent recovery months ( $df_{1,78}$ ,  $perm_{997}$ ;  $pseudo-F = 2.18$ ,  $p = 0.001$ ), supported by tight clustering of months and separation

of March sites using non-metric MDS (Fig. 3). DOC, FRP and DON, which were at elevated concentrations during the high inflow period of March (Fig. 2), correlated most strongly with community-scale shifts in bacterial community assemblage observed in the ARISA data (Fig. 4). Diminished turbidity levels and higher  $NO_x$  concentrations were also associated with temporal shifts in BCC (Fig. 4).

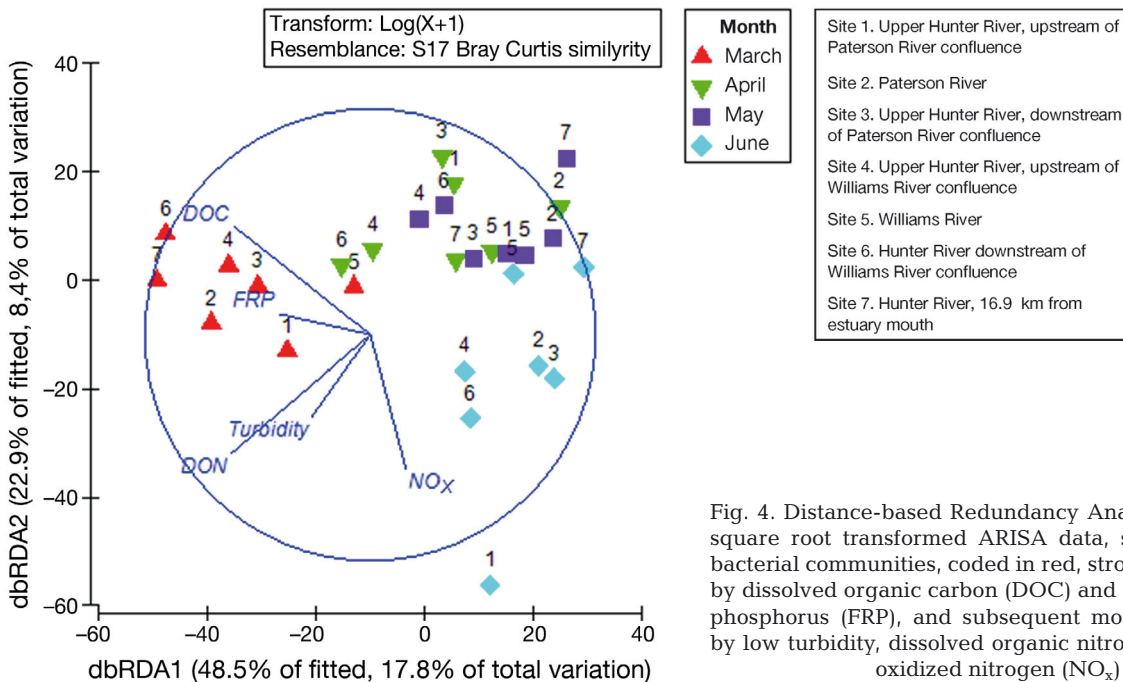


Fig. 4. Distance-based Redundancy Analysis Plot using square root transformed ARISA data, showing March bacterial communities, coded in red, strongly influenced by dissolved organic carbon (DOC) and filtered reactive phosphorus (FRP), and subsequent months influenced by low turbidity, dissolved organic nitrogen (DON) and oxidized nitrogen ( $NO_x$ )



### Bacterial phylogenetic composition

16S rRNA amplicon pyrosequencing revealed significant shifts in the bacterial community between the flood-impacted conditions in March to the recovery period in April (Fig. 5). In March, the *Proteobacteria* represented over 75% of bacterial sequences across all sampling sites (Fig. 5a), and this group was comprised predominantly of  $\beta$ -*Proteobacteria*, which made up 69% of proteobacterial sequences, followed by the  $\epsilon$ -*Proteobacteria* (12%) and  $\gamma$ -*Proteobacteria* (9%) (Fig. 5c). On the other hand, during April, *Proteobacteria* only comprised 25% of the total bacterial community (Fig. 5b), primarily as a consequence of strong increases in the relative abundance of *Cyanobacteria* (27%) and *Actinobacteria* (26%) (Fig. 5b). Furthermore, within April there was a shift in the composition of the *Proteobacteria*, with a decrease in the  $\beta$ -*Proteobacteria* (43%) relative to the  $\gamma$ -*Proteobacteria* (30%) and  $\alpha$ -*Proteobacteria* (24%) (Fig. 5d).

In terms of spatial patterns,  $\beta$ -*Proteobacteria* sequences were the most abundant proteobacterial group across all Hunter River and tributary sites (Fig. 6). In the Hunter River, the abundance of  $\gamma$ -*Proteobacteria* increased longitudinally in April from the upper Hunter River sites (Sites 1 and 3) to down river sites (Sites 4 and 6) (Fig. 6c), while  $\alpha$ -*Proteobacteria* were most abundant at Site 7 closest to the estuarine mouth, where they comprised half the proteobacterial abundance (Fig. 6d). The composition of *Proteobacteria* in Paterson and Williams rivers differed in March, whereby the  $\epsilon$ -*Proteobacteria* comprised over 30% of the community at Site 2 in the Paterson River, but was virtually absent at Site 5 in the Williams River (Fig. 6b).

Proteobacterial profiles in the Hunter River and its tributaries also differed between free-living and particle-attached bacterial size classes. Most notably, the abundance of  $\epsilon$ -*Proteobacteria* was greatest in the free-living community in the Paterson River in March and also at Hunter River sites downstream of the Paterson River (Fig. 6b). The relative importance of  $\gamma$ -*Proteobacteria* was greatest in the particle-attached communities in April where they comprised over half of the sequences across all Hunter River and tributary sites, with Site 7 (closest to the estuary mouth) being the only exception (Fig. 6c). At this time, the  $\alpha$ -*Proteobacteria* comprised over 50% of the total proteobacterial abundance in both filter size classes at Site 7 (Fig. 6c,d).

At a finer taxonomic resolution, the dominant sequences matching *Proteobacteria* in both filter size classes in March belonged to the family *Comamonadaceae*, and the genera *Limnohabitans*, *C39*, *Acinetobacter* and *Sulfurospirillum* (Fig. 7a,b). By April, the large size class was dominated by *Cyanobacteria*, including *Planktothrix* and *Synechococcus* (Fig. 7c), and the smaller size class was strongly represented by *ACK-M1* within the *Actinobacteria* phylum, which replaced the *Proteobacteria* as the dominant members of the aquatic microbial community (Fig. 7d).

Network analysis revealed that the frequency of sequences matching *Proteobacteria* in March was associated with the elevated nutrient concentrations during the inflow event (Fig. 8). DOC concentration was strongly and positively correlated with sequences matching *Dechloromonas*, *Acinetobacter*,

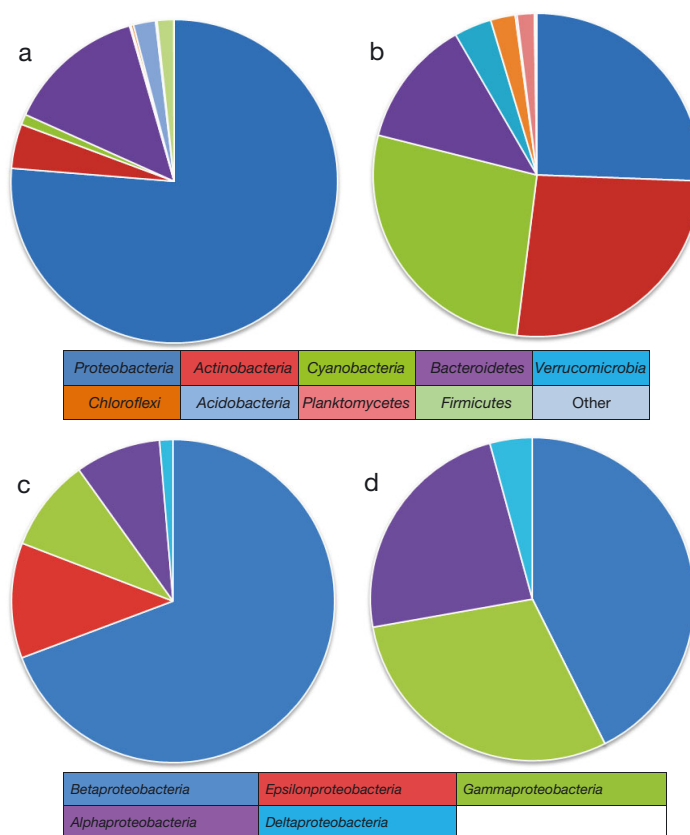


Fig. 5. Comparison between the (a,c) flood-affected period in March and (b,d) recovery period in April. Bacterial community composition data were produced by combining and averaging the sequence data from the 5 and 0.2  $\mu\text{m}$  filter size classes. Total bacterial community composition at the phylum level in (a) March and (b) April, and *Proteobacteria* at the class level in (c) March and (d) April

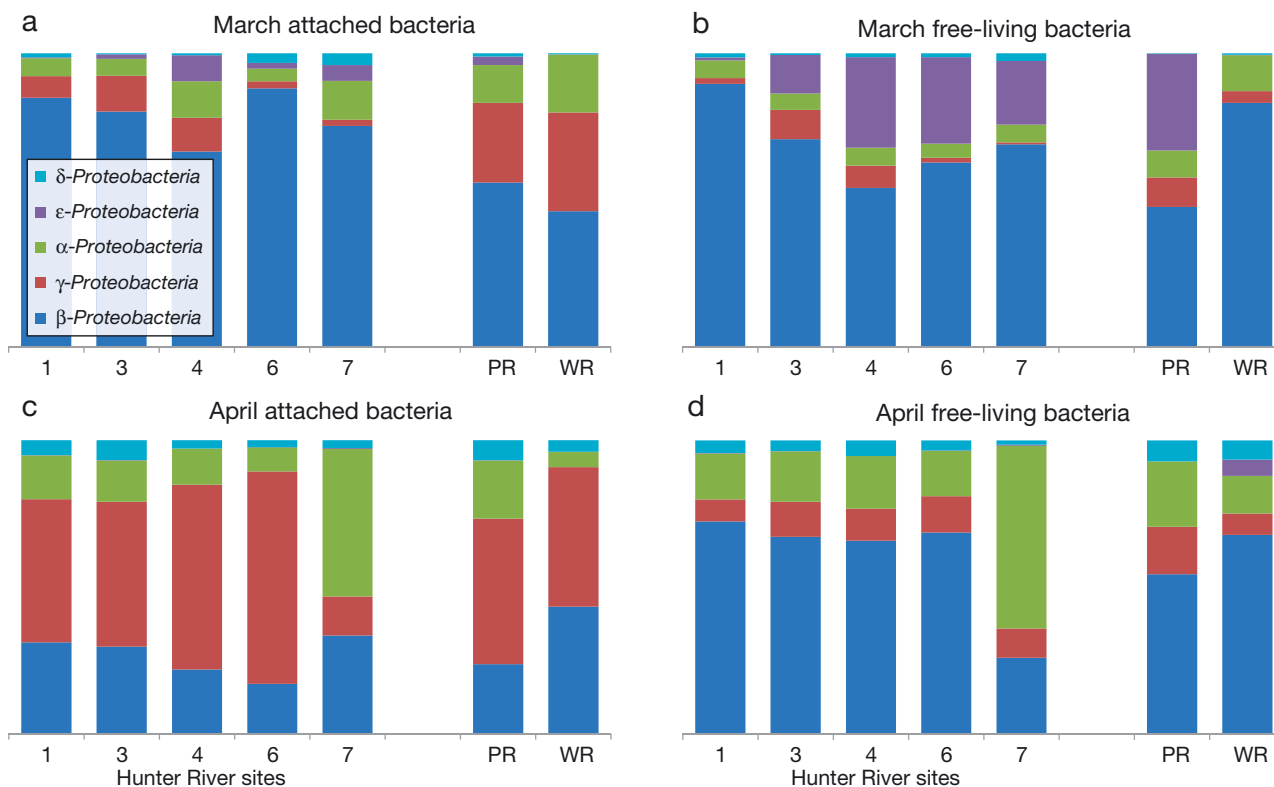


Fig. 6. Comparison of *Proteobacteria* community composition and attached and free-living communities between March and April 2013 using 454 pyrosequencing data. *Proteobacteria* composition of attached bacteria in (a) March and (c) April, and *Proteobacteria* composition of free-living bacteria in (b) March and (d) April. PR: Paterson River (Site 2); WR: Williams River (Site 5)

*KD1-23* and *Limnohabitans* (Fig. 8). Similarly, sequence matches to other *Proteobacteria* including *Dechloromonas*, *Hydrogenophaga* and *C39* were strongly and positively correlated with concentrations of DON (Fig. 8). Several sequences matching genera of *Bacteroidetes*, although in relatively lesser frequency to *Proteobacteria*, shared close association with flood-related organic nutrients, such as DOC, DON as well as turbidity. These include *Bacteroidales*, *Paludibacter*, *Flavobacterium* and sequences matching the family *Flavobacteriaceae*. Chl *a* concentration and the abundance of sequences matching *Cyanobacteria* (*Planktothricoides* and *Synechococcus*) were negatively correlated with environmental parameters characteristic of the flood event in March, particularly turbidity and DON, while positively correlated with  $\text{NO}_x$  and  $\text{NH}_4$ , respectively, which were in greater concentration during the recovery months of April to June. Taken in its entirety, the network in Fig. 8 illustrates the contrast between bacterial taxonomic groups and their nutrient associations. Genera of *Proteobacteria* and some *Bacteroidetes* were most commonly and strongly associated with the elevated organic nutrients that characterised the river condition during the flood in March, and chl *a* concen-

trations and *Cyanobacteria* were negatively associated with environmental conditions typical of flood conditions.

## DISCUSSION

Recent studies within Eastern Australian coastal river and estuarine freshwater inflows have measured patterns in bacterial abundance and biomass, and have revealed a common positive correlation between the import of allochthonous DOC and inorganic nutrients and these parameters (Hitchcock et al. 2010, Hitchcock & Mitrovic 2013, 2014). We have expanded upon these observations by identifying shifts in BCC and taxonomic diversity in response to an inflow event and have related these changes to the input of allochthonous C and inorganic nutrients. Our data revealed that shifts in community composition were strongly correlated with specific water chemistry parameters including DOC, DON and FRP, and that specific phylogenetic groups of bacteria were possibly transported from terrestrial or sediment substrate into the river or promoted or suppressed by the physicochemical conditions experienced during the

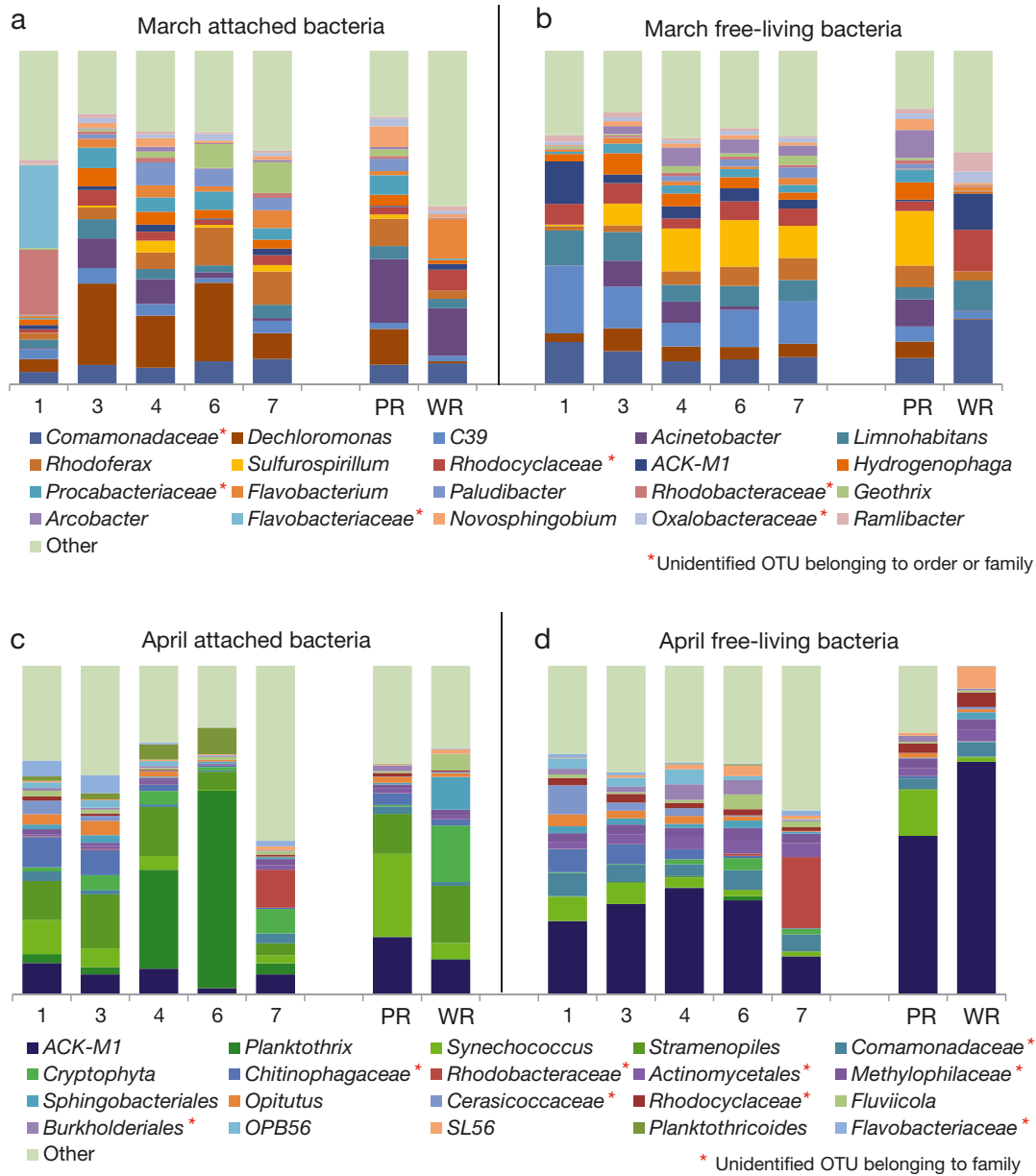


Fig. 7. Bacterial community operational taxonomic units (OTUs) clustered at 97% similarity and identified down to genus level where possible, using 454 pyrosequencing data. OTU diversity of attached communities in (a) March and (c) April, and OTU diversity of free-living communities in (b) March and (d) April. PR: Paterson River (Site 2); WR: Williams River (Site 5)

flood, while others emerged as dominant community members under the post-flood conditions.

High inflows following precipitation events transport allochthonous material into river and estuarine systems, shifting the dominant carbon and nutrient sources for bacteria from autochthonous-derived (phytoplankton exudates and zooplankton detritus) material, to labile allochthonous DOC (Sinsabaugh & Findlay 2003, Webster & Harris 2004, Farjalla et al. 2009, Petrone et al. 2009, Westhorpe & Mitrovic 2013). Evidence from nutrient addition experiments

suggests that changes in resource composition and physical conditions can temporarily uncouple bacterial dependence on autochthonous sources of carbon (Kritzberg et al. 2004, Pinhassi et al. 2006, Hitchcock et al. 2010, Hitchcock & Mitrovic 2013). This is potentially reflected in our data, where we observed increased bacterial abundance, and community-scale and taxon-specific shifts within the bacterial community, which correlated with shifts in the physical condition of the Hunter and Paterson rivers during and following a high inflow event.

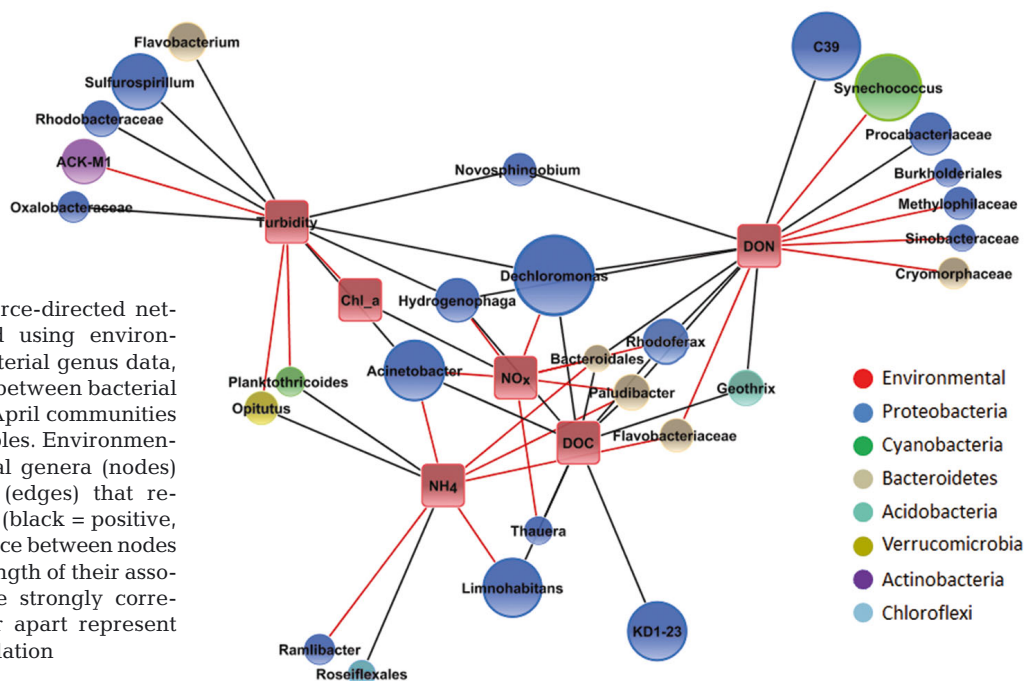


Fig. 8. Edge-weighted force-directed network analysis generated using environmental variables and bacterial genus data, showing the associations between bacterial genera from March and April communities and environmental variables. Environmental variables and bacterial genera (nodes) are connected by lines (edges) that represent their correlations (black = positive, red = negative). The distance between nodes corresponds with the strength of their association. Closer nodes are strongly correlated while those further apart represent weaker correlation

### Changes in physical and chemical conditions during inflow events

We found that shifts in water chemistry at sites in the Hunter River and its tributary (the Paterson River) were strongly influenced by the March inflow and black water event. A significant increase in organic nutrients (DOC and DON) and FRP was observed in the Hunter and Paterson rivers. Up to 8-fold increases in DOC concentrations have been observed during flood conditions in river and estuarine systems (Westhorpe & Mitrovic 2012), and we observed DOC concentrations in the Hunter River to increase from less than  $5 \text{ mg l}^{-1}$  to above  $15 \text{ mg l}^{-1}$  at Sites 3, 4, 6 and 7.

In contrast to all other sites, Site 5 on the Williams River received a much reduced inflow, and consequently did not experience the increases in nutrients or shift in DO that were observed at the other sites. Nutrient loads and composition vary between different catchments and floodplains due to hydrologic and geographic variability (Dalzell et al. 2007), and impoundments impair the transport of sediments and particulates (Baldwin et al. 2010), which may explain why the Williams River did not receive the same nutrient loads as the Hunter and Paterson rivers. The lack of inflow effects at Site 5 are likely a consequence of the presence of a weir and the discrete properties of the Williams River watershed. Due to the stability of chemical conditions at Site 5, this site

effectively acted as a negative control when considering the shifts in microbial communities observed in this study.

### Changes in bacterial abundance associated with inflow events

Both DOC and inorganic nutrients limit microbial growth in rivers and estuaries during low basal flows (Pinhassi et al. 2006, Hitchcock & Mitrovic 2013). The increase in C, N and P concentrations (DOC, TP, FRP, TN and DON) during the high inflow period coincided and possibly led to a significant increase in bacterial abundance, as has been observed in several micro-mesocosm nutrient addition experiments (Jansson et al. 2006, Pinhassi et al. 2006, Hitchcock et al. 2010, Hitchcock & Mitrovic 2013). Bacterial abundance at impacted sites during March was up to an order of magnitude higher (at  $>10^7$  cells  $\text{ml}^{-1}$ ) compared to the non-impacted Site 5 or subsequent months (April to June).

Carbon and nutrient concentrations returned to levels that resembled pre-flood conditions by the next sampling occasion in April (1 mo post inflow event). During this period, bacterial concentrations also declined substantially. These patterns reflect the capacity of riverine bacteria to rapidly utilise allochthonous pulses of dissolved organic matter during flood conditions.

### Changes in bacterial community composition during inflow events

Analyses of our ARISA data revealed that the changes in bacterial abundance between inflow and post inflow periods were accompanied by a significant shift in BCC. The dissimilarity in bacterial community assemblage was far greater between March and April, compared to dissimilarity between subsequent months, indicating that the inflow event in March profoundly altered bacterial communities beyond normal month-to-month variations. Distance linear modelling revealed that shifts in the composition of bacterial communities coincided with increased concentrations of organic and inorganic nutrients. This is suggestive of a community change related to growth responses to allochthonous nutrient inputs by copiotrophic bacteria (Palijan et al. 2008). However, there is also the possibility that the links between imported nutrients may reflect a coincidental link between water chemistry and allochthonously introduced microbes advected from adjacent floodplains, riparian zones and sediments (Miletto et al. 2008).

Notably, in addition to driving temporal variability in the composition of microbial communities, the March flood event also influenced the degree of spatial structure in microbial assemblages across the study region. Spatial homogeneity of bacterial communities in the Hunter and Paterson rivers was highest during the inflow event, and communities became more spatially heterogeneous over the 3 subsequent post inflow event months. This pattern indicates that the flood event acted to remove niche partitioning of microbial communities by homogenizing physical and chemical conditions across the Hunter River ecosystem, a process that may have further, previously unconsidered, ecological consequences. In addition, the advection of microbes from riparian and adjacent terrestrial sources may have contributed further to the apparent spatial similarity of BCC during the flood period. We suggest that the massive input of freshwater from the upper-river catchment and adjacent terrestrial zones during the flood temporarily reduced the influence of localised factors on water chemistry and biological composition of the Hunter River, which during low inflow periods would typically underpin spatial heterogeneity. In addition, the impacts of high inflow rates likely diminished tidal influence and led to reduced salinity concentrations in the estuarine portion at Sites 6 and 7 (from brackish to fresh), resulting in the more spatially homogenous physical and chemical envi-

ronment, and bacterial distributions. However by April, the re-establishment of longitudinal physicochemical gradients—ascending from upper river to down river sites (as seen in Fig. 2), likely led to the development of a longitudinal biological gradient resulting in a more spatially heterogeneous estuarine bacterioplankton community. This is indicated by the wider distribution of June data points in Fig. 3 as the impacts driven by the flood event dissipated throughout the recovery months, which is perhaps more typical of low inflow periods.

Although less pronounced in the ARISA profiles, 16S rRNA data indicated that the bacterial community at Site 5 in the Williams River differed to flood-impacted sites during March. Specifically, the  $\epsilon$ -*Proteobacteria* that were present at flood-impacted sites (*Sulfurospirillum* in particular) were virtually absent at the Williams River site in March, which had a greater proportion of freshwater bacteria compared to the flood-impacted tributary. This suggests that, consistent with the smaller shifts in physicochemical conditions at this site, the bacterial community at Site 5 was less influenced by the flood event than the other tested sites. Notably, the differences between Site 5 and the other sites were much more evident in 454 pyrosequencing data than the ARISA data, and are likely a result of the greater sensitivity of 454 pyrosequencing relative to ARISA.

Patterns in the 16S rRNA data were indicative of a shift in the microbial assemblage from a heterotroph-dominated community during the flood event, to a community with a higher proportion of autotrophic microbes in the recovery period. In April the relative proportion of sequences matching cyanobacteria was higher than during the flood event in March. A likely explanation for this pattern is the very high turbidity levels that occurred during the flood period, which will result in limited light availability to phototrophic microbes. The reduction in cyanobacteria sequences in March was mirrored by a decrease in total chlorophyll. In addition to the input of organic carbon during the flood, the turbid conditions experienced in March will have further assisted heterotrophic bacteria to outcompete phototrophs for common limiting inorganic nutrients (Almeida et al. 2005, Ameryk et al. 2005). This pattern was supported by the network analysis which revealed that the high levels of chemo-lithotrophic and chemoorganotrophic organisms, including *Sulfurospirillum*, *ACK-M1*, *Dechloromonas* and *Acinetobacter*, were strongly positively correlated with turbidity levels.

An additional driver of bacterial community patterns which likely influenced BCC in March was ter-

restrial run-off following the heavy rainfall, transporting soil and soil-associated microbes into the river water column. Sequences matching soil- and sediment-associated, chemo-lithotrophic specialist sulphate reducers and sulphur-oxidising bacteria including *Acinetobacter* (Newton et al. 2011), *Dechloromonas* and *Sulfurospirillum* (Kelly et al. 2005) comprised a major portion of the most abundant bacterial genera in the water column in March, across all flood-affected sites for both filter size classes. Network analyses indicated that these groups of bacteria were positively correlated with flood conditions, including increased concentrations of DOC, DON and turbidity levels. The decreases in DO, and links to bacterial abundance and specific OTUs observed here, could be related to either the increased abundance and activity of lotic microbes occurring as a consequence of organic and inorganic nutrient inputs, or the rise in bacterial biomass associated with allochthonous inputs of terrestrial bacteria into the system.

Endemic freshwater bacteria also responded to the flood conditions in March. Sequences matching freshwater chemo-organotrophic bacteria including *Limnohabitans*, *ACK-M1* and *C39* were abundant in the 0.2  $\mu\text{m}$  filter size class (representing the small cell sized and free-living bacteria) in March, which is consistent with previous observations that these organisms are capable of efficient assimilation of organic carbon and inorganic nutrients facilitating rapid growth in response to terrestrial water intrusions (Hahn et al. 2010, Lin et al. 2012).

In April, as the physical condition of the Hunter and Paterson river systems returned to a pre-flood state (i.e. it transitioned from an anoxic, turbid and highly eutrophic system during the flood to an aerobic, low turbidity and decreased nutrient state), substantial shifts in the composition of bacterial communities occurred. The relative importance of the *Proteobacteria* decreased significantly from March to April and this shift in total abundance was accompanied by a significant change in the composition of this group. The patterns observed here are consistent with previous studies that found *Proteobacteria* to be numerically dominant in river and lake habitats when bacterial communities are supported by allochthonous nutrient inputs, and to be less abundant when autochthonous nutrients are most important (Tang et al. 2009, Schultz et al. 2013). Most notably in March,  $\beta$ -*Proteobacteria* dominated both filter class communities. This group comprised cosmopolitan, freshwater bacteria belonging to the *Comamonadaceae* family and the genus *Limnohabitans* (Crump &

Hobbie 2005), in addition to the *Dechloromonas* genus, a group previously identified as soil-dwelling bacteria (García-Armisen et al. 2014).  $\epsilon$ -*Proteobacteria* were the second most abundant *Proteobacteria* in the free-living Hunter and Paterson river sites in March, consisting exclusively of bacteria belonging to the *Sulfurospirillum* genus, which has previously been observed to thrive in micro-aerophilic, hydrocarbon-contaminated conditions (Rossi et al. 2012). We suggest that the *Proteobacteria* composition observed in March is indicative of both a numerical increase in endemic freshwater bacteria as a result of allochthonous organic nutrient inputs, and the transport of bacteria from soil and sediment environments into the river. During April, while the  $\beta$ -*Proteobacteria* was still abundant, a substantial increase in the proportion of  $\gamma$ -*Proteobacteria* (30%) and  $\alpha$ -*Proteobacteria* groups was observed. The increase in  $\gamma$ -*Proteobacteria* was not represented by a single dominant group but accumulatively by several low abundance groups. Conversely,  $\alpha$ -*Proteobacteria* belonging to the *Rhodobacteraceae* family was more abundant in Hunter River sites closest to the estuary mouth. These observations are consistent with typical planktonic microbial communities widely reported in marine and brackish estuarine environments (Liao et al. 2007, Pujalte et al. 2014). We suggest that the change in community structure and increase in spatial heterogeneity of bacterial assemblages in April were influenced by reduced freshwater inflow, allowing the reestablishment of a longitudinal salinity gradient across the tidal pool.

We employed 2 filter size classes to discriminate bacterial sub-communities between large and attached cells, which were retrieved on the 5  $\mu\text{m}$  filter, and small and free-living cells, collected on the 0.2  $\mu\text{m}$  filter. The large filamentous cyanobacteria *Planktothrix*, and small but abundant single-celled cyanobacteria *Synechococcus* were among the most abundant organisms collected on the large filter size during April. The occurrence of the large filamentous cells (up to 8  $\mu\text{m}$  in diameter) (Entwistle et al. 1997) in this filter size class is as anticipated, but the occurrence of *Synechococcus* in this filter size, particularly in the estuarine Sites 6 and 7 during April, is likely explained by colonisation of suspended particles (Crespo et al. 2013, Thiele et al. 2014). *Planktothrix* and *Synechococcus* are obligate photoautotrophs, and their distribution and abundances across river, estuary and coastal environments are regulated by the availability of inorganic nutrients (Partensky et al. 1999, Halstvedt et al. 2007). The post-flood conditions of the Hunter and Paterson rivers were con-

ducive for *Plankothrix* and *Synechococcus* growth, whereby these organisms presumably exploited the elevated light levels and concentrations of remineralised nutrients during this period. Network analysis revealed that phototrophic genera were negatively associated with the conditions during the flood period and likely promoted by elevated inorganic nitrogen post flood in April. In addition there was a substantial increase in the relative importance of the actinobacterial group *ACK-M1* which has been observed in freshwater environments to have a strong chemotactic response to ammonium (Dennis et al. 2013) and be in high abundance during eutrophic conditions (Van der Gucht et al. 2005). Network analysis indicated that reduced turbidity levels were also a strong contributor to *ACK-M1* relative abundance.

The shifts in BCC observed here were driven by fluctuating physical and chemical conditions associated with an inflow event and are to some extent indicative of substrate-controlled succession of microbial communities within this ecosystem. While successional patterns among bacterial communities associated with algal blooms and nutrient upwellings have been widely reported in marine and lake environments (Kritzberg et al. 2004, Rösel & Grossart 2012, Teeling et al. 2012), investigations of large freshwater inflow events that describe successional changes in bacterial communities in lotic systems, particularly of the magnitude we observed in the Hunter River, are rarer. However, extrinsic, climate-related factors have previously been identified as the best predictors of seasonal microbial community patterns, whereby in a 3 year temporal study of 2 independent temperate rivers temperature and flow rates were found to be key drivers of bacterial diversity (Crump & Hobbie 2005). However, the rapid, short-term physical changes caused by large-scale floods likely override these seasonal patterns (Junk et al. 1989, Tockner et al. 2000) and lead to temporary restructuring of bacterial communities. A possible driver of rapid compositional shifts is the input of allochthonous substrate into rivers during flood events, which subsequently lead to rapid successional changes in microbial assemblages (Mašín et al. 2003, Chung et al. 2014, Santos et al. 2014).

Substrate-controlled succession occurs in bacterioplankton communities when new pulses of resources become available and are subsequently depleted by different members of the microbial assemblages. Following pulse inputs of chemical resources, specialist copiotrophic microbes that may normally persist at low abundances rapidly exploit substrate pulses and

increase in abundance (Lauro et al. 2009, Patel et al. 2014). The subsequent production of secondary metabolites and mineralised nutrients can then fuel the rise of other groups of microbes. Alternatively, as the original resource pulse is exhausted or physico-chemical conditions change the microbial community shifts further. We propose that major river inflow events provide allochthonous fuel which catalyses successional processes within river ecosystems.

Taking our data into consideration, we suggest that a typical lotic microbial response to a major inflow or flood event would be as follows: The massive introduction of allochthonous DOC and inorganic nutrients during a flood event is followed by an increase in the activity and abundance of heterotrophic bacterial populations as has been reported in several previous investigations of bacterial productivity in coastal aquatic environments (Pinhassi et al. 2006, Hitchcock et al. 2010, Pollard & Ducklow 2011). The concomitant increase in turbidity levels during the flood will restrict photoautotrophic activity, leading to a reduction in cyanobacterial and phytoplankton biomass (Henley et al. 2000). This shift in community metabolism towards heterotrophy will support the establishment of anoxic conditions and the subsequent increase in anaerobic bacterial communities (Walsh et al. 2004, Diaz & Rosenberg 2008, Zhang et al. 2010). In addition, the flood event will likely lead to the transport of non-endemic bacterial groups and anaerobic bacteria including sulphate-reducing bacteria (Miletto et al. 2008) from surrounding terrestrial sources within the catchment, into the river environment (Edwards & Meyer 1986, Crump & Hobbie 2005). Following the flood event, during the river's recovery phase, the exhaustion of allochthonous DOC and the gradual reduction in turbidity levels will allow for the re-establishment of photoautotrophic microbes, which will likely also benefit from the pool of remineralised nutrients resulting from the heterotrophic bloom during the flood. These results reveal the importance of river inflow events and floods as a significant driver of physico-chemical conditions which subsequently influence spatiotemporal patterns in the composition of bacterial communities, ultimately shaping the ecology of river ecosystems.

## CONCLUSIONS

Inflow events are likely a fundamental driver of microbial community dynamics within river ecosystems, but to date have been largely neglected. The results of this study illustrate that high inflows and

black water events can lead to profound changes in water chemistry and nutrient profiles, which stimulate sharp increases in bacterial cell abundance and phylogenetic shifts in BCC. The changes in BCC observed here are indicative of a substrate-controlled succession process, providing evidence that medium to large freshwater inflow events are an important driver of spatiotemporal heterogeneity in the bacterial communities of rivers. This research provides fundamental insights needed to understand how transportation of allochthonous nutrients via inflow events influence the bacterial communities at the base of riverine food webs, and what implications this may have for lotic ecological health.

*Acknowledgements.* We acknowledge the contributions of the New South Wales Office of Water and thank them for providing nutrient data and the use of their boat for sample collection. This project was funded by the University of Technology, Sydney - School of Environment (SoE), and the Plant Functionality and Climate Change Cluster (C3). This project was funded by the Australian Research Council Grants DP120102764 and FT130100218 to J.R.S.

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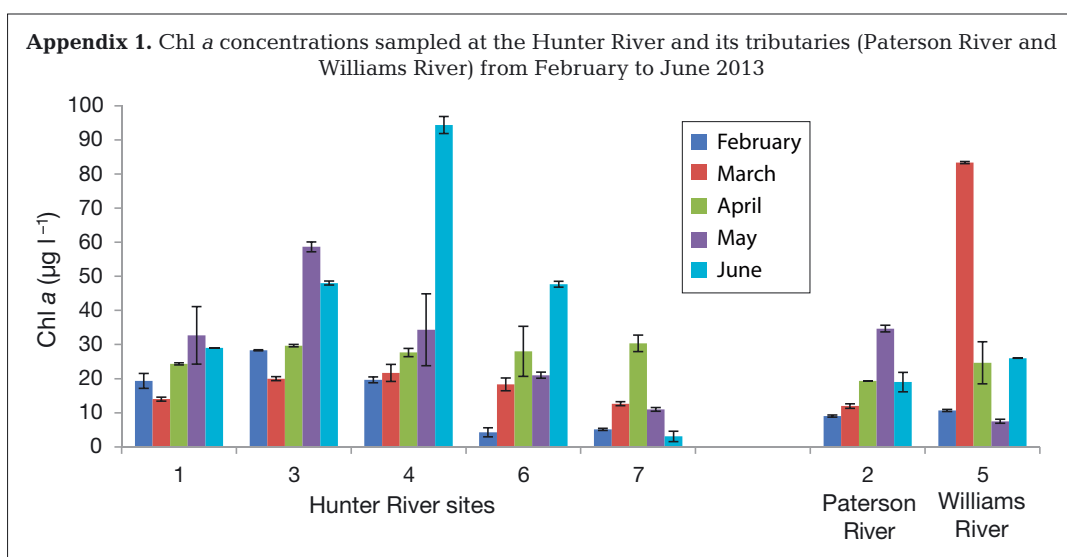
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Editorial responsibility: Tom Battin,  
Vienna, Austria

Submitted: August 4, 2014; Accepted: April 22, 2015  
Proofs received from author(s): June 24, 2015