Rehabilitating the Snowy River: The influence of environmental flow releases on dissolved organic carbon supply and utilisation

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Certificate of Original Authorship

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

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Preface

This thesis consists of six chapters. Chapters 2 to 5 have been written as separate articles that have either been published or are in preparation for submission to peer reviewed scientific journals. These papers are included as or close to their published or submitted form, and as a result, some repetition occurs. To prevent unnecessary duplication, a single reference list has been provided at the end of this thesis.

This thesis is a compilation of my original work, carried out with guidance from my academic and industry supervisors. I conceptualised this research, carried out the majority of the data collection and analysis, and wrote the manuscripts. The details of the publications arising from this thesis are provided below, and co-author contributions have been specified.

Chapter 2: Rohlfs, A., Mitrovic, S. M., Williams, S. and Coleman, D. 2015. Can tributary in-flows improve DOC regime recovery in a snowmelt river regulated by a large reservoir? *Marine and Freshwater Research*. 67 (9), pp 1338-1345, doi:10.1071/MF14230

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List of Abbreviations

ALPHA	α-1,4-glucosidase
BETA	β-1,4-glucosidase
BU	Butyl esterase
CR	Community respiration
DOC	Dissolved organic carbon
EEA	Extracellular enzyme activities
ETS	Electron transport system
FI	Fluorescence index
GPP	Gross primary productivity
INT	Iodonitrotetrazolium
LEU	Leucine amino-pepidase
NO_x	Oxides of nitrogen
PCO	Principal coordinates analysis
PHOS	Alkaline Phosphatase
SMS	Snowy Mountains Hydroelectric Scheme
SFRMM	Snowy Flow Response Monitoring and Modelling program
SRIF	Snowy River Increased Flows
SRP	Soluble reactive phosphorus
TN	Total nitrogen
TP	Total phosphorus
XYL	β-xylosidase

Abstract

Environmental flows are the quantity, timing and quality of water flow required to sustain and protect ecosystem and social values. Environmental flows delivered as managed water releases from large reservoirs often form the basis of rehabilitation programs in regulated rivers, and may benefit the aquatic food web by mobilising basal food resources, including dissolved organic carbon (DOC). However, the linkages between managed water releases, organic carbon delivery and microbial metabolic responses remain poorly understood. This thesis aimed to examine and compare the influence of dam and tributary water releases on DOC transport and microbial utilisation using field monitoring and manipulative mesocosm studies in the Snowy River in south-east Australia.

Field monitoring revealed positive, linear relationships between DOC concentration and discharge in the unregulated tributary that were absent directly downstream of the dam, and re-emerged below the tributary confluence. Variability in tributary DOC concentration was dampened downstream of a weir facilitating diversions of tributary water. These water diversions prevented approximately 80% of annual tributary DOC export from reaching the main stem. Tributary water releases supplied rapid pulses of terrestrial DOC to the regulated main stem, whereas dam releases produced low, constant DOC concentrations, and mobilised a mixture of terrestrial and microbial DOC.

The mesocosm studies showed that benthic microbial communities can respond rapidly to hydrologically-driven variations in DOC and nutrient regimes, providing a potential mechanism through which environmental flows may trigger increased rates of microbial processing. In the field mesocosm study, rapid, short duration increases in benthic metabolic respiration occurred following exposure to high-flow dam release waters. A manipulative laboratory study simulating different DOC regimes on benthic substrates found that a faster terrestrial DOC input rate facilitates stronger effects on microbial enzyme expression and bacterial taxonomic structure relative to press and control treatments.

This thesis contributes to a more comprehensive understanding of organic carbon supply and utilisation in regulated rivers, as well as the ecological mechanisms linking resource supply regime and biotic processes. These results reveal the considerable scope for

dissolved organic matter in river flows to be actively managed through environmental water delivery. In particular, these results support the wider implementation of tributary environmental water releases in river rehabilitation programs.

Chapter 1: General Introduction

1.1 Research scope and significance

Over half of the world's major rivers are regulated by impoundments that capture and store stream flow, assisting in flood mitigation, hydro-electricity generation and the provision of fresh water for agriculture, industry and human consumptive use (Nilsson *et al.*, 2005). While river regulation is critical to meeting human economic and social needs, impoundment and extraction of river flow has also contributed to a substantial decline in the ecological condition of rivers worldwide. Increasingly, scientists and river managers are modifying dam operations (Richter & Thomas, 2007) as strategy to improve ecological outcomes in regulated rivers and protect the economic, social and cultural values of our waterways into the future.

The Snowy River in South East Australia is currently the focus of an environmental rehabilitation program established to address the ecological impacts of a large, multiple-reservoir hydroelectric and irrigation diversion network. The Snowy Flow Response Monitoring and Modelling (SFRMM) program was initiated in 2000 to assess ecological responses to environmental flows. The SFRMM incorporates an adaptive management process that provides a unique opportunity to test hypotheses regarding the mobilisation of organic carbon and nutrients in response to environmental flow releases. An additional knowledge gap in this system is the extent to which tributaries contribute carbon and nutrients to the Snowy River downstream of the Snowy Mountains Hydroelectric Scheme (Rohlfs *et al.*, 2015).

Rehabilitation and restoration efforts in regulated systems like the Snowy River commonly focus on environmental flows, which are the quantity, timing and quality of water flows required to sustain and protect ecosystem and social values. Programs monitoring ecological responses to environmental flows have historically favoured metrics of community structure over ecological processes (Poff & Zimmerman, 2010) such as stream metabolism or decomposition (Young, Matthaei & Townsend, 2008). This emphasis belies the importance of ecological processes in supporting the abundance and composition of

species inhabiting a particular location. Environmental flows may only be effective if their delivery is guided by a comprehensive understanding of the key ecological processes underpinning recovery pathways of target populations (Growns, 2016), many of which are still not well clarified.

Environmental flow programs commonly aim to create a hydrological flow regime that supports a desired benthic habitat structure (Coleman & Williams, 2016), and provides abiotic environmental cues for target biota; for example, maintaining gravel beds and appropriate water temperatures to support fish spawning (Nelson, Dwyer & Greenberg, 1987). However, recent studies suggest that environmental flows may also modify the sources and transport of resources available for uptake into the food web (Cross *et al.*, 2011). For the purpose of this thesis, the term basal resources is used to describe the primary resources present in aquatic ecosystems, including organic carbon, and the major elements nitrogen and phosphorus (Elser & Hessen, 2005). Although conceptualised here as a combined pool, individual elemental resources are present in a wide variety of chemical forms (McDonald *et al.*, 2004) and their cycling is often closely interlinked (Lutz *et al.*, 2011). Differences in resource chemical composition and stoichiometry exert a strong influence on the efficiency of resource use by consumer organisms (Cross, Wallace & Rosemond, 2007; Hall *et al.*, 2012; Marcarelli *et al.*, 2011). This complexity must therefore be considered when investigating the implications of flow-mediated changes in basal resources for aquatic consumers.

Environmental flows may be used to manipulate both the quantity and quality of basal resources supplied to regulated rivers, and could offer potential benefits to target biotic communities and ecosystem process rates (Cross *et al.*, 2013). This approach rests upon the crucial role of hydrology in regulating resource transport and biological uptake (Newbold *et al.*, 1981; Moran *et al.*, 2014), which are often disrupted by the hydrological changes associated with river regulation (Miller, 2012). However, we still have a limited understanding of how impoundments affect organic carbon and nutrient transport, let alone the mechanisms by which environmental flow releases may influence resource uptake into the food web. Filling key knowledge gaps in this area would assist ecologists and river managers in predicting if and how environmental flows may be used to influence the supply and transport of basal resources in regulated rivers. Furthermore, river managers often face

pressure to justify allocating water to the environment that could be used for alternative purposes (Arthington & Pusey, 2003). Clarifying how environmental flows may modify basal resource availability, and the potential trophic consequences, may assist in demonstrating added ecological value from a limited amount of environmental water. From a broader perspective, the cumulative effect of river impoundments on carbon cycling may alter the global carbon flux from inland waters to the atmosphere (Friedl & Wuest, 2002; Regnier *et al.*, 2013). Further exploration of the effects of river impoundment on carbon storage and mineralisation will assist in improving estimates of the contribution of freshwater systems to global carbon budgets.

The scope of the following review focuses on upland rivers, reflecting the nature of the study catchment in this thesis. In formulating hypotheses regarding regulated rivers, it is informative to first examine existing models of basal resource flows and organic carbon uptake in unmodified rivers. The following sections outline the major hydrological processes driving organic carbon supply, microbial carbon use and the mechanisms of how river regulation is likely to affect these processes.

1.2 Hydrologic connectivity in river networks

Hydrologic connectivity is fundamental to the ecological integrity of river networks as it mediates the transfer of matter, energy and organisms between different ecosystem compartments (Pringle, 2003). Several key concepts in riverine ecology emphasise various aspects of hydrologic connectivity and their role in shaping resource availability. The River Continuum Concept highlights the significance of longitudinal connectivity, whereby upstream resource inputs and biotic processing can regulate the composition of organic matter available to the downstream community (Vannote *et al.*, 1980). This notion is further explored in the nutrient spiralling concept (Elwood *et al.*, 1983; Webster, Waide & Patten, 1975), which visualises the effect of stream flow as a longitudinal movement superimposed on the biogeochemical cycling of carbon and nutrients. Accordingly, streams with a high biological processing efficiency have tighter nutrient spirals, and streams with less efficient processing capacity have looser nutrient spirals, making them 'leakier' systems. Changes in biological processing efficiency can therefore alter the quantity and quality of

resources available to downstream communities. The river channel is also laterally connected to the broader landscape through riparian (Findlay *et al.*, 2001), hillslope (McGlynn & McDonnell, 2003) and groundwater (Fisher, Sponseller & Heffernan, 2004) flowpaths. Periodic inundation mobilises carbon and nutrients from beyond the river channel, which can form significant resource subsidies to the main channel and downstream river sections (Junk, Bayley & Sparks, 1989).

The degree of longitudinal and lateral connectivity within a river network varies with stream flow dynamics. Temporal flow variability is integral to riverine ecological functioning and can be understood through the paradigm of the natural flow regime (Poff *et. al.*, 1997). The natural flow regime describes the characteristic pattern of stream flow quantity, timing and variability (Palmer, Ambrose & Poff, 1997). Flow regimes differ between individual rivers but generally exhibit a pattern of relatively consistent base-flows interspersed with high-flow peaks associated with precipitation events (Poff *et al.*, 1997). These high-flow events often represent periods of strengthened longitudinal and lateral connectivity due to increased flow rates and an enlargement in the wetted area of the channel (Fisher *et al.*, 1998; Essington & Carpenter, 2000).

In montane systems, the seasonal snowmelt can also regulate lateral connectivity to the surrounding catchment, as terrestrial material is mobilised and transported to the river channel (Boyer *et al.*, 2000). Although snowmelt effects on river connectivity have been relatively well established in northern hemisphere catchments, Australian snowmelt rivers have been poorly studied. Relative to other continents, Australian rivers experience particularly high levels of flow variability (Franks & Kuczera, 2002), linked to multi-decadal climatic cycles driven by the El Nino Southern Oscillation (Verdon *et al.*, 2004). In the context of this higher flow variability, further research is needed to establish the role of the seasonal snowmelt in driving DOC fluxes in Australian montane rivers.

1.3 The role of aquatic organic carbon

Organic carbon may be present in stream water as particulate organic carbon (POC) or in solution (<0.45um) as dissolved organic carbon (DOC) (Thurman, 1985). While the magnitude of DOC and POC fluxes varies greatly between rivers (Hope, Billett & Cresser, 1994), DOC generally constitutes the largest proportion of total organic carbon export in

permanently flowing streams and rivers (Ludwig, Probst & Kempe, 1996; Dawson *et al.*, 2002; Moreira-Turcq *et al.*, 2003). Accordingly, this thesis focuses on the DOC pool, which in upland rivers are generally dominated by allochthonous, or terrestrial DOC originating from vegetation and soils in the surrounding catchment (Aitkenhead-Peterson, McDowell & Neff, 2003). The net transfer of terrestrial DOC to river networks represents a major cross-ecosystem energy subsidy from terrestrial to aquatic environments (Giling, Mac Nally & Thompson, 2015). These terrestrial subsidies are hydrologically driven, with low, consistent DOC concentrations persisting at base-flows (Boyer *et al.*, 1997; Wong & Williams, 2010), and rapid, transient DOC pulses occurring during precipitation events (Buffam *et al.*, 2001; Hornberger, Bencala & McKnight, 1994). High-flow events supply fresh, terrestrial DOC from the surrounding catchment (Mulholland, 1997) and are responsible for transporting the majority of a stream's annual DOC export (Buffam *et al.*, 2001; Boyer *et al.*, 1997; Raymond & Saiers, 2010). DOC concentration may also exhibit broader peaks associated with longer duration terrestrial inputs such as seasonal leaf-fall or the spring snowmelt (Boyer *et al.*, 1997; Agren *et al.*, 2007). Despite the existence of widely recognised mechanisms governing terrestrial DOC mobilisation, each river network is still unique with respect to the sources, magnitude and timing of the terrestrial DOC inputs it receives (Sertic Peric *et al.*, 2015). Attempts to consider the implications of DOC variability for specific management and restoration activities must therefore be guided by an empirical understanding of the DOC fluxes and underlying hydrology of the target river system.

DOC is also of particular ecological and management interest due to its active role in numerous biogeochemical processes (Jaffe *et al.*, 2008). DOC compounds such as humic and fulvic acids attenuate ultraviolet light, thereby regulating photic zone depth and in-stream primary production (Lean, 1998). DOC may also form complexes with trace metals, affecting their speciation and bioavailability, with significant implications for the transport and fate of metal contaminants (Aiken, Hsu-Kim & Ryan, 2011). An additional management concern regarding DOC is its ability to react with chlorine to form carcinogenic disinfection by products in treated water (Platikanov *et al.*, 2010). For this reason, DOC has generally been regarded as an undesirable solute by river managers, who have directed management efforts towards removing DOC from surface waters prior to treatment (Sadiq & Rodriguez, 2004).

However, DOC is also a major basal food resource in riverine environments, and represents a currency of energy flow through the aquatic food web (Tank *et al.*, 2010). Emerging research suggests that terrestrial DOC in particular supports the productivity of metazoan consumers such as macroinvertebrates (Jonsson & Stenroth, 2016) and zooplankton (Hitchcock *et al.*, 2016). This trophic perspective offers considerable scope to broaden the relevance of DOC to river management beyond considerations centred on its chemical reactivity. For example, the stimulation of ecological processing by DOC mobilised during extreme events has relevance for river restoration programs (Reich & Lake, 2015). Further clarification of the trophic implications of DOC variability is also required to support decision-makers in balancing the potential trophic benefits against the risks of undesirable biochemical outcomes.

1.4 DOC composition and bioavailability

DOC bioavailability is determined by its chemical composition, which in turn is highly dependent on the source of the DOC molecules (Findlay & Sinsabaugh, 1999). Autochthonous DOC produced by in-stream algal and macrophyte photosynthesis is dominated by low molecular weight compounds such as sugar monomers and amino acids (Sun et al. 1997, Bertilsson et al. 2003). In contrast, terrestrial DOC transported into the water column contains a large proportion of higher molecular weight humic and fulvic acids (Aitkenhead-Peterson, McDowell & Neff, 2003). Based on these chemical differences, terrestrial DOC was originally considered less bioavailable to microbes than autochthonous DOC, due to the increased energetic cost of metabolising larger, more complex molecules (Findlay & Sinsabaugh, 1999; Arnosti, 2003). It is now widely recognised that terrestrial carbon does in fact support considerable heterotrophic production, although it is utilised less efficiently in comparison to autochthonous DOC (McDonald *et al.*, 2007; Fasching *et al.*, 2014).

Numerous factors contribute to variability in DOC composition and bioavailability as it is transported through the river network. Hydrological processes govern the mobilisation of distinct DOC source pools, and drive spatial and temporal changes in DOC composition in a wide range of environments, from alpine streams (Fasching *et al.*, 2016) to forested temperate headwaters (Wilson *et al.*, 2016) to large subtropical rivers (Yang *et al.*, 2013).

Exposure to solar radiation also induces the decomposition of DOC molecules, altering their bioavailability to heterotrophic bacteria (Abboudi *et al.*, 2008; Howitt *et al.*, 2008). Furthermore, the influence of microbial degradation itself can strongly modify DOC composition (Fasching *et al.*, 2014), and has been described in the size-reactivity model of (Amon & Benner, 1996). This model proposes a bioavailability continuum whereby DOC bioavailability decreases with age, as the more easily metabolised functional groups are progressively stripped from larger molecules over time. However, more recent research from a geologically ancient, arid landscape found that older groundwater DOC was more bioavailable than more recently fixed DOC (Fellman *et al.*, 2014). These findings directly contradict the model of Amon and Benner (1996) and illustrate the need to exercise caution when extrapolating models beyond the biomes in which they were developed. Such contradictory trends in DOC bioavailability may arise from localised differences in catchment attributes such as terrestrial primary productivity (Wilson *et al.*, 2013), soils (Autio *et al.*, 2016) and land use (Petrone, Richards & Grierson, 2009). The lack of more generalised conceptual or predictive models of DOC composition and bioavailability highlights the need to collect additional data from systems that are under-represented in the literature, including the montane Australian streams that form the focus of this thesis.

A further challenge to establishing broader relationships between DOC composition and bioavailability is the high chemical heterogeneity of the riverine DOC pool, which precludes a systematic classification of DOC composition (Filella, 2010). A wide variety of methodologies have been used to fractionate various pools of riverine DOC based on its physical (Findlay & Sinsabaugh, 1999), chemical (Sun *et al.* 1997), or optical properties (Sanderman *et al.*, 2009; Hood, McKnight & Williams, 2003). Fluorescence spectroscopy is one such technique that offers a rapid method of characterising DOC composition, based on the fluorescent DOC fraction (McKnight *et al.*, 2001; Gabor *et al.*, 2014). DOC sources can be inferred by comparing the sample fluorescence patterns to those of known model substrates, whereby humic acid-like peaks can indicate terrestrial DOC and amino acid-like peaks can indicate microbial, autochthonous DOC (Fellman, Hood & Spencer, 2010). Fluorescence-based methods have provided numerous new insights into the spatiotemporal patterns of DOC composition in a wide diversity of aquatic systems (e.g Inamdar *et al.*, 2011; Fellman, Petrone & Grierson, 2011). In some cases, fluorescence characteristics have been

found to correlate strongly with DOC bioavailability, as measured by bacterial biomass turnover time (Kaartokallio *et al.*, 2016). However, other studies have failed to find consistent relationships between DOC fluorescence characteristics and bioavailability (Baldwin & Valo, 2015), illustrating the need for caution when interpreting fluorescence data. Nevertheless, fluorescence methods have useful management applications in tracing different DOC sources, for example, in tracking sewage inputs (Baker & Inverarity, 2004). There is considerable scope to employ fluorescence methodologies more widely in management and rehabilitation applications, including tracing DOC sources mobilised by environmental flow releases.

1.5 Linking microbial processing, resource supply and hydrology

DOC is a primary metabolic substrate for aquatic heterotrophic microbes such as bacteria, fungi and archaea, which employ a range of strategies to exploit the ambient DOC supply. Bacteria and fungi respond to DOC variability by rapidly synthesising extracellular enzymes targeting specific DOC molecules and functional groups, such as carbohydrates, amino acids and phenols (Thurman, 1985; Arnosti, 2003). The resulting patterns of enzyme expression can be used to identify which components of the DOC pool may be limiting bacterial metabolism (Romani & Sabater, 1999; Clinton, Edwards & Findlay, 2010). Bacteria may also respond to qualitative variations in the DOC supply through shifts in community taxonomic composition (Findlay *et al.*, 2003). Bacterial communities contain a substantial reserve of taxonomic and functional capacity, and may shift to favour taxa that are better able to metabolise a given DOC source. Several studies have shown shifts in community structure on exposure to new DOC sources (Covert & Moran, 2001; Judd, Crump & Kling, 2006). Furthermore, the response of bacterial activity and community composition to changes in DOC quality has been used to explore broader ecological questions surrounding the interrelationship between structure and function (Judd, Crump & Kling, 2006). Heterotrophic microbial transformation of aquatic DOC contributes to several fundamental ecosystem processes. Microbes are primary agents of organic matter decomposition (Lindeman, 1942), and microbial mineralisation of organic matter to carbon dioxide often represents a large contribution to net ecosystem metabolism (Cole *et al.*, 2000). At a global scale, the carbon mineralised through microbial respiration is a significant pathway of

carbon transfer from freshwater systems to the atmosphere (Algesten *et al.*, 2004; Cole *et al.*, 2007). Microbes also facilitate organic carbon uptake in to the aquatic food web through detrital food webs (Wetzel, 1995). These food webs provide a pathway of energy flow to consumers at higher trophic levels (Hall & Meyer, 1998), particularly in smaller streams and shaded upland rivers, where terrestrial carbon inputs are likely to be high relative to in-stream carbon fixation (Meyer, 1994; Wilcox *et al.*, 2005).

DOC variability may therefore exert a bottom-up control on spatial and temporal variability in microbial process rates (Lennon & Pfaff, 2005). McClain et al. (2003) explore the linkages between resource variability and biotic process rates using a conceptual model of biogeochemical 'hot moments' (Fig 1.2). McClain et al. (2003) define hot moments as 'short periods of time that exhibit disproportionately high reaction rates relative to longer intervening time periods'. These periods are triggered by the supply of a missing or limiting reagent, frequently via a hydrological flow-path (Fig 1.2).

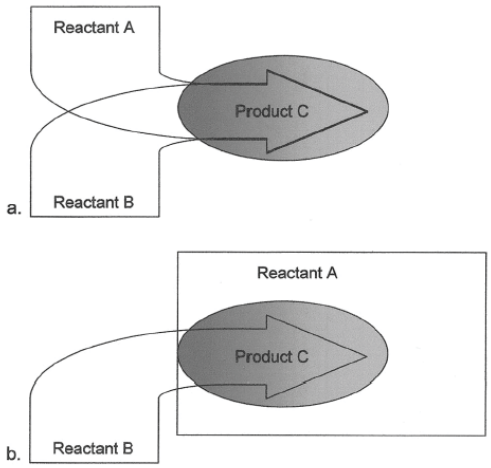


Figure 1.2: Hot spots and hot moments occur when limiting reactants are combined through a) two flow-paths converging or b) when a flow path carries one reactant into a location where another is present (McClain et al. 2003).

Empirical evidence from field observations and manipulative studies further supports the notion that hydrologically-driven DOC fluctuations have the capacity to trigger microbial hot moments. Increased community respiration rates may be examples of hot moments triggered by DOC pulses transported during flood events (Zoppini *et al.*, 2010). Hot moments may also occur in benthic habitats, with high rates of epilithic bacterial enzyme

activity coinciding with peaks in DOC concentration during high-flow events in a Mediterranean stream (Romani & Sabater, 1999). Further evidence of DOC-mediated microbial hot moments can be seen in a study by Lennon & Cottingham (2008), who amended lake mesocosms with equivalent doses of leaf leachate, added as either one single pulse treatment, or as a series of daily 'press' treatments. Cumulative bacterial productivity under the pulse treatment was two to five times higher, depending on DOC source quality (Lennon & Cottingham, 2008). The pulsed DOC additions used in this experiment are analogous to the pattern of DOC input that occurs in streams during storm flows, suggesting that the high rates of DOC input provided by hydrological events are important in stimulating biogeochemical hot moments.

1.6 DOC in regulated rivers

Reservoir construction imposes artificial lakes on the stream network, disrupting longitudinal organic matter transport (Ward & Stanford, 1983). During prolonged storage within the lake, DOC may be removed from the river network through microbial degradation or sequestration in bottom sediments (Acuna & Tockner, 2010; Cole *et al.*, 2007). These processes may substantially reduce DOC export from lake outflows (Algesten *et al.*, 2004; Larson *et al.*, 2007). Primary production within lakes may also contribute highly bioavailable algal DOC to the downstream river, producing a detectable difference in DOC composition (Hood, Williams & McKnight, 2005 and references therein). Additionally, river regulation homogenises the natural flow regime (Poff *et al.*, 1997), often decreasing the frequency and magnitude of high-flow events (Poff & Zimmerman, 2010). The reduction or loss of high-flow events may reduce lateral DOC inputs to the downstream river by inhibiting floodplain inundation and the expansion and contraction of the wetted channel area. Reservoirs may also temporally disconnect downstream rivers from seasonal DOC inputs, such as the spring snowmelt (Larson *et al.*, 2007; Boyer *et al.*, 1997).

Tributary inflows contribute to the recovery of many water quality gradients downstream of impoundments and help restore longitudinal connectivity to headwater streams (Stanford & Ward, 2001; Growns *et al.*, 2009). Additionally, inflowing tributary water is an important source of organic and immigrant biota (Kiffney *et al.*, 2006), which can affect ecological

functions in the regulated main stem (i.e. the primary channel downstream), such as stream metabolism and invertebrate recruitment. There is considerable scope for tributary inflows to be used to facilitate a more natural pattern of DOC quantity and variability below dams, as smaller headwater streams tend to be tightly connected to hydrologically-driven terrestrial DOC inputs (Agren *et al.*, 2007). However, the relative influence of a given tributary on main-stem water quality is dependent on the ratio of tributary to main stem discharge (Benda *et al.*, 2004), which may also vary temporally with precipitation (Wallis, Mac Nally & Lake, 2009). Further studies of tributaries feeding regulated rivers are required to determine the extent to which tributaries may influence DOC dynamics below large reservoirs, as well as the associated ecological implications of management actions that modify tributary inflows to regulated rivers.

1.7 Study area

This study was carried out in the montane reaches of the Snowy River, an iconic river of high economic and cultural significance. The Snowy River is one of the largest snowmelt rivers in Australia; it rises near Mount Kosciuszko in the Australian Alps, and flows for approximately 350 km to join the Tasman Sea in Victoria (Fig 1.3). The sites used in this study were located near the town of Jindabyne (-36°24’58”, +148°37’30”) in southern NSW. The Snowy River has been regulated by the Snowy Mountains Hydroelectric Scheme (SMS), a large hydroelectricity and irrigation diversion scheme since 1967. The SMS in the Snowy River catchment consists of four large reservoirs and a network of aqueducts, weirs and tunnels that capture and redirect snowmelt inland to the Murray and Murrumbidgee catchments. Jindabyne Dam is the most downstream reservoir on the Snowy River, and has a maximum capacity of 894.1 GL, a mean depth of 37 m, and was retrofitted with a multi-level offtake in 2005 that delivers epilimnial water from the top 5 m of the dam.

Erskine and Webb (2000) classified the Snowy River below Jindabyne into nine distinct geomorphic macro-reaches. The three geomorphic reaches down to the Delegate River junction (i.e. Jindabyne Gorge, Dalgety Uplands and the Burnt Hut Gorge) are the reaches most severely impacted by the Snowy Scheme, and comprise the spatial extent of this study (approx. 90 km below Jindabyne Dam).

The Jindabyne Gorge is a high gradient reach, vegetated with native Eucalypt woodland. In the lower gradient depositional reach of the Dalgety Uplands (Coleman & Williams, 2016) the Snowy River is largely cleared for grazing interspersed with some forested areas, and the in-channel vegetation primarily comprises aquatic emergent macrophytes. Beyond the Dalgety Uplands, the Snowy River enters a wilderness area and flows through the Burnt Hut Gorge, which is the location of the most downstream site used in this study. The riparian zones at the Snowy River sites include a mixture of native eucalypt woodland, *Poa* spp, *Juncus* spp*., Melaleuca* spp. and invasive herbaceous weeds.

Some study sites were also located on the Mowamba River, which is the highest snowmelt tributary to join the Snowy River below Jindabyne Dam, with 15.1 km^2 and 5% of the catchment above the snowline. The Mowamba River joins the Snowy River approximately 2 km downstream of Jindabyne Dam, and is the first major tributary entering the Snowy River below the SMS. The Mowamba catchment is approximately 220 km^2 and is forested in the upper reaches but predominantly cleared for cattle grazing in the mid to lower catchment.

After the Mowamba River, the next major perennial river to join the Snowy River below Jindabyne is the unregulated Delegate River some 65 km downstream of Jindabyne Dam. The hydrology of the Delegate River is strongly influenced by winter rainfall and coastal weather patterns, rather than snowmelt processes. With the exception of the Mowamba and Delegate Rivers, most of the tributaries joining the Snowy River in the study area are strongly intermittent, due to the location of the Snowy catchment between Jindabyne Dam and the Dalgety Uplands in a rain shadow of the Australian Alps.

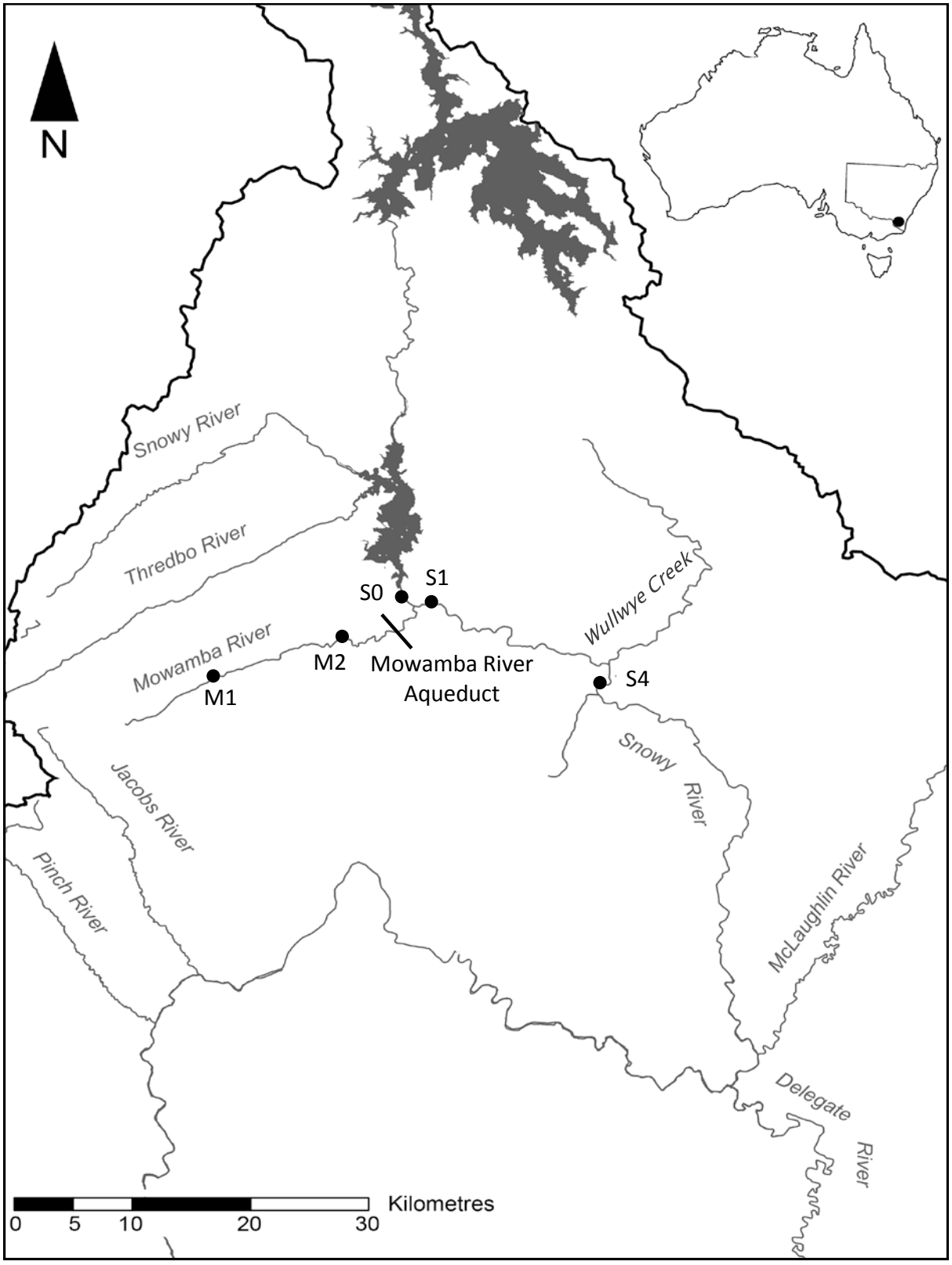


Figure 1.3. The study area showing Jindabyne Dam and the Upper Snowy River catchment. Study sites were located on the Mowamba (M) and Snowy (S) Rivers.

1.8 Snowy River environmental flows

Construction of the SMS heavily impacted the natural hydrology of the Snowy River, resulting in the diversion of 99% of mean annual natural flow below Jindabyne Dam. In particular, river regulation reduced the magnitude and frequency of high-flow events below Jindabyne Dam, including the seasonal winter rainfall and spring snowmelt floods. Consequently, the ecological health of the Snowy River declined considerably (Gilligan & Williams, 2008), including a loss of channel habitat area, accumulation of fine sediment (Coleman & Williams, 2016) and the encroachment of terrestrial vegetation into the river channel.

In 2002, the Commonwealth, New South Wales and Victorian governments formally set a new annual discharge target of up to 21% of the mean annual natural flow to the Snowy River. Delivery of environmental flows to the Snowy River was initially slow due to the gradual acquisition of water entitlements to provide the releases, and a severe drought in South East Australia, which broke in 2009. Increased licenced water allocations allowed for larger flushing releases to be made from Jindabyne Dam, which aimed to reinstate a spring snowmelt flood signal. This study coincided with the introduction of annual larger flushing flow events, which took place in spring 2010, 2011 and 2012 (Williams, 2015b).

The primary environmental flow releases to the Snowy River are typically sourced from Jindabyne Dam. Flows up to 5000 MLd^{-1} are released through a cone valve, and higher flow rates are released via radial gates down a spillway. The Mowamba River is a secondary environmental water source, which is regulated by a weir that diverts water through an aqueduct and into Jindabyne Dam (Fig 1.3). This configuration allows for a portion of the Snowy River environmental water allocation to be released via the Mowamba tributary instead of Jindabyne Dam. This is achieved by 'turning out' the aqueduct to suspend tributary water diversion, allowing 100% of natural tributary flow to overtop the weir and join the Snowy River directly, without being routed through Jindabyne Dam. The maximum diversion rate at the Mowamba aqueduct is 523 MLd^{-1}. This represents a flow percentile of 98% at the gauging station, and typically captures smaller to medium high-flow events.

1.9 Conceptual Framework

The riverine DOC pool is highly heterogeneous with respect to its spatiotemporal dynamics and chemical composition. In examining the effects of river regulation and environmental flows on the downstream DOC pool, it is useful to have a framework to aid in the comparison of specific aspects of the DOC supply under different management scenarios. For the purposes of this thesis, the term 'DOC regime' is defined as the pattern of quantitative and qualitative variation in the DOC pool of a given stream or river. The following framework includes aspects of the DOC regime that have been selected on the basis of their role in driving microbial metabolic activity rates. According to this framework, the DOC resource regime can be separated into three distinct components;

- *Quantity* refers to the amount of DOC present in a given waterway. The quantity of DOC available represents a theoretical maximum of the total amount of material present in the system that may be exploited by consumers. Measures of resource quantity can include the DOC load or mass, DOC export as a mass per unit time, or also the ambient DOC concentration at a given point in time.
- *Input rate* is the rate of change in ambient DOC concentration. Some studies indicate that faster DOC loading rates can be significant in triggering bacterial metabolic responses (section 1.6).
- *Composition* describes the chemical make-up of the DOC pool, which is linked to its bioavailability. Measures of DOC composition include direct assessment of its physical or chemical properties, as well as measurement of microbial metabolic activities to infer the bioavailability of either bulk stream water DOC or specific DOC substrates.

A conceptual model of two distinct types of DOC regime can be derived by considering the likely characteristics of each of these three components in unmodified and regulated rivers. In unregulated upland rivers, DOC is predicted to show low concentrations at base-flows, punctuated by rapid pulses during high-flow events, and DOC composition is likely to be predominantly terrestrial in origin. Downstream of a large dam, the DOC concentration is anticipated to reflect that of the lake water, show a smaller range of variability, and be

predominantly microbial in origin due to algal production in the dam. The DOC supply can be conceptualised as a variable natural DOC regime in an unmodified river, and as a more homogenous, regulated river DOC regime in reaches downstream of large reservoirs.

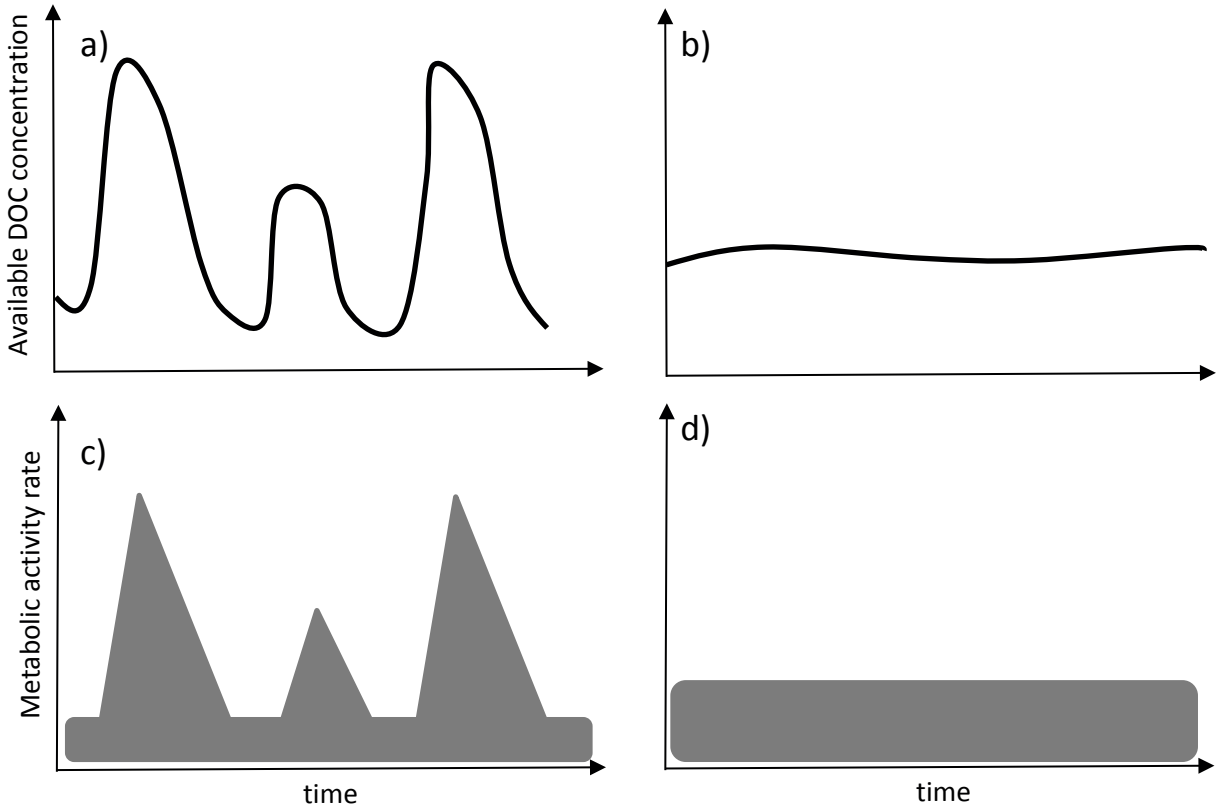


Figure 1.4. Conceptual model of a) a natural DOC regime and b) a regulated river DOC regime, and corresponding microbial process rates under c) natural and d) regulated regimes.

These distinct DOC regime types may also drive variability in heterotrophic microbial metabolic processes. Accordingly, the conceptual model of natural and regulated DOC regimes also addresses associated patterns of microbial metabolic activities. The theoretical basis for the proposed microbial responses to natural and regulated DOC regimes draws on the biogeochemical 'hot moments' concept of McClain et al. (2003). Under a natural resource regime, the rapid DOC inputs delivered during high-flow events trigger more frequent biogeochemical 'hot moments' of accelerated microbial processing. Conversely, the less variable but more bioavailable regulated DOC regime results in the absence or reduction of microbial hot moments, but potentially sustains higher background rates of microbial metabolic activity.

This conceptual model of natural and regulated DOC regimes forms a theoretical foundation for generating testable hypotheses regarding the linkages between hydrology, DOC dynamics and the microbial community. In regulated systems, environmental flow releases can be used to manipulate river discharge, providing opportunities to actively manage the DOC regime. For example, pulsed release events from reservoirs may have the capacity to trigger microbial hot moments by mobilising DOC pulses to the downstream river. In terms of the DOC regime model, delivery of pulsed dam releases can be conceptualised as a strategy to shift the DOC regime from a regulated state towards a natural state. Alternative environmental water sources such as tributaries may also be used to achieve a similar outcome by reconnecting the regulated river to DOC pulses transported from upstream. Ultimately, differing patterns of microbial processing arising from natural and regulated DOC regimes may have significant implications for broader ecosystem scale processes and the flow of basal resources to consumers at higher trophic levels.

1.10 Thesis aims and overview

The overarching objective of this thesis is to examine the effects of environmental flows on the transport and utilisation of DOC in the Snowy River. The separate studies within this thesis examine a range of scientific and management questions concerning various components of pulse and press DOC regimes. The specific aims and hypotheses are summarised below;

Chapter 2: Can tributary in-flows improve the recovery of the dissolved organic carbon regime in a snowmelt river regulated by a large reservoir?

This chapter aimed to identify whether tributary in-flows can increase DOC concentration, load and variability in the Snowy River downstream of Jindabyne Dam. This monitoring study also addressed river management requirements regarding the contribution of the Mowamba River tributary to the DOC regime in the Snowy River below Jindabyne Dam.

Hypothesis 1: DOC concentration and discharge below Jindabyne Dam are more strongly correlated downstream of the Mowamba tributary confluence than above the Mowamba tributary confluence.

Hypothesis 2: That tributary flow diversion at the Mowamba Weir significantly reduces annual tributary DOC delivery to the Snowy River below Jindabyne Dam relative to modelled natural tributary inflows.

Chapter 3: Dissolved organic carbon delivery from managed flow releases in a montane snowmelt river.

This study aimed to identify and compare the concentration, load and composition of DOC delivered to the Snowy River by managed high-flow release events made from a large reservoir and a tributary weir.

Hypothesis 1: DOC concentration and load in the Snowy River below Jindabyne Dam will increase during dam and tributary releases, relative to pre-event conditions.

Hypothesis 2: DOC composition will be altered and reservoir releases will contain protein-like DOC fluorophores whereas the tributary releases would be characterised by humic-like DOC fluorophores.

Chapter 4: Functional responses to environmental flows: does resource delivery from pulsed dam releases influence microbial metabolism?

In this study, we examined the potential role of shifting basal resource availability in driving benthic microbial metabolic activity by controlling flow velocity using a stream-side mesocosm system.

Hypothesis 1: Exposure to dam release water would significantly increase epilithic biofilm respiration

Hypothesis 2: Exposure to dam release water shifts the relative expression of carbon and nutrient-acquiring enzymes by river sediment bacteria.

Chapter 5: Terrestrial DOC supply regime effects on bacterial functioning and community structure in an epilithic biofilm

This laboratory mesocosm experiment employed a range of functional and structural metrics to distinguish potential mechanisms of bacterial response to differing DOC supply regimes. We performed a laboratory mesocosm experiment to investigate the effect of input rate on terrestrial DOC utilisation within an epilithic biofilm community.

Hypothesis 1: Addition of a terrestrial DOC source would significantly increase heterotrophic bacterial metabolic activity rates and shift bacterial taxonomic composition within the biofilm.

Hypothesis 2: A DOC treatment added at a rapid input rate would elicit higher rates of bacterial metabolic activities and a faster shift in bacterial community structure than an equivalent DOC treatment added at a slower input rate.

Chapter 6: General Discussion

To provide a general synthesis of the results of this thesis and suggest management recommendations and future studies based on these findings.

Chapter 2: Can tributary in-flows improve DOC regime recovery in a snowmelt river regulated by a large reservoir?

2.1 Abstract

Although tributary inputs can accelerate the recovery of many physical and chemical gradients below large reservoirs, their contribution to the dissolved organic carbon (DOC) regime in regulated rivers remains poorly studied. In some regulated tributaries, flow volumes can be manipulated, potentially influencing DOC supply to the main stem. This study examines how tributary water diversion affects DOC supply to a snowmelt river regulated by large reservoirs. DOC concentration was measured at tributary and main stem sites, and tributary DOC export was estimated under different tributary flow diversion scenarios. DOC concentration and discharge were not significantly related directly below the dam, but were positively correlated in the unregulated tributary, and below the tributary confluence. Irrespective of water diversion practices, tributary in-flows reconnected the regulated main stem to a more variable DOC regime driven by catchment flushing processes. However, tributary water diversion dampened the tributary signal by reducing DOC pulse frequency and total DOC export to the regulated river. These aspects of the DOC regime may influence basal resource availability and ecosystem functioning in the regulated main stem. This study illustrates how an ecologically valuable tributary function can be addressed and quantified to guide the management and rehabilitation of a regulated river system.

2.2 Introduction

Large dams disrupt the longitudinal river continuum by altering the hydrological flow regime, trapping sediment and interrupting nutrient spiralling (Ward & Stanford, 1983). Whilst reservoirs are known to block the downstream transport of particulate organic matter (Ward & Stanford, 1995), the influence of impoundments on longitudinal transport of the dissolved organic carbon (DOC) fraction has received less attention. This is despite DOC being the dominant form of organic carbon in many river ecosystems, and therefore

playing a key role in driving ecosystem metabolism (Hadwen *et al.*, 2010), structuring food webs (Wilcox *et al.*, 2005) and mediating inorganic nutrient cycling (Bernhardt & Likens, 2002).

Dams can potentially affect aquatic DOC dynamics through altering DOC quantity and delivery pattern. Whilst trapped in a reservoir for extended time periods, DOC can be consumed through heterotrophic respiration, photodegraded, and sequestered in benthic sediments (Cole *et al.*, 2007). These processes may contribute to decreased DOC concentrations in impoundment outflows; an effect which has been documented downstream of natural lakes (Larson *et al.*, 2007). The storm and snowmelt events responsible for delivering large pulses of terrestrial DOC into river systems are often reduced or eliminated by river regulation (Poff & Zimmerman, 2010), altering the input pattern, frequency and timing of organic matter delivery to the downstream river (Goodman, Baker & Wurtsbaugh, 2011).

The critical role of tributary in-flows in the recovery of rivers regulated by large reservoirs has long been recognised (Ward & Stanford, 1983). Among the benefits provided by tributaries are the inclusion of components of the pre-regulation flow regime, the supply of colonist species and increased habitat complexity in the tributary confluence zone (Rice *et al.*, 2008). Tributaries may also contribute to the recovery of dissolved constituents such as DOC, restoring a more natural regime where changed conditions due to dams are apparent. The impacts of large reservoirs on carbon supply, transport and uptake are rarely addressed in river management and rehabilitation programs (Stanley *et al.*, 2012). In affected areas below dams, regulated tributaries may be managed to provide a more natural DOC regime characterised by increased DOC concentration and temporal variability.

In 1967 the Snowy River in South-East Australia was reduced to approximately 1% of mean annual natural flow at Jindabyne, due to the construction of the Snowy Mountains Scheme, a multiple-reservoir hydroelectricity and irrigation system. Since 2002, environmental water releases have either occurred from the Jindabyne Dam, the most downstream reservoir in the Snowy River Catchment or the Mowamba River, the first tributary below the Jindabyne Dam. However, the mean concentration, load and variability of DOC in the Snowy River

below Jindabyne Dam has remained significantly lower than in unregulated reference streams (Coleman & Williams, 2016). Tributary in-flows below Jindabyne Dam could potentially increase DOC load, concentration and variability in the regulated Snowy River. However, DOC regime recovery through this mechanism may be limited because the first major tributary to join the Snowy River below the dam is regulated by the Mowamba Weir and Aqueduct, which diverts a large proportion of tributary flow (all flows < 12th percentile) to storage reservoirs.

This study aims to identify whether tributary in-flows can increase DOC concentration, load and variability in the Snowy River downstream of a large reservoir. An additional aim is to estimate the impact of tributary water diversion on tributary DOC export. We hypothesised that DOC concentration and discharge below the reservoir would be more strongly correlated downstream of the first major tributary confluence than above the tributary confluence. We further hypothesised that tributary flow diversion at the Mowamba Weir significantly reduces annual tributary DOC export, relative to modelled natural tributary in-flows.

2.3 Methods and materials

2.3.1 Study area and hydrology

The Snowy River extends from the Australian Alps to the southern Australian coastline (Fig 2.1). The upper catchment is predominantly montane to sub-alpine, with alpine areas at the highest elevations. Following several decades of heavy flow regulation, the introduction of environmental flow releases has gradually increased river discharge at Jindabyne from 1% to approximately 16% of mean annual natural flow in 2011. The Snowy River catchment below Jindabyne Dam is predominantly grazing land with native vegetation cover occurring at higher altitudes.

The Mowamba River is the first major tributary of the regulated Snowy River, joining it approximately 2 km downstream of Jindabyne Dam (Fig 2.1). The Mowamba River hydrology is primarily storm-driven, but it also receives a small spring snowmelt signal as approximately 5% of the catchment is above the snow-line of 1400 m. Land use in the Mowamba catchment is a mixture of undisturbed forest in the higher reaches and grazing

land towards the middle and lower reaches. The Mowamba River is regulated by the Mowamba Weir and an aqueduct that diverts tributary flows into Jindabyne Dam (Fig 2.1). Throughout the study period, the Mowamba Aqueduct had a maximum diversion rate of 6.05 m^3s^{-1}. The next Snowy River tributary is the rain-shadow affected, intermittent Wullwye Creek which joins the Snowy River about 22 km downstream of Jindabyne Dam.

2.3.2 Study design

Mowamba River sites M1 and M2 located upstream of the Mowamba Weir (Fig 2.1) were used to characterise DOC dynamics in the unregulated tributary. Three sites on the Snowy River were monitored to identify the influence of tributary in-flows on DOC dynamics in the main stem (Fig 2.1). Snowy River sites S0 and S1 are located in a steep, confined channel in the Jindabyne Gorge, about 0.5 km and 3 km from Jindabyne Dam respectively. Site S4 is 24 km downstream from Jindabyne Dam in the flatter, more sinuous Dalgety Uplands. Site S0 is upstream of the tributary confluence and sites S1 and S4 are about 1 km and 24 km downstream of the Mowamba River confluence. Site locations and hydrological attributes are specified in Table 2.1.

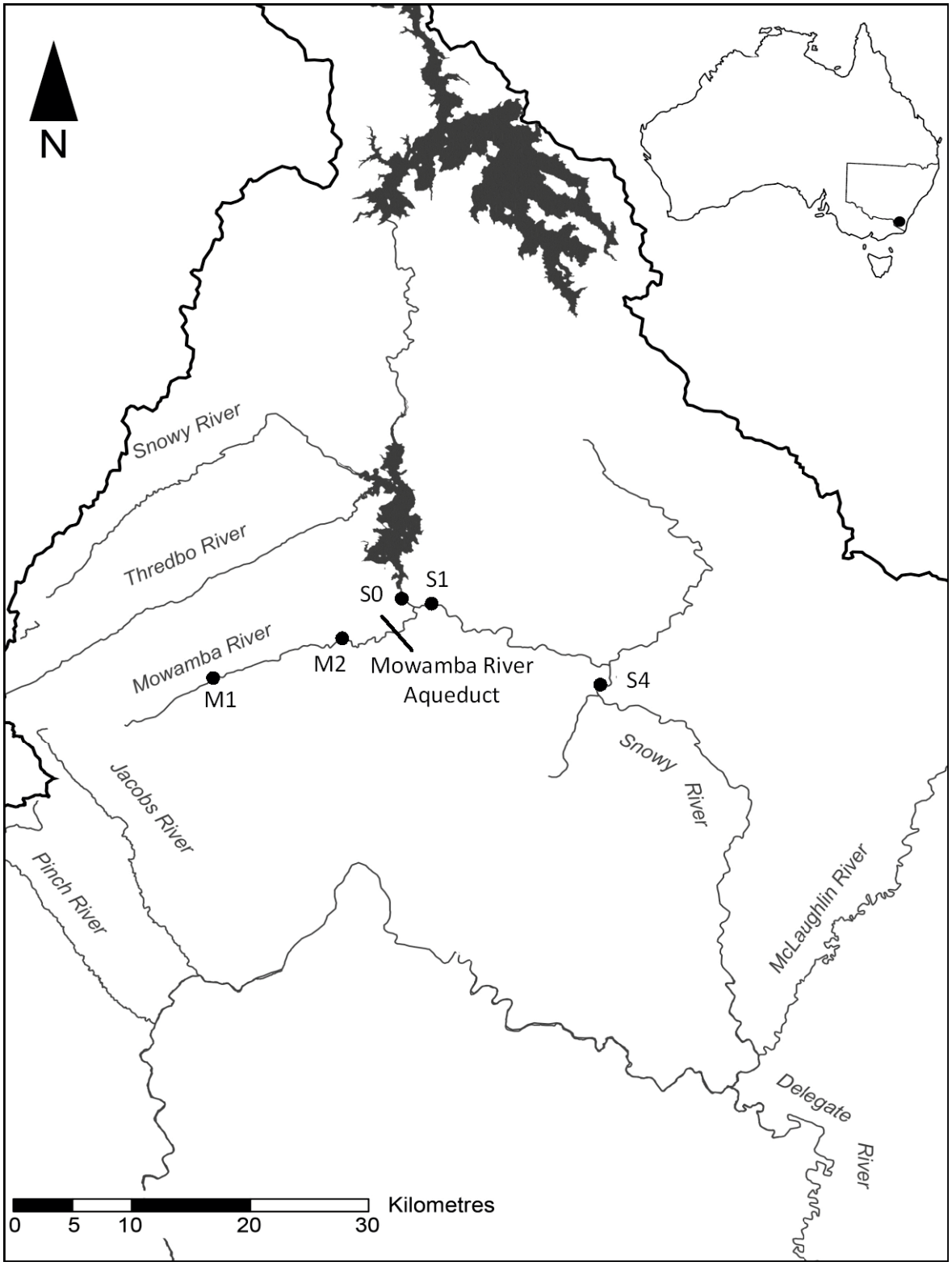


Figure 2.1: Map of study area showing tributary sites (M1, M2) and Snowy River sites (S0, S1, S4).

Table 2.1: Study site locations and hydrological attributes.

Site ID	Site name	Geographic Coordinates	Mean annual flow (m^3s^{-1})
Regulated sites			
S0	Snowy River downstream of Jindabyne Dam	36° 26' 25"S, 148° 38' 00"E	3.49
S1	Snowy River downstream of Mowamba River junction	36° 26' 46"S, 148° 39' 13"E	3.65
S4	Snowy River at Blackburn Creek	36° 31' 26"S, 148° 50' 18"E	3.98
Unregulated Sites			
M1	Mowamba River at Moonbah Hut	36°30' 5.9"S, 148° 28' 54"E	1.18
M2	Mowamba River at the Barry Way	36° 28' 43"S, 148° 35' 20"E	1.18

2.3.3 Sample collection

Snowy River discharge data were obtained from rising stage discharge gauges located at sites S0 (gauge 222 020, Snowy Hydro Ltd.) and S4 (222 026, NSW Office of Water). Discharge in the unregulated tributary was estimated by adding discharges recorded at rising stage gauges in the Mowamba aqueduct off-take (600 179, Snowy Hydro Ltd.) and below the Mowamba Weir (222 546, Snowy Hydro Ltd.). This calculation assumes constant storage in the Mowamba Weir pool. The influence of water diversion on high-flow event frequency was determined by enumerating the total number of high-flow events above and below the Mowamba Weir. High-flow events in the Mowamba River were arbitrarily defined as all events ≥ the 25th flow percentile, as measured at site M2. DOC was sampled at the Mowamba tributary sites M1 and M2 and at Snowy River sites S0, S1 and S4 approximately monthly to fortnightly from January 2010 to March 2012. DOC surface grab samples were collected in pre-combusted amber glass bottles from a well-mixed part of the stream. DOC samples were collected in triplicate and acidified in the field with 1 mL 2N HCl except for those collected prior to August 2010. DOC samples from January to August 2010 were collected as part of a separate monitoring program so only single, non-acidified DOC

replicates were taken. These data were still considered comparable as pilot studies showed negligible differences in DOC concentration between acidified and non-acidified samples (unpublished data), and because subsequent triplicates showed low variability (SE <0.2 mgL^{-1} DOC). Following collection, DOC samples were refrigerated, filtered to 0.45 µm and analysed by high-temperature combustion with a Shimadzu TOC-VCSH analyser (APHA, 2005).

2.3.4 Data analysis

All DOC data collected during high flow environmental water releases from the Jindabyne Dam were excluded to avoid bias from dilution effects. A mean was calculated where triplicate DOC samples were taken. For each site, DOC concentration and stream discharge data were natural log transformed, checked for normality and where normally distributed, linear correlations were performed using SPSS version 21 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA).

2.3.5 DOC export model

DOC concentration data from the unregulated Mowamba River site M2 were partitioned into snow-melt (August to November) and non-snowmelt (December to June) seasons. DOC concentration data and mean daily discharge at site M2 were natural log transformed and rating curves were developed from linear regression models (Cooper & Watts, 2002; Quilbe *et al.*, 2006). The rating curves were used to model daily DOC concentrations, which were multiplied by mean daily flow rate to generate daily DOC load estimates for the unregulated Mowamba River, and for the aqueduct off-take. Daily DOC estimates were summed to estimate total annual DOC export for the unregulated Mowamba River, and the annual DOC load removed from the tributary due to water diversion.

2.4 Results

2.4.1 Discharge

During the study period, the Snowy River experienced flows of approximately 1.1-4.6 m^3s^{-1}, punctuated by three controlled flood releases from Jindabyne Dam and one large natural flood event (Fig 2.2a). The Mowamba Aqueduct was operational throughout the study period, with the exception of two experimental releases in 2011 from March 14th to April 8th and November 28th to December 23rd when water diversion was suspended and all tributary discharge flowed directly to the Snowy River (Fig 2.2b). Over the study period, water diversion at the Mowamba Weir reduced the frequency of tributary high-flow events from 39 at site M2, to 9 events downstream of the Mowamba Weir.

2.4.2 DOC concentration

Mean sampled DOC concentration was lowest directly below the Jindabyne Dam at site S0 (2.52 mgL^{-1}) and highest in the unregulated tributary at Mowamba site M2 (4.91 mgL^{-1}) (Table 2.2). DOC concentration was least variable at S0 and most variable at M2 (Table 2.2). In the Snowy River, DOC concentration and variability increased longitudinally with distance from the Jindabyne Dam (Fig 2.3, Fig 2.4). DOC concentration in the Snowy River above and below the Mowamba tributary confluence remained fairly similar during base-flow conditions in the tributary when most tributary discharge was diverted into the aqueduct (Fig 2.3). On a few occasions, DOC concentration was markedly higher downstream of the confluence at S1 than above the confluence at S0. These larger increases in DOC concentration below the tributary confluence coincided with storm events in the Mowamba tributary that overtopped the weir (Fig 2.3).

Significant, positive correlations between log DOC concentration and log river discharge existed at all sites except S0, immediately downstream of Jindabyne Dam (Fig 2.4a-e). DOC concentration and discharge were most strongly correlated at Mowamba River site M2 (r^2=0.59) and Snowy River site S4 (r^2=0.36). In the Snowy River, DOC concentration and stream discharge became more closely correlated with increasing distance from Jindabyne Dam (Fig 2.4).

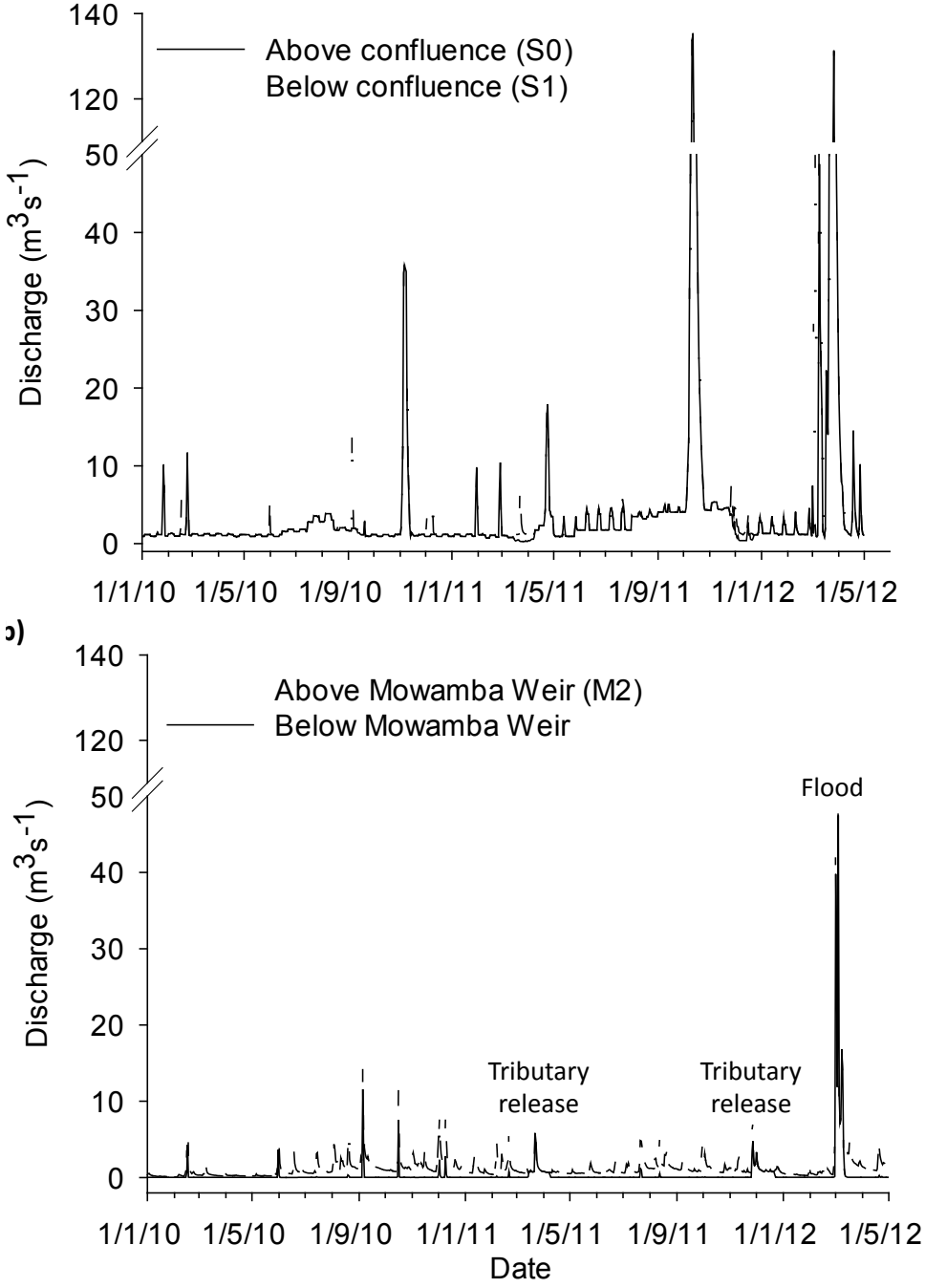


Figure 2.2: Hydrographs of a) the Snowy River above and below the Mowamba River confluence and b) the Mowamba River above and below the Mowamba Weir.

Table 2.2 Descriptive statistics for DOC concentration (mg L^{-1}) in the Snowy River and Mowamba tributary. Data collected January 2010 to March 2012.

Site	*n*	Mean	Median	Std.Dev.	Range
Regulated sites					
S0	54	2.52	2.50	0.54	3.06
S1	53	3.40	2.87	1.86	10.17
S4	49	4.26	3.60	2.26	9.40
Unregulated Sites					
M1	17	3.13	2.96	1.06	3.33
M2	54	4.91	3.90	2.49	11.30

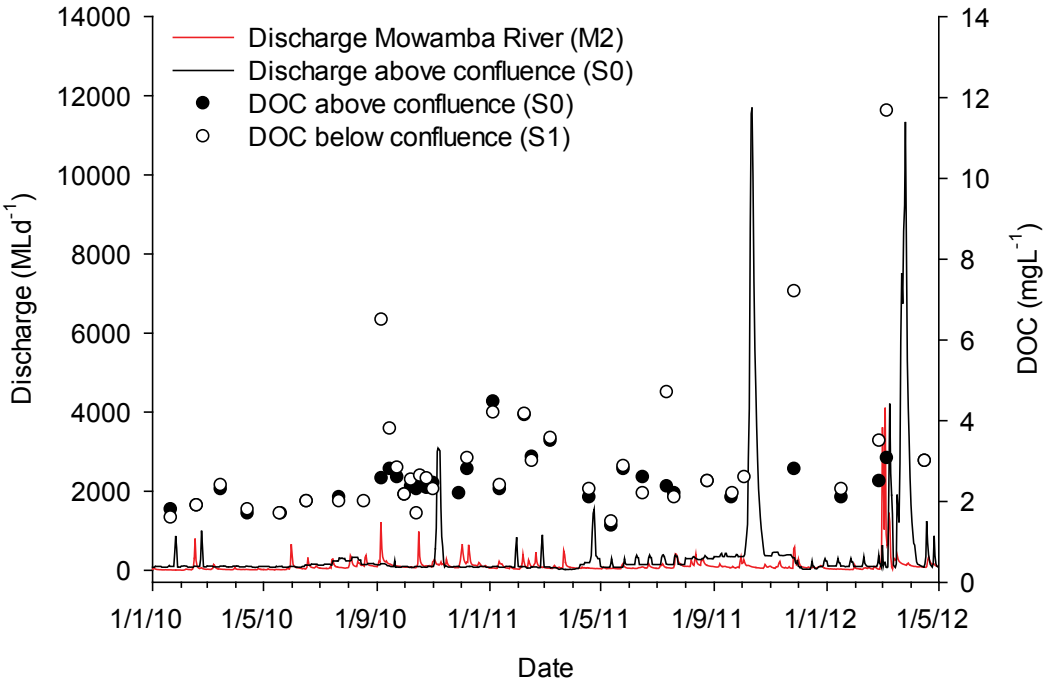


Figure 2.3. DOC concentration in the regulated Snowy River above (S0) and 1 km below (S1) the Mowamba tributary confluence. Discharge in the unregulated Mowamba River at Site M2 and in the Snowy River above the tributary confluence at Site S0 are shown for reference.

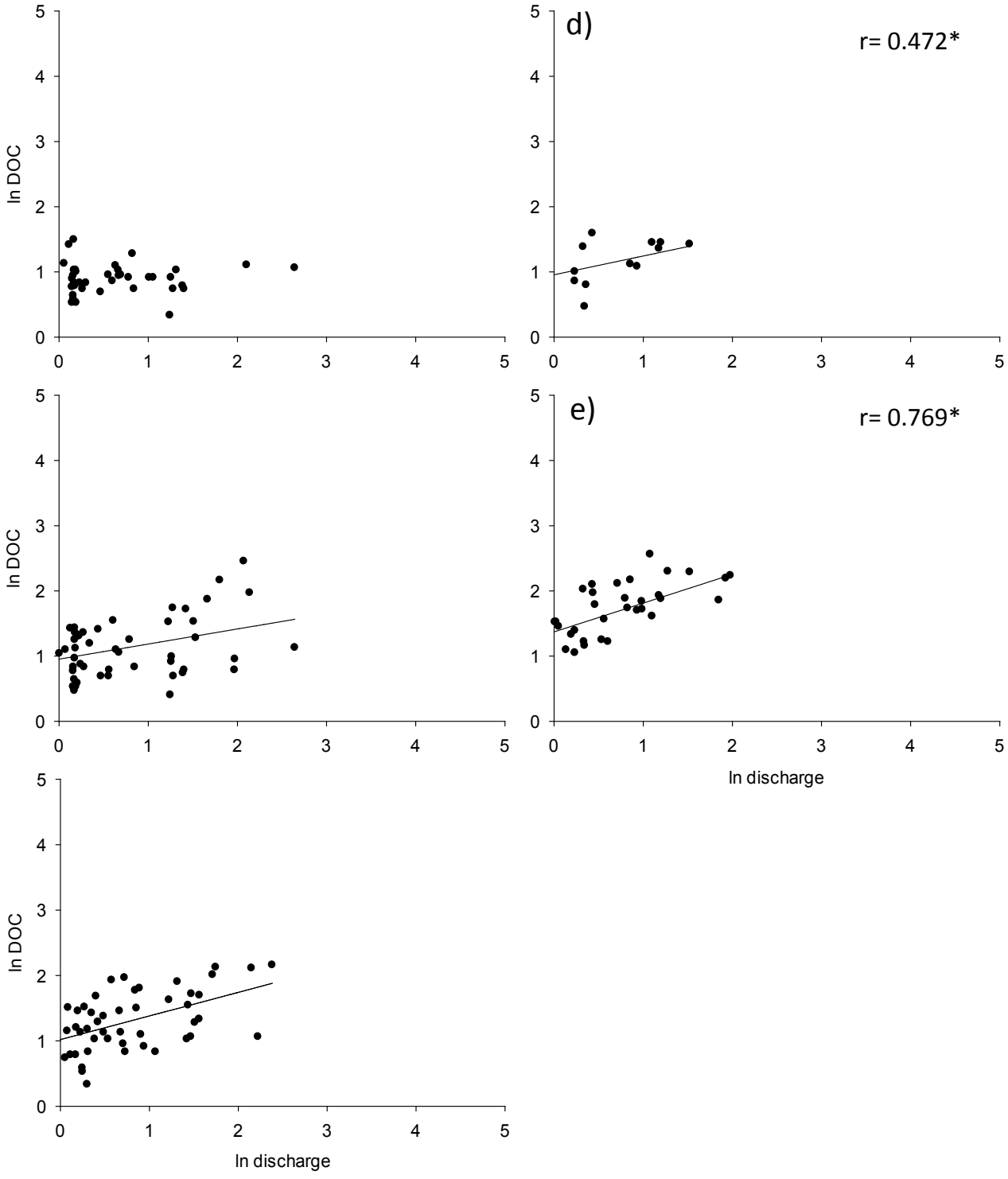


Figure 2.4. DOC concentration from 2010 to 2012 at regulated Snowy River sites a) S0 (n=52), b) S1 (n=53) c) S4 (n=49) and unregulated Mowamba tributary sites d) M1 (n=17) and e) M2 (n=54). * denotes significant correlations (p<0.05). Excludes data collected at Snowy River sites during the high flow spring environmental flow releases from Jindabyne Dam (1/11/10 to 13/11/10 and 4/10/11 to 26/10/11).

2.4.3 Mowamba tributary DOC load

In the unregulated Mowamba River, a large proportion of variation in DOC concentration was explained by mean daily discharge, both in the snowmelt (r^2=0.66) and non-snowmelt (r^2=0.76) seasons. This strong relationship between natural log transformed DOC concentration and discharge allowed annual DOC load in the unregulated Mowamba River and the aqueduct off-take to be modelled using the rating curve method. Model results showed that under existing water diversion practises, approximately 78% of the total annual tributary DOC export and 86% of total annual stream discharge was diverted into the Mowamba aqueduct (Table 2.3). A much greater proportion of DOC was transported to the Snowy River in 2012 due to a large natural flood in March which spilled the Mowamba Weir (Table 2.3).

Table 2.3: Water diversion and estimated DOC export in the Mowamba River tributary under active and suspended flow diversion at the Mowamba Weir. Annual mean calculated from 2010 and 2011 only.

	2010	**2011**	**2012 (Jan-Mar)**	**Mean**
Volume diverted at Mowamba Weir (ML)	33,750	30,791	10,787	33,770
(% total annual flow)	*(90%)*	*(82%)*	*(40%)*	*(86%)*
Annual DOC export, active weir diversion (t)	40.5	40.3	282.8	40.4
Annual DOC export, no weir diversion (t)	201.5	176.1	295.3	188.8
DOC diverted at weir (%) (t)	79.8	77.0	4.2	78.4

2.5 Discussion

These results support the hypothesis that DOC concentration and discharge below the Jindabyne Dam are more strongly correlated downstream of the Mowamba tributary confluence, suggesting that tributary in-flows reconnect a regulated river to a more natural storm-driven DOC supply. DOC load modelling suggests that tributary water diversion limits the capacity of the Mowamba tributary to increase DOC concentration and variability in the DOC-poor Snowy River below Jindabyne Dam.

2.5.1 DOC concentration and export

Mean DOC concentration was higher in the Mowamba River relative to the Snowy River above the tributary confluence, confirming the tributary augments DOC concentrations in a DOC-poor section of the Snowy River below Jindabyne Dam. Even while the Mowamba Weir was diverting tributary water, a DOC signal from the Mowamba River was detectable as an increase in mean DOC concentration and range below the Mowamba tributary confluence. The strong correlation between DOC concentration and discharge at unregulated Mowamba River sites (Fig 2.4d-e) is consistent with observations from other upland streams in the Northern Hemisphere (Hinton, Schiff & English, 1997; Buffam *et al.*, 2001). In these systems, the increase in DOC concentration with stream discharge is attributed to the temporary activation of shallow soil flowpaths during storm and snowmelt events, which mobilise terrestrial soil DOC and deliver it into the stream (Boyer *et al.*, 1997; Inamdar, 2011). The strong correlation between DOC concentration and discharge observed in the Mowamba River therefore indicates a close linkage to terrestrial DOC sources.

In contrast, the consistently low DOC concentration and lack of significant DOC-discharge relationship at site S0 (Table 2.2, Fig 2.4a) suggest that the DOC pool immediately downstream of the dam is disconnected from the hydrology-driven supply pathways that typically dominate upland rivers and streams. These results support a study by Miller (2012), who showed that a large reservoir disrupted a longitudinal continuum of DOC load and concentration in a snowmelt river, and caused a marked reduction in DOC variability immediately below the reservoir. By comparison, DOC concentration in the unregulated Snake river increased approximately five-fold on the rising limb of the snowmelt peak

(Hornberger, Bencala & McKnight, 1994). In mixed rainfall- snowmelt rivers like the Snowy River, reduced DOC variability in lake out-flows may be due to the capture and processing of the spring snowmelt associated DOC pulse within the lake (Goodman, Baker & Wurtsbaugh, 2011). Goodman et al. (2011) further suggest that the DOC concentration of lake outflows is more strongly influenced by lake water residence time and in-lake processes such as primary production rather than the hydrological processes that drive DOC concentration in lake in-flows.

The re-emergence of significant, positive correlations between DOC concentration and discharge below the tributary confluence (Fig 2.4a-c) suggests that tributary in-flows restore some connectivity with a DOC source driven by catchment hydrology, potentially contributing to the recovery of the DOC regime below Jindabyne dam. In addition to re-establishing longitudinal connectivity to lower-order streams, hydrologic variability of tributary in-flows may facilitate lateral DOC mobilisation below large reservoirs by contributing to water level changes and greater riparian inundation.

An influence of the tributary was detectable in Snowy River DOC dynamics downstream of the tributary confluence at both monitored sites. However, water diversion practices at the Mowamba Aqueduct dampen some aspects of the tributary DOC signal. Whilst the aqueduct was actively diverting water, the total annual tributary DOC export was reduced by 70-80% (Table 2.3). This was mainly due to the trapping of many smaller flow events rather than the less frequent, larger events, which still spill the Mowamba Weir and deliver DOC pulses to the Snowy River. Approximately 75% of high-flow storm events were trapped by the Mowamba Weir, which would have reduced the frequency and magnitude of associated DOC pulses that reached the regulated Snowy River. As only one tributary and confluence was included in the study design, only tentative broader conclusions can be drawn regarding tributary DOC inputs to rivers with upstream dams.

2.5.2 Ecological implications

Tributary in-flows below major dams restore connectivity to terrestrial organic matter supplies from headwater streams, which constitute an important energetic subsidy for downstream food webs (Wipfli, Richardson & Naiman, 2007; Vannote *et al.*, 1980), and this can be demonstrated below Jindabyne Dam on the Snowy River. The substantial reduction in tributary DOC export caused by a weir diversion diminishes the organic matter resource base in an already DOC-poor section of the Snowy River. As the heterotrophic microbial community includes the primary consumers of DOC (Meyer, 1994), microbially mediated ecosystem processes such as metabolism (Findlay & Sinsabaugh, 2003), and carbon and nutrient cycling (Johnson *et al.*, 2012) could potentially be affected by tributary DOC inputs. In upland streams such as the Snowy River, heterotrophic microbes are a major pathway of carbon uptake to higher trophic levels (Meyer, 1994). Fluctuations in the DOC supply from a tributary may therefore contribute to cascading effects on other components of the downstream food web, such as macroinvertebrates (Hall & Meyer 1998), in the reaches influenced by the tributary.

2.5.3 Tributary DOC in river rehabilitation

Rice *et al.* (2008) emphasise the critical role of tributary inputs in structuring biotic communities and regulating stream functioning in river systems with dams. Our study suggests that one function performed by tributaries is the acceleration of longitudinal DOC regime recovery by increasing DOC load, concentration and frequency of DOC pulses downstream of a large reservoir. Regulated tributaries like the Mowamba River may be managed to optimise DOC delivery to a regulated main stem by temporarily suspending water diversion, effectively providing a 'tributary release' event. Increased tributary flows could be timed to occur in seasons where higher DOC export is anticipated, such as during deciduous leaf-fall (Mulholland, 1997). Unlike many Northern Hemisphere systems, Australian catchments do not experience autumnal leaf-fall and the associated release of DOC into the aquatic environment. For the Snowy River system, DOC export from tributaries may be instead be higher if releases are timed immediately prior to the onset of snowmelt (Boyer *et al.*, 1997), or in seasons where a longer inter-storm period may deliver a higher DOC load per unit discharge (Wilson *et al.*, 2013). The strong association between

tributary DOC inputs and storm activity means that tributary release duration would also be an important determinant of the quantity of DOC that such a release could supply. In the Mowamba catchment, high-flow events occurred at approximately 3 week intervals, so water diversion would need to be suspended for a minimum of 4-6 weeks to maximise the likelihood of capturing at least 1-2 storm-associated DOC pulses within the release period.

The net improvement in ecological health that may be achieved specifically by supplying DOC from tributary in-flows is uncertain, as any benefits may be negated or obscured by other elements of the abiotic environment. For example, the magnitude of tributary influence is partially dependent on the size ratio of the tributary to the main stem (Benda *et al.*, 2004). Tributary-driven DOC signals may be weak below junctions with a low tributary to main stem ratio, or temporarily obscured during periods of higher discharge in the main stem, such as artificial flood releases from a reservoir. Further research examining linkages between tributary DOC inputs and ecological functional responses in regulated rivers is required to determine how tributary management activities may be used to achieve specific river restoration objectives. The spatial extent of tributary influence is also poorly studied (Kiffney *et al.*, 2006), and should be investigated when determining the potential contribution of DOC inputs from a given tributary to the rehabilitation of the main stem.

Tributary inputs should also be considered within the broader context of other rehabilitation objectives. For example, a tributary within an agricultural catchment may deliver elevated suspended solid (Terry *et al.*, 2014) and dissolved nutrient (Jordan, Correll & Weller, 1997) loads, which may offset the benefits of DOC delivery to the main stem. This may necessitate supplementary management activities to address catchment-derived sources of undesirable inputs and improve water quality in tributary out-flows.

2.6 Conclusions

This study examined the contribution of tributary in-flows to the longitudinal recovery of an artificial DOC regime downstream of a large reservoir. The re-emergence of significant, positive correlations between DOC concentration and discharge below the tributary confluence indicate that tributary in-flows reconnect a regulated river to a storm-flow driven DOC supply, even whilst tributary water diversion is occurring. However, water diversion dampened the tributary DOC signal by substantially decreasing annual tributary DOC export and reducing the frequency of storm-associated DOC pulses supplied to the Snowy River. These results suggest that re-introducing a more natural tributary hydrology by altering water diversion practices would assist in shifting the DOC regime below a large reservoir closer to that of an unregulated river. Further studies are required to determine how tributary environmental water delivery may affect multiple aspects of ecological functioning and contribute to the long-term rehabilitation of a regulated river. This study illustrates how a potentially ecologically valuable tributary function can be quantified to guide the management of a regulated river system, and is an example of the growing consideration of DOC dynamics (Stanley *et al.*, 2012) in environmental water delivery and long term river rehabilitation.

Chapter 3: Dissolved organic carbon delivery from managed flow releases in a montane snowmelt river

3.1 Abstract

Managed flow releases are increasingly being utilised in the rehabilitation of regulated rivers to improve physical habitat condition and restore spatial connectivity. However, the potential for managed flow releases to influence basal resource availability to the downstream food web has received less attention. This study investigated dissolved organic carbon (DOC) delivery from managed flow releases from Jindabyne Dam and a regulated tributary to the Snowy River; a mixed rainfall-snowmelt river in south-east Australia. DOC concentration and load were monitored downstream of Jindabyne Dam during two high-flow dam releases and two month-long tributary releases provided by temporarily suspending tributary weir diversions. DOC chemical composition in the downstream Snowy River was characterised using fluorescence spectrophotometry. A negligible change or decrease in DOC concentration occurred at all monitored sites during both dam releases. In contrast, pulsed increases in DOC concentration concomitant with natural high-flow events were observed in the Snowy River during the tributary releases. The estimated DOC load delivered by the larger dam release increased 1.5-fold between sites 2 km and 22 km downstream of the Jindabyne Dam. Reservoir release waters contained both humic-like and protein-like DOC fluorophores, whereas tributary releases contained only humic-like DOC fluorophores. Collectively, these results suggest that changes in DOC quantity and composition during managed dam releases reflect localised wetting and DOC mobilisation from the riparian zone whilst tributary releases deliver storm-associated pulses of terrestrial DOC flushed from the catchment. The unique DOC regimes associated with dam and tributary water releases may influence ecosystem functioning in the downstream river.

3.2 Introduction

A common consequence of river regulation by dams is a decline in flow variability, particularly a reduction in the magnitude and frequency of high-flow events (Poff & Zimmerman, 2010). High-flow events drive key riverine processes such as sediment transport, floodplain inundation and the cycling of carbon and nutrients (Palmer, Ambrose & Poff, 1997). Reductions in flow variability can consequently have adverse effects on biodiversity and ecological functioning downstream of impoundments (Bunn & Arthington, 2002). To mitigate these impacts, water managers are increasingly incorporating managed flow releases into river restoration and rehabilitation programs by releasing pulses of stored reservoir water (Konrad *et al.*, 2011; Arthington & Pusey, 2003; Robinson & Uehlinger, 2008).

Many managed flow releases aim to shift biotic community structure in regulated rivers by restructuring the physical habitat (Patten *et al.*, 2001) or by restoring spatial connectivity to facilitate species recruitment and dispersal (King *et al.*, 2010). However, there is an emerging recognition of the need for managed flow releases to influence broader ecosystem functions such as food web dynamics and metabolism by modifying basal resource availability in the downstream ecosystem (Chester & Norris, 2006; Kelly *et al.*, 2013; Davie & Mitrovic, 2014). Whilst these studies generally focus on the role of benthic algae as a primary food resource (e.g. Uehlinger, Kawecka & Robinson, 2003), less attention has been given to understanding how managed flow releases influence dissolved organic carbon (DOC). This is despite the fact that DOC is typically the largest pool of organic carbon in riverine systems (Ludwig, Probst & Kempe, 1996; Dawson *et al.*, 2002). DOC is a primary metabolic substrate for heterotrophic microbes and is closely linked to microbial community dynamics, ecosystem metabolism and inorganic nutrient cycling (Findlay & Sinsabaugh, 1999). The delivery of DOC from managed flow releases can therefore constitute an important ecosystem function and energetic resource for aquatic food webs (Meyer, 1994; Tank *et al.*, 2010).

Dams can substantially alter the quantity and chemical composition of DOC as it is transported through a river network (Miller, 2012), and thus influences the amount and form of DOC delivered to downstream food webs. In unregulated upland rivers, DOC

dynamics are driven by catchment flushing processes, with the majority of DOC exported in pulses associated with high-flow events such as storms (Buffam *et al.*, 2001), and the seasonal snowmelt (Boyer *et al.*, 1997). Optical analyses of DOC composition indicate that natural high-flow events deliver terrestrially derived, humic-like carbon that is metabolised slowly by heterotrophic bacteria (Nguyen, Hur & Shin, 2010). However, in regulated systems inflowing DOC can be trapped in reservoirs for extended periods where it is subjected to photodegradation and metabolism by lake microbes, producing lake outflows with an altered DOC concentration and chemical composition (Minor & Stephens, 2008; Larson *et al.*, 2007). Additionally, algal DOC production within the reservoir can introduce high bioavailability, protein-like substrates to the DOC pool (Nguyen *et al.*, 2005). Imposing a reservoir on the river continuum may also alter the seasonality of DOC pulses transported to downstream reaches by dampening rainfall and snowmelt-associated DOC pulses and supplying elevated concentrations of algal DOC in summer (Goodman, Baker & Wurtsbaugh, 2011). Managed flow releases delivered from reservoirs are therefore likely to provide a different quantity and quality of DOC compared to the natural events they are intended to simulate, potentially influencing carbon flow through the downstream aquatic ecosystem. Few studies have examined DOC delivery from managed flow releases in detail, although Henson et al. (2007) reported a pulse in DOC concentration from 0.5 mgL^{-1} to 3.6 mgL^{-1} up to 54 km downstream of Camanche Dam on the rising limb of a managed flow release on the Mokelumne River, California.

The Snowy River in southeast Australia is a mixed rainfall-snowmelt river regulated by the Snowy Mountains Scheme (SMS), a large, multiple-reservoir hydroelectric and irrigation diversion scheme. The Snowy River is unique in that many of its tributaries are also regulated by aqueducts that divert tributary water into the storage reservoirs, thus increasing the reservoir catchment area and water yields. This configuration allows environmental water to be released directly from a dam, or in the more unconventional form of a tributary release, achieved by temporarily suspending tributary water diversions at an offtake aqueduct. This is important because the quantity and quality of DOC present in tributary water is likely to be influenced by whether or not it is routed through the reservoir. The most downstream reservoir in the SMS is Jindabyne Dam, which delivers outflows characterised by a consistently low DOC concentration (Rohlfs *et al.*, 2015).

A more thorough understanding of DOC delivery from both reservoir and tributary-sourced water releases would reveal how organic matter delivery from managed flow releases may differ from natural events. This may have implications for bacterial, benthic algal and macroinvertebrate community structure, and downstream ecosystem functioning (Cross *et al.*, 2011). These different ecological responses to the delivery of DOC will have significant implications for river management and rehabilitation strategies. Our study aims to identify and compare the concentration, load and composition of DOC delivered to the Snowy River by managed high-flow release events made from a large reservoir and a tributary weir. We predicted that DOC concentration and load in the Snowy River below Jindabyne Dam would increase during both release types, relative to pre-event conditions. We further hypothesised that the reservoir releases would contain protein-like DOC fluorophores whereas the tributary releases would be characterised by humic-like DOC fluorophores.

3.3 Methods and materials

3.3.1 Study area and hydrology

The Snowy River rises in the Australian Alps and flows 352 km south to Bass Strait. The study area is located in a montane to temperate part of the upper catchment, and includes sites on the Snowy River between Jindabyne Dam and Burnt Hut Gorge, and on its tributary, the Mowamba River (Fig 3.1). The Mowamba River joins the Snowy River approximately 2 km downstream of Jindabyne Dam and is the first mixed rainfall-snowmelt tributary flowing into the Snowy River below the SMS.

The Mowamba River is regulated by the Mowamba Weir and an aqueduct that, while operational, diverts tributary flows between the 99th and 2nd flow percentiles, (i.e. 0.034 - 6.05 m^3s^{-1}) into Jindabyne Dam. Land use in the Mowamba River catchment consists of approximately 40% native vegetation cover and 60% pasture, with forested areas concentrated at the higher elevations. The Snowy River catchment below Jindabyne Dam is predominantly cleared for grazing.

The Snowy River is regulated by the SMS; a complex network of 16 interconnected dams that redirect water inland to the Murray and Murrumbidgee River catchments for hydropower generation and irrigation diversions. From 1967-2002, the SMS reduced the

Snowy River to 1% of mean annual natural flow (MANF) at Jindabyne. This study coincided with a transition to higher environmental water allocations to the Snowy River at Jindabyne, which increased from about 38 GLy^{-1} (~4% MANF) in 2002-2009, to 69 GL (~7% MANF) in 2011 (Reinfelds *et al.*, 2013). During the study period, the annual environmental water allocation was divided into prescribed daily release targets from Jindabyne Dam in accordance with a 'building blocks' strategy (King & Louw, 1998) that prioritised delivering an annual flushing flow event. Although designed to loosely replicate the timing of the historic spring snowmelt flood, the flushing flows from Jindabyne Dam were not specifically linked to actual precipitation in the upper Snowy catchment (Reinfelds *et al.*, 2013).

Four managed flow releases were monitored during the study period (Fig 3.2); they are subsequently referred to as dam release 1 (November 2010), dam release 2 (October 2011), tributary release 1 (April 2011) and tributary release 2 (December 2011). The dam releases were pulsed events from Jindabyne Dam, designed to provide a seasonal 'snowmelt' flood to the regulated Snowy River. The managed tributary releases were made by suspending diversion of Mowamba River flows to Jindabyne Dam, allowing the river to run its normal course and join the Snowy River just downstream of the Jindabyne Dam. Hydrological details of the four managed releases are provided in the results section.

Snowy River discharge was recorded by rising stage gauges located at S0 (gauge 222 020, Snowy Hydro Ltd.), S4 (gauge 222 026, NSW Office of Water) and S5 (gauge 222 013, NSW Office of Water). Discharge at S1 was estimated as the combined discharge recorded at S0 and at the Mowamba River below the Mowamba Weir (gauge 222 546, Snowy Hydro Ltd.). Mowamba River discharge at M2 was estimated by adding discharges recorded at rising stage gauges in the Mowamba aqueduct off-take (gauge 600 179, Snowy Hydro Ltd.) and below the Mowamba Weir.

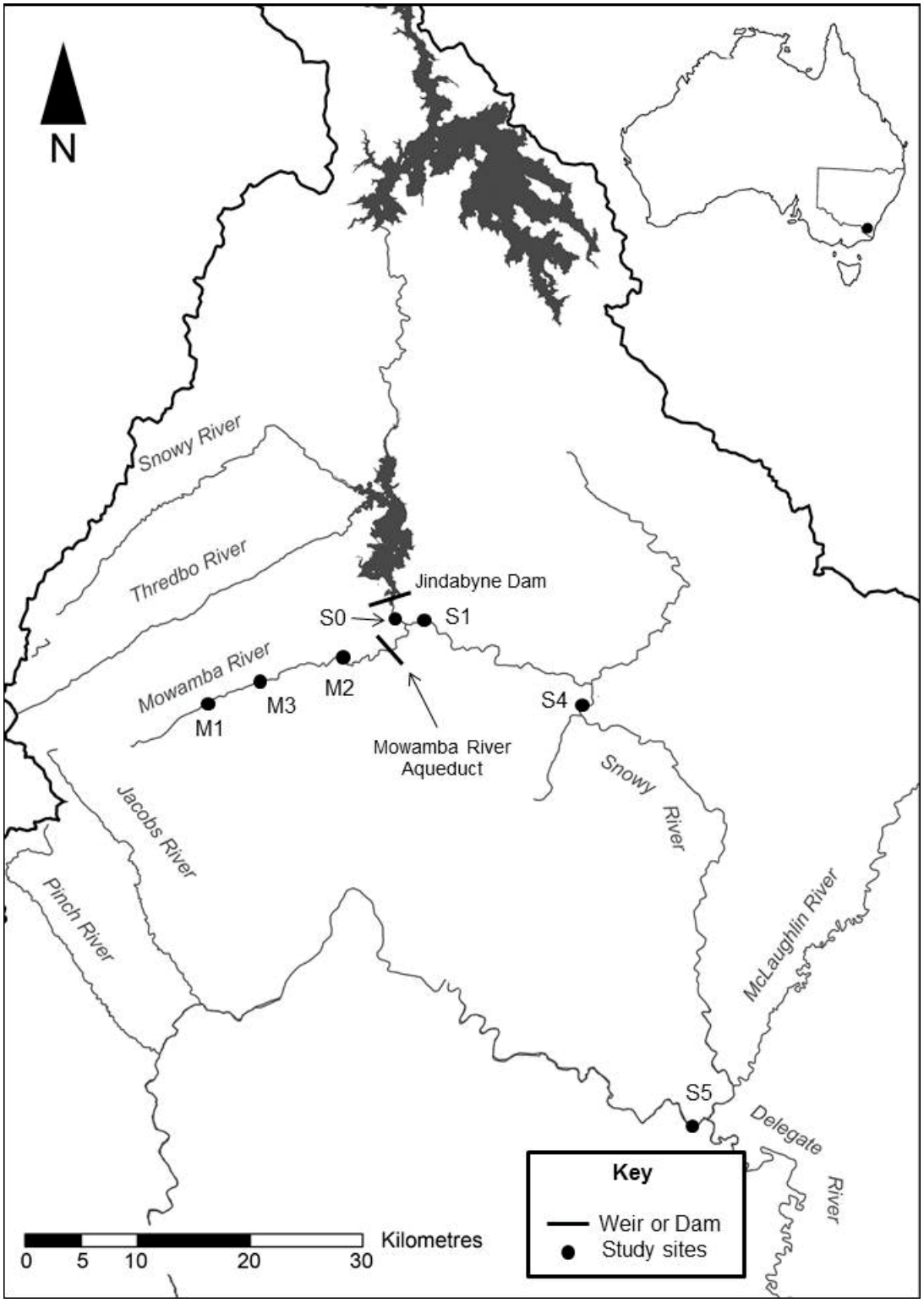


Figure 3.1: Map of study area showing Mowamba River (tributary) sites (M1, M2, M3) and Snowy River sites (S0, S1, S4, S5).

3.3.2 Site descriptions

A total of four sites on the Snowy River were sampled to examine longitudinal changes in the DOC regime in response to reservoir and tributary water releases (Fig 3.1). Site S0 is upstream of the Mowamba River confluence, whilst sites S1 and S4 are below the Mowamba River confluence. Sites S0 and S1 are 0.5 km and 3 km downstream of Jindabyne Dam, and are located in a constricted channel section of the Jindabyne Gorge. Site S4 is 24 km below Jindabyne Dam, and is located in the Dalgety Uplands, a lower gradient, more sinuous river section. Site S5 is located 92 km from Jindabyne Dam in the higher gradient Burnt Hut Gorge and is downstream of the confluence with the Delegate River, a major non-snowmelt tributary of the Snowy River, which is often influenced by coastal rainfall patterns. In addition, three unregulated Mowamba River sites were sampled to characterise tributary DOC inputs (Fig 3.1). Geographic details for the study sites are given in Table 3.1.

3.3.3 DOC concentration

DOC concentration was measured during dam releases 1 and 2, and tributary releases 1 and 2. Sampling frequencies and locations for each of the four experimental releases are given in Table 3.2. For both tributary releases and dam release 1, duplicate surface water DOC samples were collected by hand in pre-combusted amber borosilicate glass bottles and acidified with 1 mL of 2N HCl upon collection. Acidified samples were stored at 4ºC in the dark until analysis. During dam release 2, automated refrigerated water samplers were used to collect water into HDPE bottles (Teledyne ISCO) and DOC subsamples were preserved and stored as described above after no longer than 48 hours of dark storage at 4ºC in the refrigerated sampler. The automated samplers allowed the sampling frequency to be increased for release 2 (Table 3.2). DOC samples were filtered through a 0.45 µm pore-sized membrane filter and processed according to Eaton and Franson (2005), using a Shimadzu TOC-VCSH analyser.

Table 3.1: Study site names and locations. Geomorphic regions for Snowy River sites are described following Erskine et al. (1999).

Site ID	Site name	Geographic Coordinates	Geomorphic region
Snowy River Sites			
S0	Snowy River downstream of Jindabyne Dam	36° 26' 25"S, 148° 38' 00"E	Jindabyne Gorge
S1	Snowy River downstream of Mowamba River junction	36° 26' 46"S, 148° 39' 13"E	Jindabyne Gorge
S4	Snowy River at Blackburn Creek	36° 31' 26"S, 148° 50' 18"E	Dalgety Uplands
S5	Snowy River at Burnt Hut Gorge	36° 26' 38"S, 148° 56' 16"E	Burnt Hut Gorge
Mowamba River Sites			
M1	Mowamba River at Moonbah Hut	36°30' 5.9"S, 148° 28' 54"E	Mowamba valley
M2	Mowamba River at the Barry Way	36° 28' 43"S, 148° 35' 20"E	Mowamba valley
M3	Mowamba River at Wollondibby Rd	36° 29' 32"S, 148° 31' 15"E	Mowamba valley

Table 3.2: DOC concentration sampling frequency and locations for each of the four experimental releases.

Event	Sites sampled	Sample method	Sample frequency
Dam release 1	S0, S1, S4	Grab samples	Twice daily
Dam release 2	S1, S4, S5	Autosampler	4 to 6 hourly
Tributary release 1	S0, S1, S4	Grab samples	Weekly
Tributary release 2	S0, S1, S4	Grab samples	Week 1: twice daily Weeks 2-4: twice weekly

3.3.4 DOC load

The total DOC load, $L_{release}$ (t), delivered to each site during each of the release events was calculated as:

$$L_{release} = \text{Sum of } (Q_d C_d), \text{ from } d = 1 \text{ to } N \quad (3.1)$$

where Q_d (m^3d^{-1}) is the daily mean site discharge, C_d (gm^{-3}) is the daily DOC concentration and N is the number of days in each release period. For the dam releases, C_d was calculated as the mean of all DOC samples taken at each specified site during each respective event. Due to the higher sampling frequency, finer-scale temporal changes in DOC export from the dam releases were analysed by calculating the DOC export during each sampling interval (L_i), which ranged from 4-24 hours (Table 3.2):

$$L_i = Q_i C_i \quad (3.2)$$

Where Qi is the mean discharge rate during the sampling interval (MLd^{-1}), based on hourly gauge data, and C_i is the sampled DOC concentration for the given interval.

For the experimental tributary releases, C_d was calculated using rating curves developed from a 2.5-year set of monthly to fortnightly DOC concentration data collected from M2 (further details in Rohlfs et al. (2015). The data were partitioned into non-snowmelt (tributary release 1) and snowmelt (tributary release 2) seasons and used to develop two rating curves of the form:

$$\ln C_d = b_0 + b_1 \ln Q_d \quad (3.3)$$

with model coefficients b_0 and b_1 (R; R Core development team, 2015). The residuals of both rating curves were normally distributed and homeoscedastic, as assessed using residual plots. The daily concentration values generated from the models were used to calculate daily DOC loads using equation 1. DOC load estimates derived from discharge models will always have some uncertainty due to the influence of additional factors including antecedent precipitation, groundwater DOC inputs and exhaustion of catchment DOC sources (Cooper & Watts, 2002). This uncertainty was quantified in terms of 95% confidence intervals generated for load estimates for each of the release events. For the

dam releases, upper and lower confidence intervals were calculated from the standard error of the mean measured DOC concentration from each of the dam releases. Bootstrapping with 1000 resamplings was used to determine 95% confidence intervals for the tributary release load estimates (R; R Core development team, 2015).

3.3.5 DOC composition

DOC composition was examined on the receding limb of dam release 2 at sites S1, S4, M1, M2 and M3 on 13/10/11 and at S5 on 14/10/11. Tributary release 2 was sampled on the receding limb of a storm-pulse on 01/12/14 at sites S0, S1, S4, M1, M2 and M3. DOC composition samples were collected in pre-rinsed HDPE bottles, filtered through 0.2 µm pore-sized filters and stored frozen in the dark until thawed prior to analysis.

Fluorescence spectrophotometry was used to characterise the chemical composition of DOC mobilised by each release type. This method uses electromagnetic emission patterns to distinguish fluorescent DOC components (Fellman, Hood & Spencer, 2010). A Cary Eclipse spectrofluorometer was used to generate three-dimensional excitation-emission matrices (EEMs) by scanning excitation wavelengths 230-465 nm and emission wavelengths 260-600 nm. Each sample was dispensed into a quartz cuvette and scans were performed in ratio mode, using a bandpass width of 5 nm and scan speed of 120 nms^{-1}. Each EEM was corrected according to Coble et al. (1996) and normalised to Raman Units (Lawaetz & Stedmon, 2009). Triplicate scans were performed for each sample and averaged for determination of EEM peaks. The resultant EEMs were visually inspected to identify major DOC fluorophores based on the location of fluorescence intensity maxima (Coble, 1996).

No inner filter correction was applied as the fluorescence measurements were intended to provide only a rapid survey of major DOC fluorophores. Inner filtering effects result in reduced fluorescence intensity and the shifting of fluorescence peaks to longer wavelengths (Kothawala *et al.*, 2013). Therefore, major DOC fluorophores can still be distinguished in uncorrected spectra even when some inner-filter effects are present, (e.g. Baker & Inverarity, 2004). Furthermore, it was considered unlikely the absorbance of the samples was high enough to completely preclude interpretation of the fluorescence spectra, due to their relatively low DOC concentration and lack of visible colouration.

3.3.6 Statistical analysis

The mean DOC concentration at S1 was calculated from all available DOC concentration values recorded during each of the dam and tributary releases. For comparative purposes, the mean DOC concentration in the 2 months prior to each release event was also estimated using data collected at S1 as part of a separate sampling program described in Rohlfs et al (2015). Any data collected during other release events within the 2-month pre-event period were excluded, resulting in a minimum of 3 data points being used to estimate mean pre-event DOC concentration. Independent t-tests (SPSS version 21, IBM Corp.) were used to compare mean DOC concentration in the pre-event and event period for each of the four releases, after first verifying normal distribution of the sample data using normality plots and the Komolgorov-Smirnov test ($p>0.05$). The relationship between DOC concentration and discharge for the two release types was further analysed graphically and using linear correlations (SPSS version 21, IBM Corp.). Where a significant correlation was found, the relationship was defined using linear regression after first checking residuals plots to ensure the data met the relevant assumptions.

3.4 Results

3.4.1 Hydrology

During the study period, the Snowy River experienced relatively constant, low flows between 0.9-3.5 m^3s^{-1} punctuated by several managed flow releases from Jindabyne Dam, the largest of which were in November 2010 (34.7 m^3s^{-1}) and October 2011 (138.9 m^3s^{-1}) (Fig 3.2). Tributary water was diverted at the Mowamba aqueduct, allowing a base passing flow of 0.34m^3s^{-1} throughout the study period, with the exception of two controlled release events delivered by temporarily suspending water diversion and allowing the weir to spill (Fig 3.2). The Mowamba weir also spilled naturally during several larger storm events (Fig 3.2). Dam release 2 was significantly larger than any of the other tributary or dam releases, with the peak discharge for each tributary releases only a fraction ($1/25^{th}$) of dam release 2 (Table 3.3).

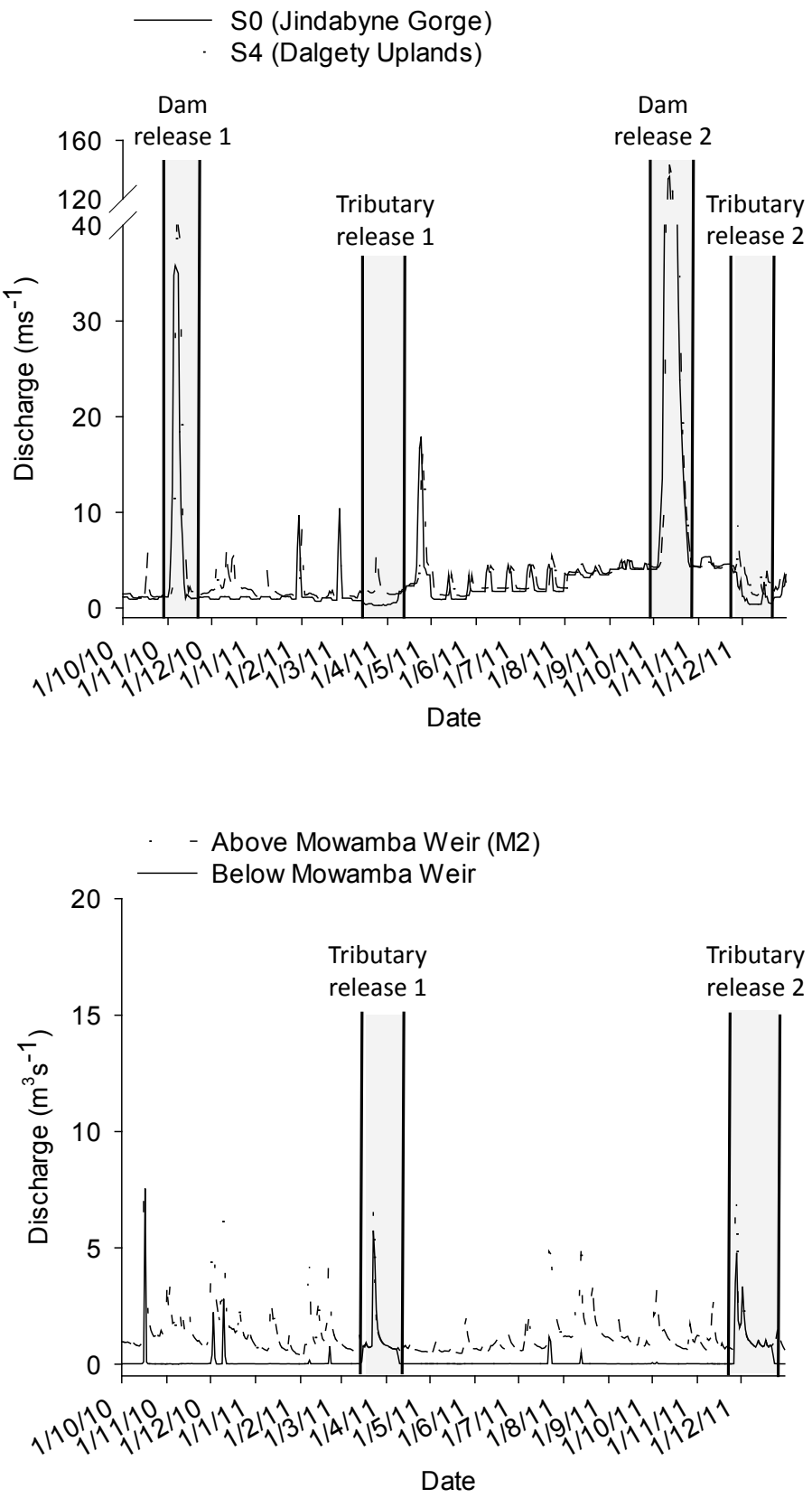


Figure 3.2 Mean daily stream discharge over the study period in the a) regulated Snowy River and b) Mowamba River above and below the Mowamba Weir.

3.4.2 DOC concentration

During both tributary releases, distinct DOC pulses were measured below the tributary confluence at S1 and S4 (Fig 3.3) that were not detected above the confluence at S0. For both tributary releases, DOC concentration maxima at Snowy River sites coincided with rainfall events and increased tributary inflows from the Mowamba River. Maximum recorded DOC concentrations were 8.7 mgL^{-1} at S1 and 6.9 mgL^{-1} at S4 during tributary release 1, and 7.2 mgL^{-1} at S1 and 8.3 mgL^{-1} at S4 during tributary release 2. DOC remained between 2.2 mgL^{-1} and 3.6 mgL^{-1} at S0 on both occasions.

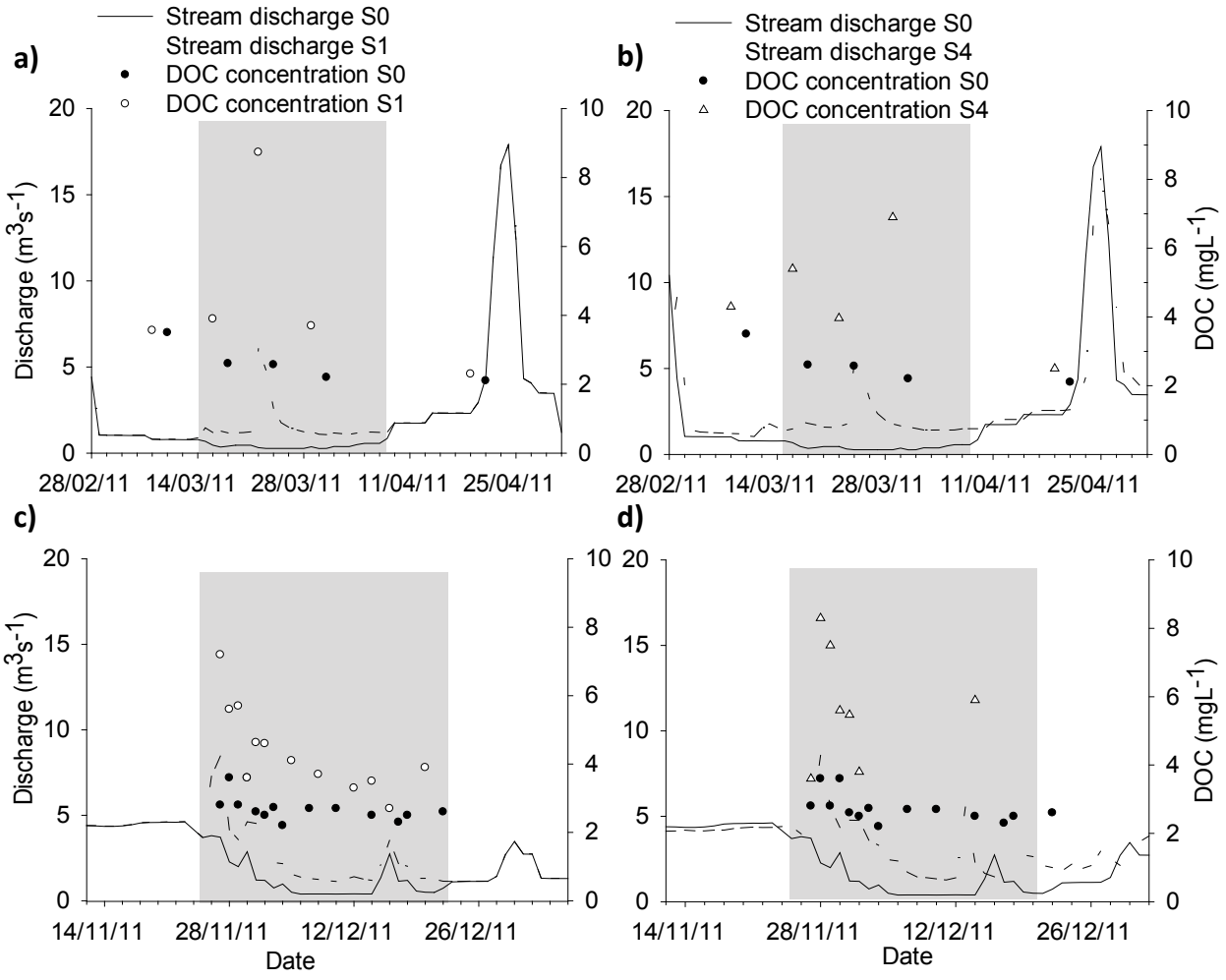


Figure 3.3 Increase in DOC concentration below the Mowamba River confluence during tributary release 1 at a) S1 and b) S4; and during tributary release 2 at c) S1 and d) S4. Shaded grey bars indicate tributary release periods.

During dam release 1, DOC concentration at all Snowy River sites remained within ±1 mgL^{-1} of pre-event conditions, although a slight increase in DOC concentration was observed on the rising limb of the flow peak at site S4 (Fig 3.4a-c). Maximum DOC concentrations recorded during dam release 1 were 3.0 mgL^{-1}, 2.7 mgL^{-1} and 3.4 mgL^{-1} at sites S0, S1 and S4 respectively. In the larger and more intensively sampled dam release 2, DOC concentration also remained within ±1mgL^{-1} of pre-release levels at sites S1 and S4 (Fig 3.4d-e). However, further downstream at S5, DOC concentration decreased from a maximum of 5.0 mgL^{-1} immediately preceding the release to a minimum of 2.7 mgL^{-1} during the receding flow limb (Fig 3.4f).

Relative to pre-flow conditions, mean DOC concentration at S1 increased during both tributary releases, but this difference was statistically significant only for tributary release 2 ($t = -2.726$, $p<0.05$). Mean DOC concentrations did not differ significantly from pre-flow levels during either dam release 1 ($t = 1.691$, $p>0.05$) or dam release 2 ($t = 0.399$, $p>0.05$) (Table 3.3). Furthermore, DOC concentration was more variable during the tributary releases than during the dam releases (Table 3.3). When pooled by flow release type, a significant, positive correlation between DOC concentration and discharge was only apparent at S1 during the tributary releases (Fig 3.5, $R^2=0.74$, $p<0.05$).

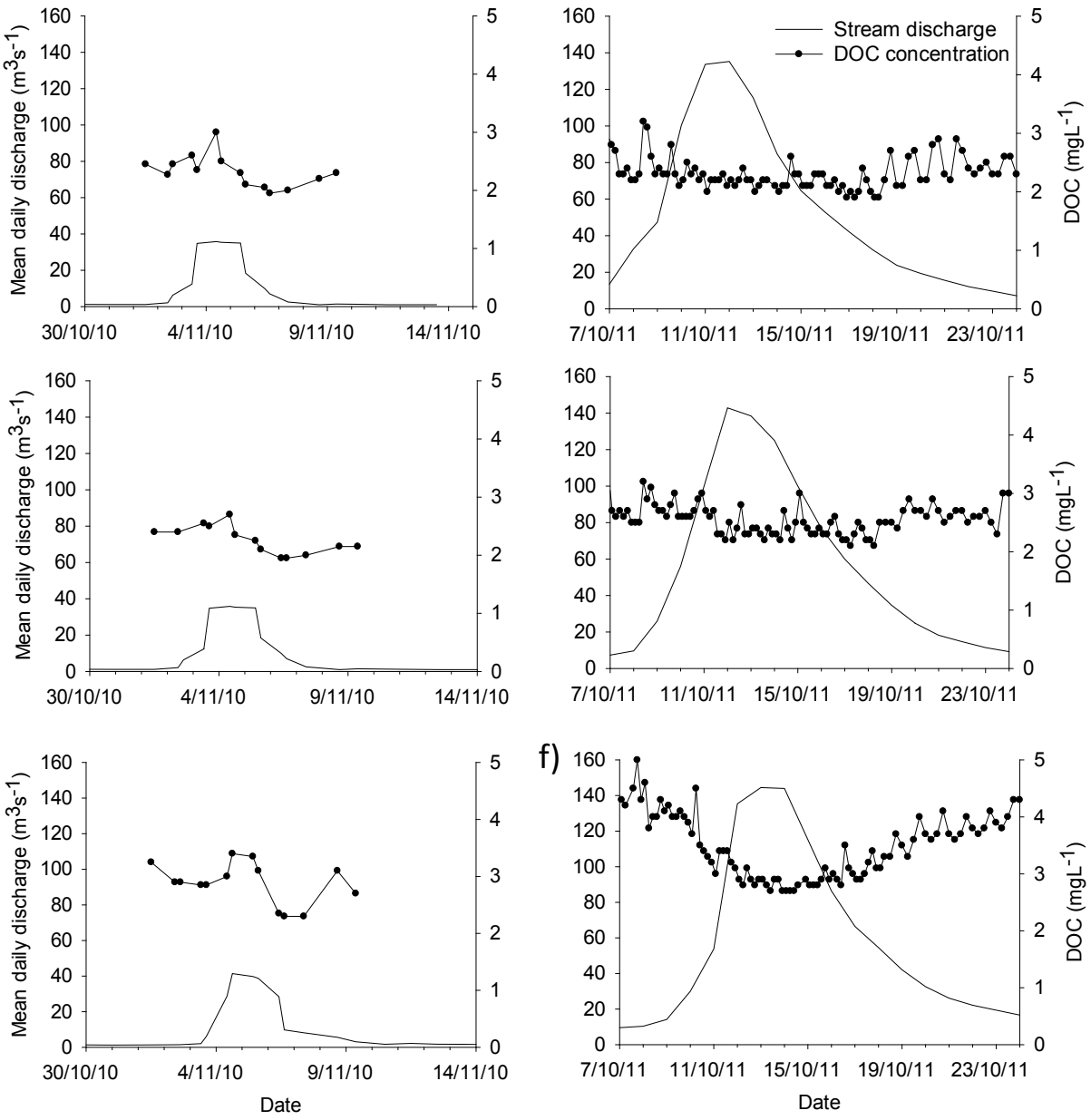


Figure 3.4 DOC concentration and stream discharge during dam release 1 at a) S0, b) S1 and c) S4 and during dam release 2 at d) S1, e) S4 and f) S5.

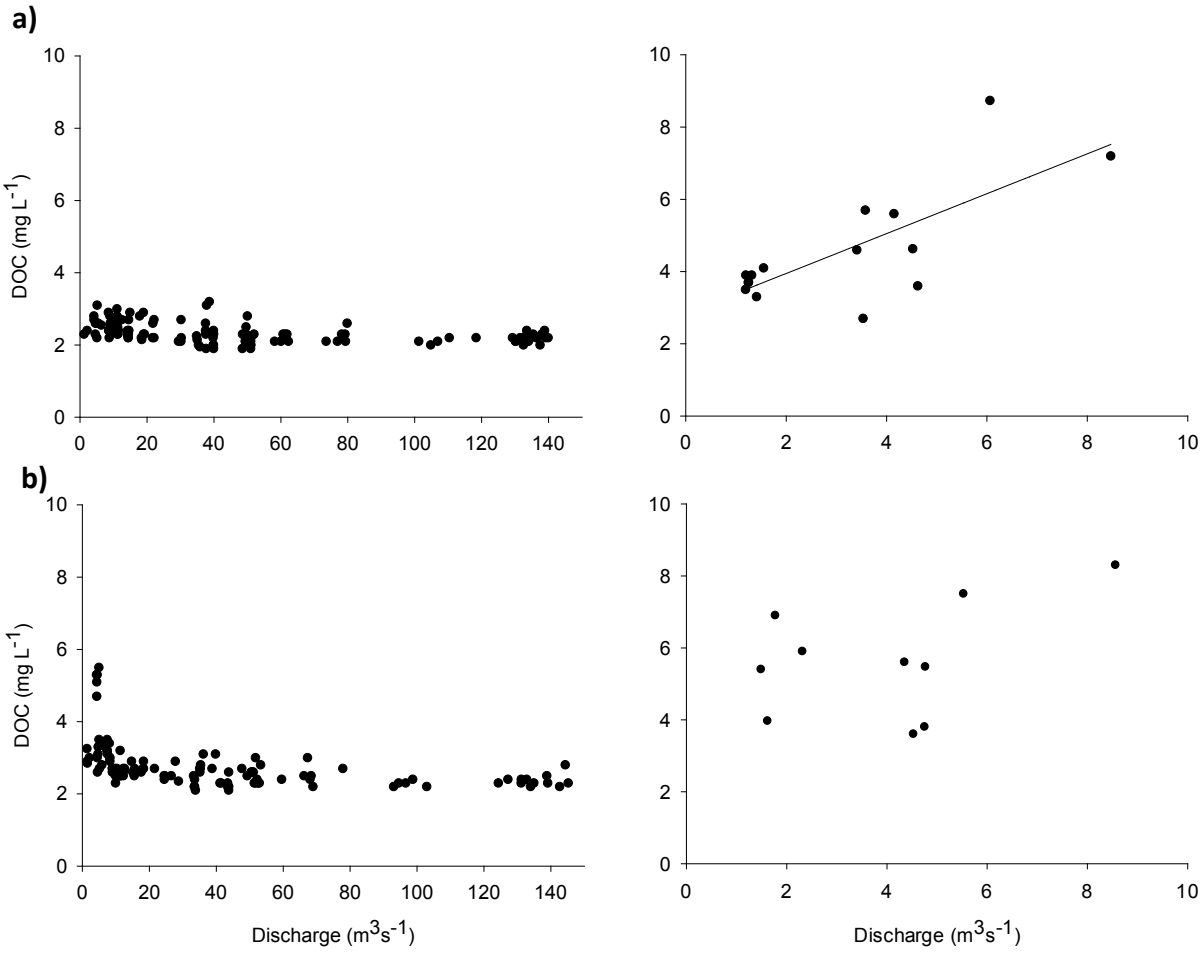


Figure 3.5: DOC concentration and mean daily discharge rate from dam releases 1 and 2 at a) S1 and b) S4, and from tributary releases 1 and 2 at c) S1 and d) S4. The regression line in c) is defined as [DOC] = 2.84 + 0.552 x discharge (p = 0.01, R^2 = 0.74) and is shown where DOC and discharge are significantly correlated (p<0.05).

3.4.3 DOC load

The rating curves adequately represented variability in DOC concentration at M2 in both the snowmelt (R^2 = 0.77, F=137.395, p<0.05) and non-snowmelt (R^2 = 0.665, F =67.529, p<0.05) seasons, such that

Snowmelt season: $\ln C_d = -1.625 + 0.615 \ln(Q)$

Non –snowmelt season: $\ln C_d = -0.549 + 0.450 \ln (Q)$.

The total DOC load delivered by dam release 1 was 36.42±1.01 t, 35.44±0.99 t and 40.05±1.54 t at S0, S1 and S4 respectively. DOC export from dam release 1 is potentially an

underestimate, as only the first 8 days of the event were sampled. The total DOC mobilised by dam release 2 increased longitudinally from 195.77±0.02 t at S0 to 204.55±0.06 t at S4 and 325.88±0.06 t at S5 (Fig 3.6). The dam releases delivered a larger total DOC load to the Snowy River than the tributary releases, which was a function of the larger water volumes allocated to the dam releases (Table 3.3).

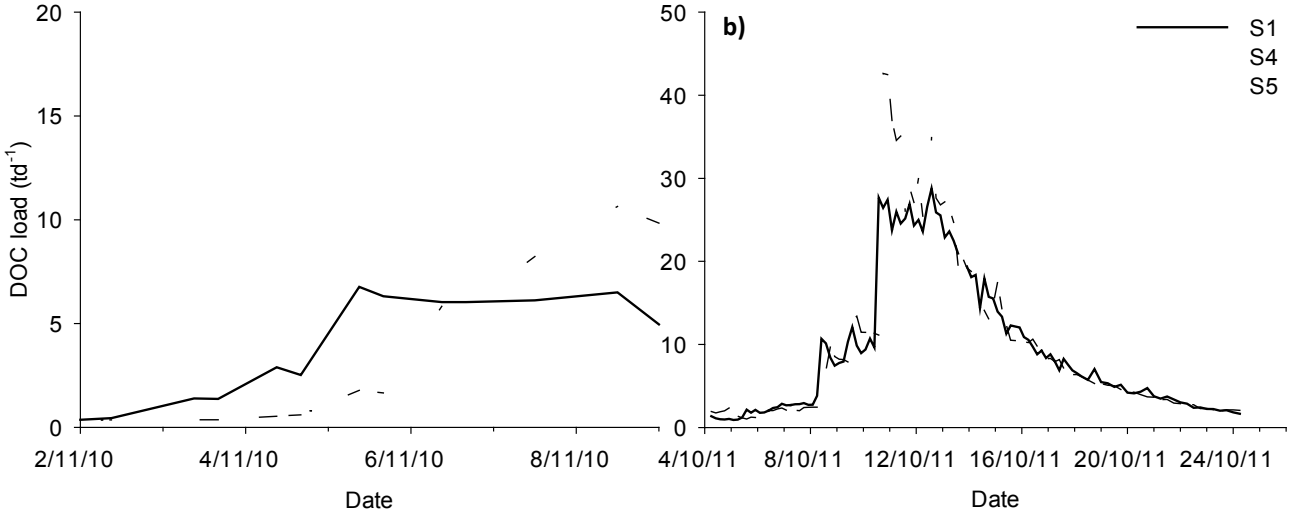


Figure 3.6 DOC load in the Snowy River during a) dam release 1 at S1 and S4 and b) dam release 2 at S1, S4 and S5.

3.4.4 DOC composition

Two humic-like fluorescence peaks were evident in EEMs across all sites, from both tributary and reservoir releases (Figs 3.7 and 3.8). These were identified as peak A (ex_{max} <230 nm, em_{max} 400-450 nm) and peak C (ex_{max} 300-340 nm, em_{max} 400-450 nm) (Coble, 1996). During tributary release 2, the intensity of peaks A and C was lowest immediately downstream of Jindabyne Dam at S0, and increased with distance from the dam (Fig 3.7). During dam release 2, the relative intensity of peaks A and C varied among sites, but did not exhibit any consistent longitudinal change in either the tributary or Snowy River (Fig 3.8). The tryptophan-like fluorophore peak T (Coble, 1996) was present only at Snowy River sites during dam release 2, with primary and secondary peaks at ex_{max} 290 nm, em_{max} 340-360 nm, and ex_{max} <230nm, em_{max} 330-350 nm respectively (Fig 3.8). The intensity of Peak T was highest at S4, the middle site below Jindabyne Dam, with only a very weak presence at S1 and S5.

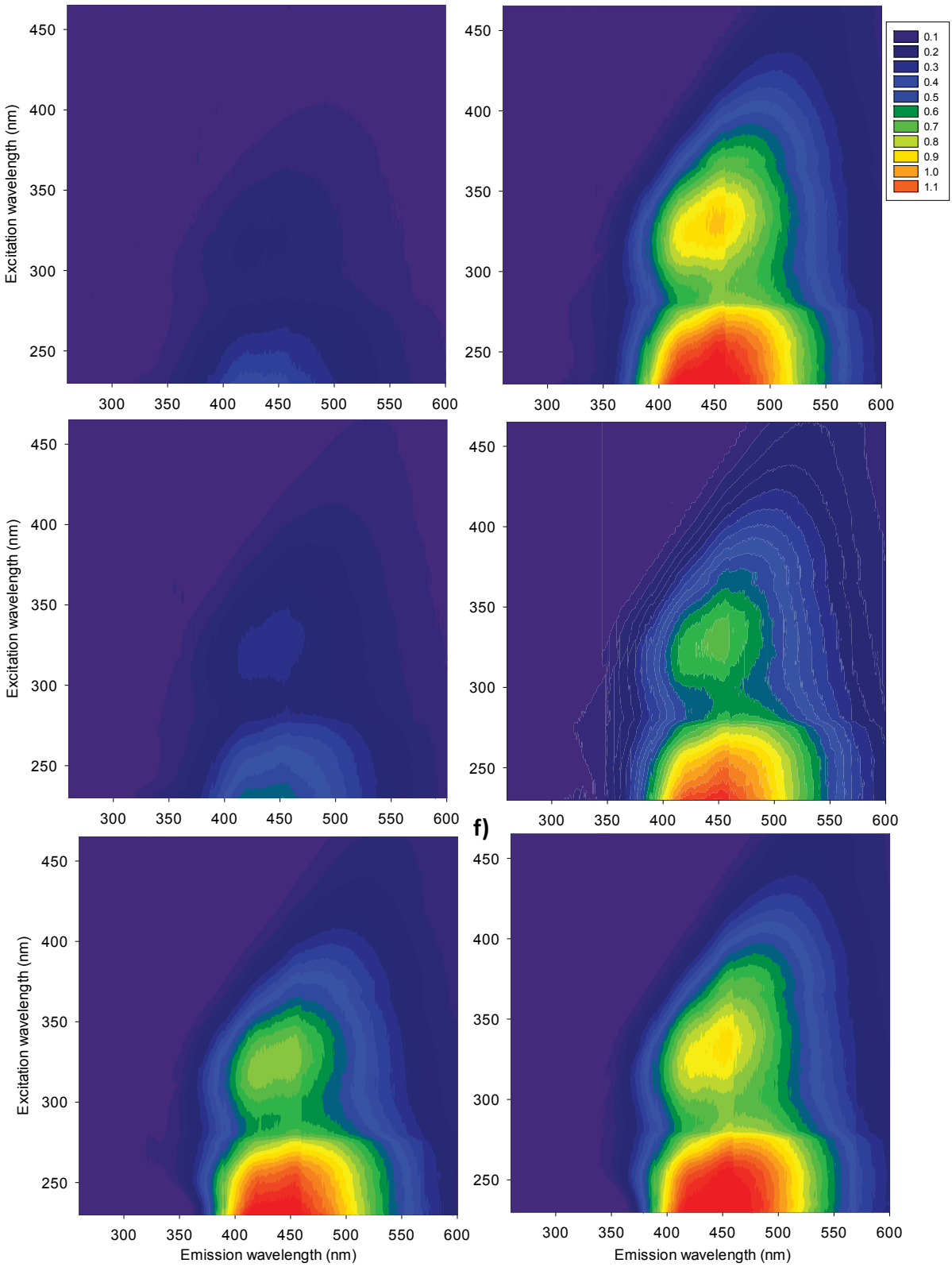


Figure 3.7: Excitation-emission matrices for samples collected during tributary release 2 at Snowy River sites a) S0, b) S1, c) S4 and at Mowamba River sites d) M1, e) M2, f) M3.

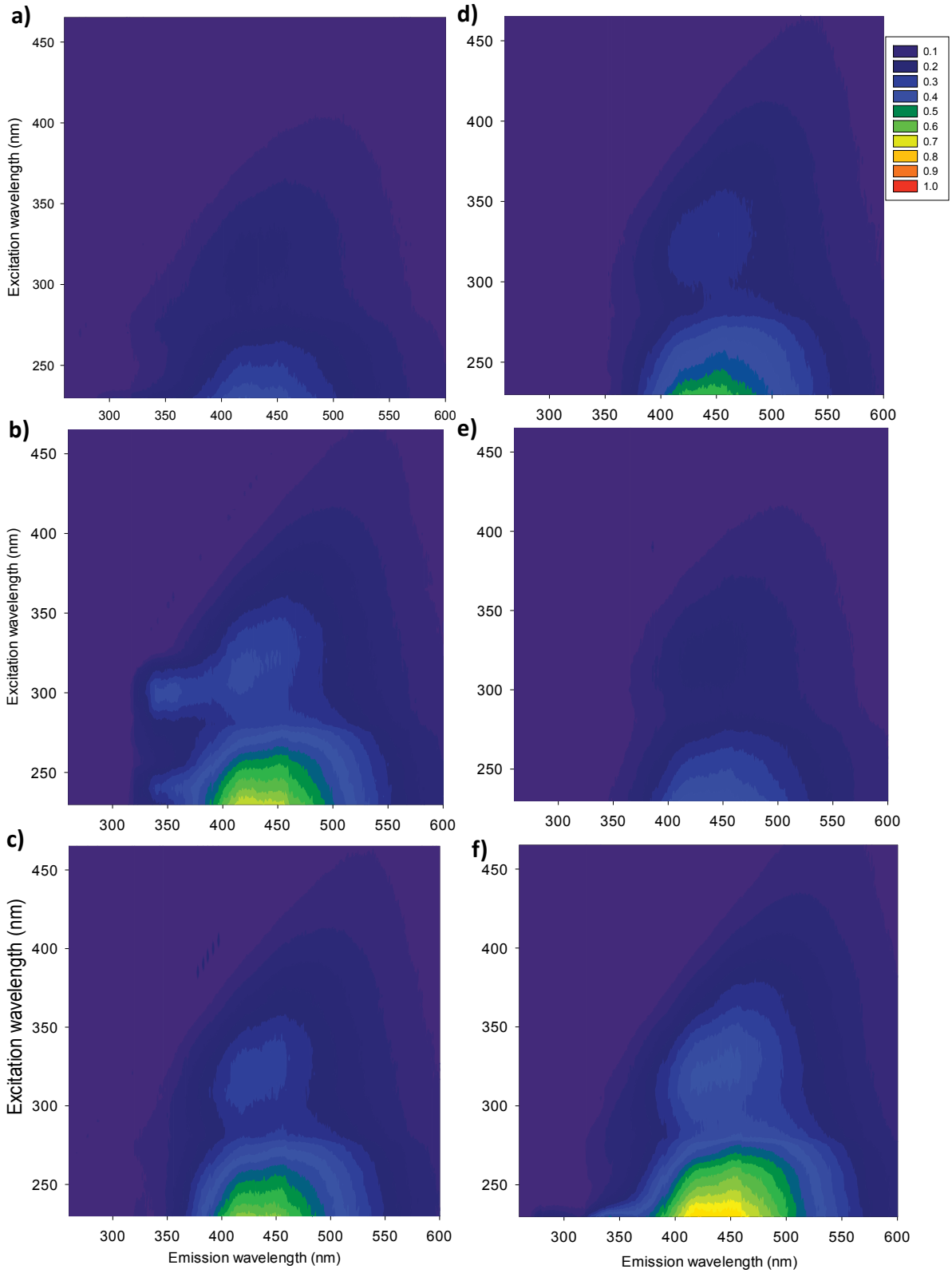


Figure 3.8: Excitation-emission matrices for samples collected during dam release 2 at Snowy River sites a) S1, b) S4, c) S5 and at Mowamba River sites d) M1, e) M2, f) M3.

3.5 Discussion

3.5.1 DOC regime characteristics

The initial hypothesis that reservoir releases would increase DOC concentration was not supported, with only negligible changes or decreases in DOC concentration observed at all Snowy River sites during both dam releases (Fig 3.4). Whilst our results clearly show that DOC concentration was not related to discharge during Jindabyne Dam releases (Fig 3.5), the literature report inconsistent relationships between DOC concentration and discharge in dam tailwaters, with DOC concentration increasing with discharge below some dams (Henson *et al.*, 2007; Ulseth & Hall Jr, 2015), and an insignificant relationship below others (Ulseth & Hall Jr, 2015). Although Henson et al. (2007) and Ulseth et al. (2015) do not specify the spatial extent of riparian and floodplain inundation in their studies, it is possible that the relatively small area inundated by the dam releases in our study precluded large changes in DOC concentration, as only the lower river benches were wetted. We propose that the small areal extent of riparian inundation released only a limited quantity of carbon that was rapidly diluted by the large volume of low-DOC water from Jindabyne Dam. Any potential DOC pulse generated by dam release 1 may also have been too rapid to be captured by the twice-daily sampling frequency. In contrast to the experimental dam releases, the tributary releases delivered pulses of terrestrial DOC to regulated Snowy River sites (Table 3.3, Fig 3.3), with a significant, positive relationship between DOC concentration and discharge evident immediately below the tributary confluence (Fig 3.5). These rapid increases in DOC concentration usually occur as a result of catchment DOC flushing during natural high-flow events (Hornberger, Bencala & McKnight, 1994) and are consistent with the highly variable DOC regime characteristic of unregulated upland streams (Hinton, Schiff & English, 1997).

3.5.2 DOC supply from dam releases

Despite the delivery of a similar water volume to all Snowy River sites, DOC load was substantially higher at S4 than at S1 during both dam releases (Fig 3.6), suggesting the mobilisation of an additional DOC source between these two sites. In the absence of large tributary in-flows during the Jindabyne dam releases (Fig 3.2, unpublished data), this

additional DOC may have been liberated from the riparian soils and vegetation inundated by rising water levels, which can be rich sources of DOC (Sanderman *et al.*, 2009). DOC is also often generated and stored in bed sediments (Schindler & Krabbenhoft, 1998) and their resuspension during the dam releases may have contributed additional DOC to the water column. The greater surface area of river benches inundated between S1 and S4 (approximately 22 km compared to 2.2 km from Jindabyne Dam to S1) may have also contributed to the larger DOC load at S4. The higher intensity of humic-like and protein-like fluorophores relative to the upstream S1, (Fig 3.8a-b) indicates that the increased DOC input at S4 may have been accompanied by a compositional shift in the DOC pool.

Although it was hypothesised that outflowing dam water would contain protein-like DOC fluorophores, peak T was only very weakly present closest to the dam wall at S1 during dam release 2 (Fig 3.8a). The protein-like DOC pool is highly dynamic, and microbial consumption and photodegradation of DOC within the reservoir (Stedmon & Cory, 2014) may have contributed to the marginal protein-like DOC signal in the outflowing dam water. Additionally, protein-like DOC fluorophores may only be detectable during phytoplankton bloom conditions (Stedmon & Markager, 2005), which were unlikely to have occurred given the low nutrient levels immediately below Jindabyne Dam during the study period (unpublished data). The tryptophan-like fluorophore was strongest further downstream at S4 (Fig 3.8e). We hypothesise that this protein-like DOC was mobilised from inundated river benches, benthic algae and bed sediments disturbed by the dam release. Agricultural sources are also likely to have contributed to the tryptophan-like DOC observed at S4 (Baker & Inverarity, 2004), as stock access to this section of the Snowy River is unrestricted and the surrounding catchment is heavily influenced by grazing and cropping.

3.5.3 Ecological significance

The hydrologically induced DOC concentration peaks observed during the tributary releases may contribute to biogeochemical 'hot moments' (McClain *et al.*, 2003), whereby rapid DOC additions temporarily stimulate heterotrophic microbial growth and metabolic activity rates. In particular, the pulsed input pattern of tributary DOC may be crucial in triggering microbial responses in the downstream river, as cumulative bacterial productivity can be several-fold higher in response to pulsed DOC additions relative to equivalent DOC additions made at a

constant rate (Lennon & Cottingham, 2008). Although this study did not measure microbial functioning directly, significant increases in microbial metabolic activity have been widely documented in the literature in response to similar rainfall and snowmelt-associated DOC pulses (McKnight *et al.*, 1993; Buffam *et al.*, 2001). Our study shows that characteristic DOC pulses can be absent from managed dam releases, which may consequently have a reduced capacity to trigger the biogeochemical 'hot moments' that occur during natural high-flow events. Variations in DOC quantity and composition arising from a managed release event may therefore affect microbially-mediated riverine processes such as community metabolism (Findlay *et al.*, 2003), nutrient cycling (Bernhardt & Likens, 2002) and food web dynamics (Wilcox *et al.*, 2005). Fluctuations in the DOC regime within year may also be particularly important in influencing food web structure in impounded rivers, which often have reduced stocks of particulate terrestrial detritus (Ward & Stanford, 1983), potentially leading to increased reliance on autochthonous DOC as an energetic resource base (Hall & Meyer, 1998).

3.5.4 Management implications

This improved understanding of DOC delivery from managed flow releases is an important step towards inclusion of the organic matter regime as a primary consideration for the rehabilitation and management of impounded rivers. In most regulated river basins, the volume of water available for environmental flow releases is limited. Our results show that particular components of the DOC regime can be maintained in regulated rivers by modifying the water source, duration, timing and discharge pattern of release events. Additional studies examining DOC delivery and associated microbial responses from a greater range of water sources and events of differing hydrological characteristics would clarify how rehabilitation activities may influence river processes such as ecosystem metabolism, which are increasingly being incorporated into environmental water management strategies (Fellman *et al.*, 2014).

Furthermore, DOC delivery from a dam release event is likely to be dependent upon reservoir hydrologic characteristics and the particular release strategy employed. Larger reservoirs with a relatively long retention time such as Jindabyne Dam (~12 years) are likely to buffer short-term fluctuations in inflowing solutes such as DOC (Ahearn, Sheibley &

Dahlgren, 2005). However, the DOC supply from dams with a shorter retention time may be more heavily influenced by flow release strategy and the variations in the inflowing DOC signal. The dam releases in the present study were not triggered by antecedent precipitation, and may be less likely to contain the pulses of terrestrial organic matter than releases linked to inflow conditions, such as transparent or translucent releases (Growns & Reinfelds, 2014). Future releases from Jindabyne dam will be implemented under an alternative 'natural scaling' strategy whereby daily flow targets will be derived by scaling available water allocations to a 2-3 year prior daily flow sequence from an unregulated reference river (Reinfelds 2013). Although still disconnected from upstream precipitation, a natural scaling strategy would yield more numerous, smaller magnitude flow peaks relative to the single large peak characteristic of the dam releases in the present study. Further studies are required to elucidate the net effect of this strategy on in-stream DOC delivery, for example by increasing the frequency but reducing the areal extent of riparian inundation.

Whilst our study only examined DOC dynamics within a fairly limited spatial extent (<92 km downstream of the dam), the humic-like DOC fluorophores mobilised by both tributary and reservoir releases may be transported much longer distances before being metabolised (Cory & Kaplan, 2012) or photo-degraded (Moran & Covert, 2003). Further studies linking carbon delivery from managed flow events to food web responses at greater spatial scales are required to establish their potential influence on fish recruitment and the productivity of downstream river and estuarine ecosystems. Additionally, the tryptophan-like DOC fluorophore detected during dam release 2 has been linked to agricultural sources, and is often considered an indicator of lower water quality (Baker & Inverarity, 2004). Collectively, our results highlight the need to further consider complementary management strategies to improve riparian condition to provide more locally derived terrestrial carbon from within the channel and to minimise the mobilisation of potentially undesirable DOC sources by high-flow in-channel releases.

3.6 Conclusion

Our study showed that the water source used for release events (i.e. reservoir vs. tributary water) produces markedly different DOC regimes with respect to DOC concentration, load, input pattern and composition in a heavily regulated section of the Snowy River. The tributary releases supplied rapid pulses of increased DOC concentration, whereas dam releases are characterised by a fairly constant DOC concentration with minimal variation. Spectrophotometric analysis of DOC composition showed that reservoir releases contained both humic-like and protein-like flurophores, whereas only humic-like fluorophores were detected during tributary releases. The distinct DOC supply regimes observed in this study can be explained by the differing hydrological characteristics and DOC sources mobilised by each of the release types. We highlight the ability of a mixed rainfall -snowmelt tributary to supply increased DOC concentration and variability to a regulated channel compared with managed flow releases from a reservoir.

Chapter 4: Functional responses to environmental flows: does resource delivery from pulsed dam releases influence microbial metabolism?

4.1. Abstract

River regulation by large reservoirs disrupts the hydrologically-driven supply of resources such as dissolved organic carbon and nutrients. Pulsed dam releases may mobilise some resources to the downstream river, but may not necessarily provide the same resource quantity, quality or supply pattern as that of the natural high-flow events that they are intended to simulate. Microbial metabolic processes may potentially be used as a metric of carbon and nutrient utilisation in regulated rivers, but their responses to pulsed dam releases remain poorly understood. This study examined the effect of shifting resource availability on benthic microbial metabolic activities during a series of experimental pulsed dam releases to a montane river. We hypothesised that exposure to dam release waters would increase a) epilithic biofilm respiration and b) sediment extracellular enzyme activities. Epilithic biofilm metabolism was quantified by exposing biofilm-colonised tiles to dam release waters in a streamside mesocosm, allowing the effects of physical disturbance to be controlled. Activity of a range of sediment enzymes was measured and used to infer changes in carbon and nutrient availability to the benthic microbial community. Tile biofilm respiration increased significantly ($p<0.05$) during two of the three release events. Bacterial enzyme profiles also shifted during two release events, although these changes were decoupled from the observed pulses in biofilm respiration. Our results indicate that pulsed dam release waters can trigger transient shifts in epilithic biofilm community metabolism via a mechanism that operates independently from the physical disturbance effects of increased flow velocities. Although microbial responses could not be conclusively linked to variations in DOC or nutrient concentrations in this study, further studies incorporating measurements of DOC bioavailability may potentially resolve the underlying mechanism for the observed biofilm metabolic response. The results of this study contribute to an improved understanding of the pathways through which pulsed high-flow dam releases may influence ecological processes in regulated rivers.

4.2 Introduction

River regulation by large reservoirs reduces the frequency, magnitude and volume of natural high-flow events, which disrupts the longitudinal and lateral transport of materials and energy to the downstream reaches of the river (Poff *et al.*, 2007). Conventionally, the ecological effects of river regulation and associated rehabilitation actions have been quantified using community structural metrics such as species abundance and diversity (Poff & Zimmerman, 2010; Gillespie *et al.*, 2015). However, there is growing awareness that the rehabilitation of key ecological processes is essential in supporting the recovery of target species at higher trophic levels (Beechie *et al.*, 2010), such as birds, fish and platypus. Accordingly, metrics of ecosystem functioning are increasingly being adopted in rehabilitation and subsequent monitoring strategies of regulated rivers (Colangelo, 2007; Robinson & Uehlinger; Williams, 2015a).

Below large dams, recovery of key ecosystem functions may be achieved using high-flow pulsed releases of reservoir water, which are commonly designed to restore physical processes such as sediment transport and benthic scouring (e.g. Patten *et al.*, 2001; Coleman & Williams, 2016). Regulation by dams also disrupts organic matter and nutrient delivery to the downstream reaches of rivers, but the potential for pulsed dam releases to contribute to the recovery of these key ecosystem functions has only recently been recognised (Doi *et al.*, 2008; Cross *et al.*, 2011; Rohlfs *et al.*, 2015). While pulsed dam releases do have the potential to mobilise some resources, they may not necessarily provide the equivalent resource quantity, quality or supply pattern as that of natural high-flow events of an equivalent magnitude (Rohlfs *et al.*, 2016).

One resource of particular importance in river ecosystems is dissolved organic carbon (DOC), which is directly consumed by heterotrophic microbes. In unregulated streams and rivers, high-flow events including storms and the seasonal snowmelt typically deliver DOC and nutrient pulses to the receiving waterways (Hinton, Schiff & English, 1997). This increased resource availability can release heterotrophic microbes from nutrient limitation (Hitchcock & Mitrovic, 2013), triggering temporarily accelerated rates of respiration (Roberts, Mulholland & Hill, 2007) and biomass production (Lennon & Cottingham, 2008). These episodes can be conceptualised as biogeochemical 'hot moments'; defined by

McClain *et al.* (2003) as periods of increased process rates, triggered by the delivery of limiting reactants. Although short in duration, a large proportion of the net movement or transformation of major elements can occur during these periods (Boyer *et al.*, 2000). River regulation can produce a more homogenous downstream DOC regime lacking the characteristic pulses found in unregulated river systems (Rohlfs *et al.*, 2015), potentially eliminating a trigger for the occurrence of microbial hot moments. Whilst this potential effect is relatively unstudied, delivery of limiting resources from environmental flow releases may stimulate microbially-mediated ecosystem processes such as community respiration, and even gross primary productivity, as respired carbon is cycled through autotrophic components of the biofilm community (Cook *et al.*, 2015).

While microbial processes offer much potential as functional indicators of river rehabilitation efforts, their usefulness as a metric of carbon and nutrient limitation in regulated rivers has received little attention. The few existing studies which measure microbial responses to dam releases report inconsistent results; for example, benthic community respiration was enhanced by pulsed flow events with peak magnitudes of 55 m^3s^{-1} and 13 m^3s^{-1} in a European montane river (Uehlinger, Kawecka & Robinson, 2003) but showed an inconsistent response to release events of 1.15 m^3s^{-1} – 1.77 m^3s^{-1} from a reservoir in south-east Australia (Reid, Thoms & Dyer, 2006). These discrepancies are unsurprising given that microbial processes respond to numerous environmental gradients that are dependent on release hydrology and catchment conditions, such as flow velocity (Augspurger & Kusel, 2010) and benthic substrate type (Romani & Sabater, 2001) as well as resource supply (Findlay *et al.*, 2003). These variables may also have interactive or threshold effects; for example, flow velocity enhances diffusion of dissolved nutrients, stimulating benthic metabolism up to a threshold beyond which it can be rapidly reduced by physical scouring effects (Davie & Mitrovic, 2014). Additionally, microbial process rates are often measured at monthly or pre and post-event scales (e.g. Rees *et al.*, 2005; Reid, Thoms & Dyer, 2006) that may fail to capture rapid microbial responses that can occur at temporal scales of hours to days (Kreutzweiser & Capell, 2003). An improved understanding of the specific mechanisms through which dam releases may influence microbial metabolic processes would support the more widespread inclusion of metabolism as a functional indicator in river rehabilitation and management programs.

An experimental flow regime was implemented in the Snowy River to test microbial metabolic responses to a series of pulsed dam releases below a multiple reservoir hydroelectric and water diversion network. In this study, we examined the potential role of shifting carbon and nutrient availability in driving benthic microbial metabolic activity by controlling flow velocity using a stream-side mesocosm system. We hypothesised that exposure to dam release water would significantly increase epilithic biofilm respiration and shift the expression of carbon and nutrient-acquiring enzymes by river sediment bacteria.

4.3 Materials and Methods

4.3.1 Study area and hydrology

This study was carried out in the Snowy River, approximately 22 km downstream of Jindabyne Dam, in a montane to cool temperate region of New South Wales, Australia. Jindabyne Dam has a maximum capacity of 688 GL and is the most downstream dam on the Snowy River. Jindabyne Dam forms part of a large network of interconnected dams that divert water for hydropower and irrigation to the Murray-Darling Basin. The catchment surrounding the study site is in the Dalgety Uplands region, and contains highly erosive soils and is primarily used for cattle and sheep grazing. Following construction of the Snowy Scheme, high levels of catchment erosion combined with a lack of regular high-flow events have caused extensive sediment armouring of the original cobble substrate, producing a bed dominated by finer mud and sand particles interspersed with cobbles and boulders (Williams and Coleman 2016).

This study followed benthic metabolic responses to three pulsed experimental flow releases with peak mean daily flows of 10.7 $m^3 s^{-1}$, 10.1 $m^3 s^{-1}$ and 12.1 $m^3 s^{-1}$, created by releasing epilimnetic water from Jindabyne Dam. The experimental releases were equivalent to the 3^{rd} exceedance flow percentile, and were interspersed by flows of 0.92 $m^3 s^{-1}$ and 1.15 $m^3 s^{-1}$, representing the 95^{th} and 80^{th} flow percentiles respectively. These characteristics of three events were generally the same, except that Event 2 (i.e. 11.57 $m^{-3} s^{-1}$) had a planned slightly quicker recession than Events 1 and 3 (i.e. 6.94 $m^3 s^{-1}$) from Day 1 to Day 2 (Table

4.1, Fig 4.1). Events of this magnitude allowed for the inundation of the lower vegetated channel benches and produced flow velocities sufficient to mobilise fine sediment on the river bed (Williams pers. comm). The experimental releases took place on 22/10/12, 4/12/12 and 16/4/13, corresponding to the Austral spring, summer and autumn (Fig 4.1). These events are subsequently referred to as Event 1 (October 2012), Event 2 (December 2012) and Event 3 (April 2013) (Fig 4.1 and 4.2). Discharge was measured at a rising stage gauge (DPI gauge 222026) located at Dalgety, approximately 2 km downstream of the study site.

Table 4.1: Hydrological characteristics of the experimental release events. Percent exceedance values are expressed relative to a baseflow of 1.2 m^3 s^{-1}. Data are calculated from discharge values recorded at the Dalgety NSW Office of Water gauge 222026.

Event	Duration (days)	Volume (ML)	Maximum mean hourly discharge (m^3 s^{-1})	% exceedance of base flow
Event 1	5	3150	14.6	1216
Event 2	5	2827	18.4	1533
Event 3	6	3303	18.4	1533

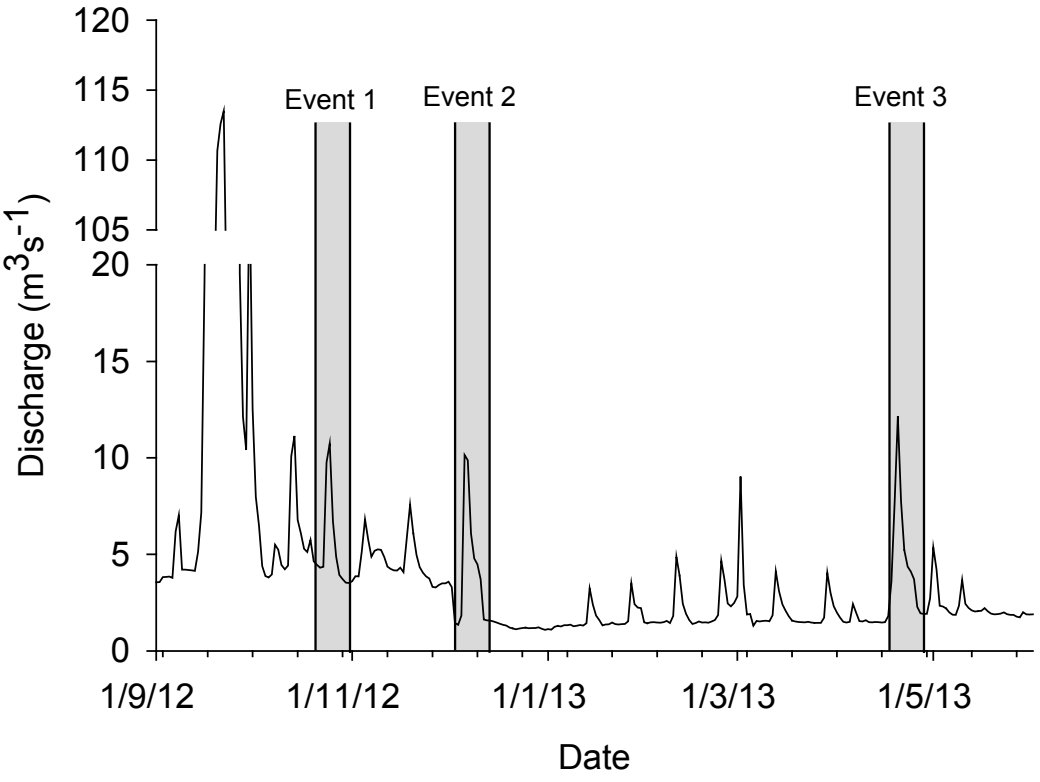


Figure 4.1: Mean daily stream discharge measured 2 km downstream of the study site (NSW DPI gauge no 222026), showing the three experimental releases monitored in this study.

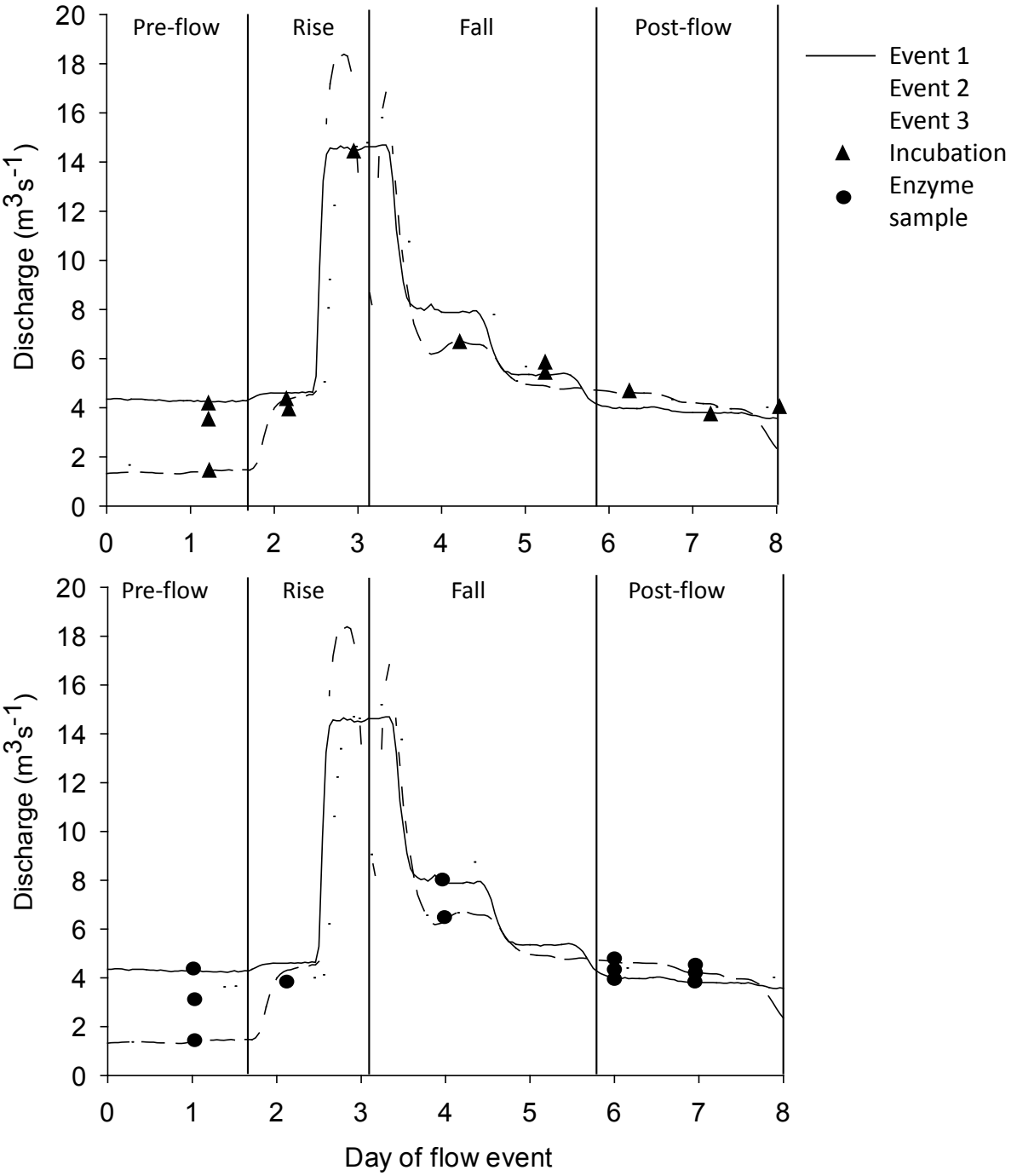


Figure 4.2: Metabolism incubation and enzyme sampling schedule for the three release events. Figure shows mean hourly discharge, measured 2 km downstream of the study site (NSW DPI gauge no 222026).

4.3.2 Physicochemistry

Throughout each release event, stream water temperature was measured twice daily onsite using a Hydrolab Surveyor and MS5 Sonde probe. Turbidity was also measured twice daily using a Hach Turbidimeter. Water for chemical analyses was sampled in duplicate by collecting surface water grab samples into pre-rinsed polyethylene bottles twice daily throughout the release events. Water for DOC measurements was filtered on collection through a 0.45 µm pore size membrane and the filtrate acidified with 2 N HCl and stored at -4°C prior to analysis using the wet combustion method (APHA, 2005), to a detection limit of 0.5 mg L^{-1}. Nutrient samples were stored -20°C and nutrient concentrations were quantified according to standard methods (Eaton & Franson, 2005), using unfiltered water for total nitrogen (TN) and total phosphorus (TP) analyses, and water filtered through a 0.45 µm pore size filter for analysis of soluble reactive phosphorus (SRP) and nitrogen oxides (NO_x).

4.3.3 Field mesocosm system

A stream-side mesocosm system was used to measure biofilm community metabolic responses to changing resource availability during the release events. The mesocosms consisted of a modified trailer fitted with four open-topped PVC flumes through which stream water was continually pumped to reflect real-time changes in stream water chemistry. The mesocosms allowed for metabolism to be measured throughout the flow pulse when access to the river benthos was restricted, and enabled the specific influence of resource supply to be examined separately from physical scouring processes in the river channel. Prior to each event, a set of unglazed 100 cm^2 ceramic tiles was incubated in situ at the sample site for three weeks. The tiles were relocated to the stream-side mesocosms and allowed to acclimate in the flumes for 48 h prior to commencing metabolism measurements.

4.3.4 Biofilm metabolism

Gross primary productivity (GPP) and community respiration (CR) were quantified by incubating colonised tiles in sealed light and dark chambers (Bott *et al.*, 1997). Four metabolism incubation experiments were conducted during each release event, with one incubation experiment in each flow stage (Fig 4.2). Each chamber consisted of an airtight

2.7 L polyethylene container fitted with a perspex lid panel and a 12 V pump (Whale) which circulated water internally to prevent boundary layer effects. A dissolved oxygen probe (ProODO, YSI) was sealed inside each chamber and logged temperature and dissolved oxygen concentration and percentage saturation at 10-minute intervals. Prior to each incubation experiment the flumes were flushed to expose tiles to river water for 1.5 h. Two tiles were placed into each chamber, which was then filled with river water, carefully sealed and incubated in the flumes for 3-7 h. Six pairs of chambers were used for each incubation experiment, with one of each pair wrapped in black plastic to exclude light. Temperature in the flumes was maintained throughout the incubation experiments by filling the trailer with stream water to a level that allowed the flumes to be immersed but not completely submerged. Upon termination of each incubation experiment, the tiles were removed from the chambers and gently placed back into the flumes. At the end of the final incubation experiment for each release event, periphyton was scraped from the tile pairs in distilled water and a subsample of the resulting slurry was filtered onto GF/C filters and used for determination of chlorophyll a using the grinding technique and an acetone extraction solvent with correction for phaeophytin (APHA, 2005).

Some chamber replicates were excluded from analyses due to chamber leakage or probe failure, resulting in a final dataset of 5 replicate light and dark chambers for each incubation experiment. CR and net ecosystem productivity (NEP) were calculated for dark and light chambers respectively, as the rate of change in dissolved oxgyen concentration over the straight section of the logged oxygen curve:

$$\Delta DO = \frac{\sum(DO_{t_{final}} - DO_{t_{initial}})}{t} . V \quad (4.1)$$

where DO is dissolved oxygen concentration (mgL^{-1}), t_{final} and $t_{initial}$ bracket each 10 minute logging interval, t is the total incubation time for the linear section of the oxygen curve and V is the chamber volume in litres. Gross primary production (GPP) was calculated by summing the net change in oxygen concentration from paired light-dark chambers (Bott, 2006). Where oxygen supersaturation occurred in light chambers any subsequent data

points were excluded. GPP and CR were expressed as areal rates by dividing total estimates by the total area of upper and lower tile surfaces.

4.3.5 Extracellular enzyme activity

Extracellular enzyme activities (EEA) of surface sediments were assayed to provide an index of the specific substrates limiting in-stream benthic metabolic responses to the experimental release events (Findlay *et al.*, 1993). Sediment EEA were measured as the experimental mesocosm system did not have the capacity for sufficient tiles to allow destructive sampling of biofilm EEA throughout each release event. During Events 1 and 2, sediments were sampled four times, including during the pre-flow, falling flow and post-flow stages (Fig 4.2). Event 3 had a slightly slower rate of flow rise and fall than that of Events 1 and 2, and consequently was sampled during the flow rise rather than the falling flow stage, due to logistical constraints in accessing the river benthos (Fig 4.2). On each sampling day, five replicate sediment cores were collected using sterile polyethylene vials from the top 5 cm of the stream substrate, in a permanently submerged section of the river channel. The cores were immediately frozen at -20 °C, which is a common method of preserving sediment samples prior to EEA analysis (Giles *et al.*, 2015).

For EEA analysis, sediment cores were thawed and homogenised and 0.5 g of wet sediment was weighed out into sterile centrifuge tubes. A known volume of sterile milli-Q water was added and the resulting slurry homogenised by sonication for 30 s at ≈ 40 W (Vibracell, Sonics Materials) to achieve more even dispersal of sediment. Activities of 6 extracellular enzymes were assayed for each sample; butyl-esterase (BU), alkaline phosphatase (PHOS), α-1,4-glucosidase (ALPHA), β-1,4-glucosidase (BETA), leucine aminopeptidase (LEU) and β-xylosidase (XYL). Potential extracellular enzyme activities were quantified using fluorescent methylumbelliferyl (MUB)-linked substrates (Sigma-Aldrich) prepared in either 5 mM $NaHCO_3$ (PHOS, ALPHA, BETA, XYL) or 5 mM $NaPO_4$ (BU, LEU) buffer. The slurry was incubated in sterile 96-well plates with MUB-linked substrates mixed to a predetermined saturating concentration of 300 mM. Fluorescence was recorded periodically at 365 nm excitation and 445 nm emission in a microplate reader (Infinite® 200 Pro, Tecan, Switzerland) over a 3 h incubation period at 25 ˚C. A set of boiled sample blanks was also assayed to account for substrate degradation over time. All fluorescence readings were

converted to enzyme production rates using a standard curve plotted from MUB solutions mixed to 0.1 µmol L^{-1}, 0.5 µmol L^{-1}, 1 µmol L^{-1}, 2 µmol L^{-1}, 3 µmol L^{-1} and 5 µmol L^{-1} in 5 mM $NaHCO_3$ buffer. Abiotic fluorescence quenching due to sample turbidity was estimated by incubating 150 µL slurry with the MUB standard solutions, and a correction factor applied to the sample enzyme activity rates by subtracting the slope of the quench curve from the slope of the standard curve.

4.3.6 Statistical analysis

Mean CR and GPP rates were determined for each daily metabolism incubation experiment, and the GPP:CR ratio was calculated as a measure of the relative trophic state of the benthic tile communities. GPP and CR rates were checked for normality and variance homogeneity and logx+1 transformed where appropriate. Repeated measures ANOVA was used to test for temporal changes in GPP and CR during each of the three release events. Sphericity was checked using Mauchley's test and uncorrected p-values were used as sphericity was not violated for any of the three release events. Mean daily GPP and CR rates from the three release events were pooled and linear regressions were performed to examine relationships between biofilm metabolism rates and stream water DOC and nutrient concentrations. Linear regressions were also performed for other potentially influential environmental variables including mesocosm water temperature, hourly discharge at the time of tile flushing, photosynthetically active radiation and stream water turbidity. One-way ANOVA was used to test for differences in biofilm chlorophyll-a concentration between tile sets at the end of each release event.

The mean potential activity rate for each enzyme was calculated for each sampling day. The enzyme dataset was screened for multivariate outliers by converting the Mahalanobis distance for each sample to a probability value based on the chi-square distribution and eliminating all samples with a p value of <0.001. Absolute enzyme rates were standardised to maximum values and PCA analysis was performed on the standardised dataset using SPSS (version 21, IBM) using a correlation matrix to eliminate the effect of differing variances from each enzyme activity on the overall solution.

4.4 Results

4.4.1 Hydrology and chemistry

Release events 1 and 3 were unimodal, with maximum hourly discharge rates of 14.6 m^3s^{-1} and 18.4 m^3s^{-1} respectively, while release event 2 had dual flow peaks of 18.4 m^3s^{-1} and 16.83 m^3s^{-1} resulting from a natural precipitation event during the release period. (Fig 4.2). Hydrological attributes of the release events are specified in Table 4.1. Mean stream temperatures were 14.9± 0.2°C, 19.7± 0.6°C and 14.7 ±0.5°C during release events 1, 2 and 3 respectively.

DOC reached maximum concentrations of 3.8 mgL^{-1}, 3.2 mgL^{-1} and 3.8 mgL^{-1} during release events 1, 2 and 3 respectively. These increases in DOC concentration occurred on the rising limb of each release event, before returning to pre-event levels in the falling flow stage (Fig 4.3 a-c). Distinct pulses in dissolved N and P concentrations were also evident during the rising limb of all three release events (Fig 4.3d-f). TN concentrations peaked at 0.43 mgL^{-1}, 0.31 mgL^{-1} and 0.33 mgL^{-1} during release events 1, 2 and 3 respectively, representing an approximate doubling in TN concentration from pre-event levels. Secondary peaks in TN concentration were also evident during the falling flow stages of all three release events (Fig 4.3d-f). Maximum TP concentrations of 0.035 mgL^{-1}, 0.041 mgL^{-1} , and 0.094 mgL^{-1} occurred during release Events 1, 2 and 3 respectively. Events 1 and 2 exhibited dual peaks in TP concentration, occurring on the rising and falling flow limbs, whereas only one single peak in TP was evident on the rising limb of release event 3 (Fig 4.3d-f).

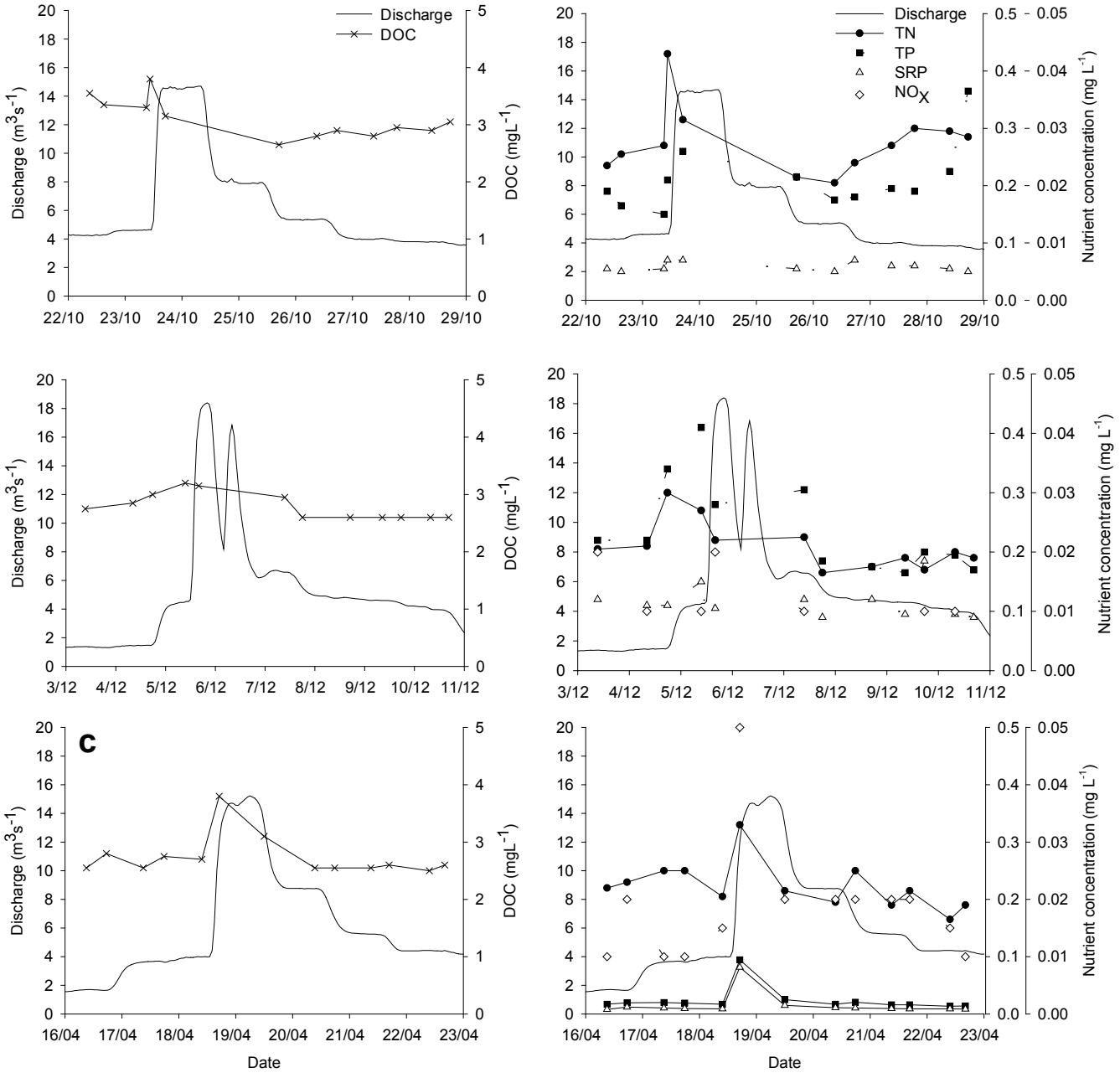


Figure 4.3: DOC and nutrient concentrations during Event 1 (a,d), Event 2 (b,e) and Event 3 (c,f). Symbols with dashed lines are plotted on the second y axis. NO_x remained below the detection limit of 0.01 mg L^{-1} throughout Event 1.

4.4.2 Incubation experiments

Gross primary productivity of tile biofilm communities was highly variable within and between individual flow stages, and did not respond consistently to the release events (Fig 4.4a-c). In release event 1, average GPP increased compared to pre-event levels, but during release events 2 and 3 a decrease was detected. Within each of the three release events, biofilm GPP showed no response to changes in flow ($p>0.05$). When pooled across all three events, mean biofilm GPP was negatively related to TP, SRP and stream water turbidity (Table 4.2).

Mean CR increased significantly during release events 1 ($F=6.579$, $p<0.05$) and 2 ($F=14.31$, $p<0.05$) (Fig 4.4d-f). CR was higher during the rising flow stage of release event 3 (Fig 4.4f), although this difference was not significant ($F=1.061$, $p>0.05$). These increases in CR drove a clear shift from net autotrophy to net heterotrophy during all three release events (Fig 4.5). GPP:CR ratios decreased to <1 during the falling flow stage of release events 1 and 3, and during the rising and peak flow stages of release event 2. Biofilm CR rates were not linearly related to any of the measured dissolved resources or environmental parameters (4.3).

Mean tile biofilm chlorophyll-a concentration differed significantly between the three release events ($F=27.81$, $p<0.05$), with a higher chlorophyll-a concentration recorded in release event 3 (0.7 ± 0.05 gm^{-2}) compared with release events 1 (0.37 ± 0.02 gm^{-2}) and 2 (0.45 ± 0.03 gm^{-2}).

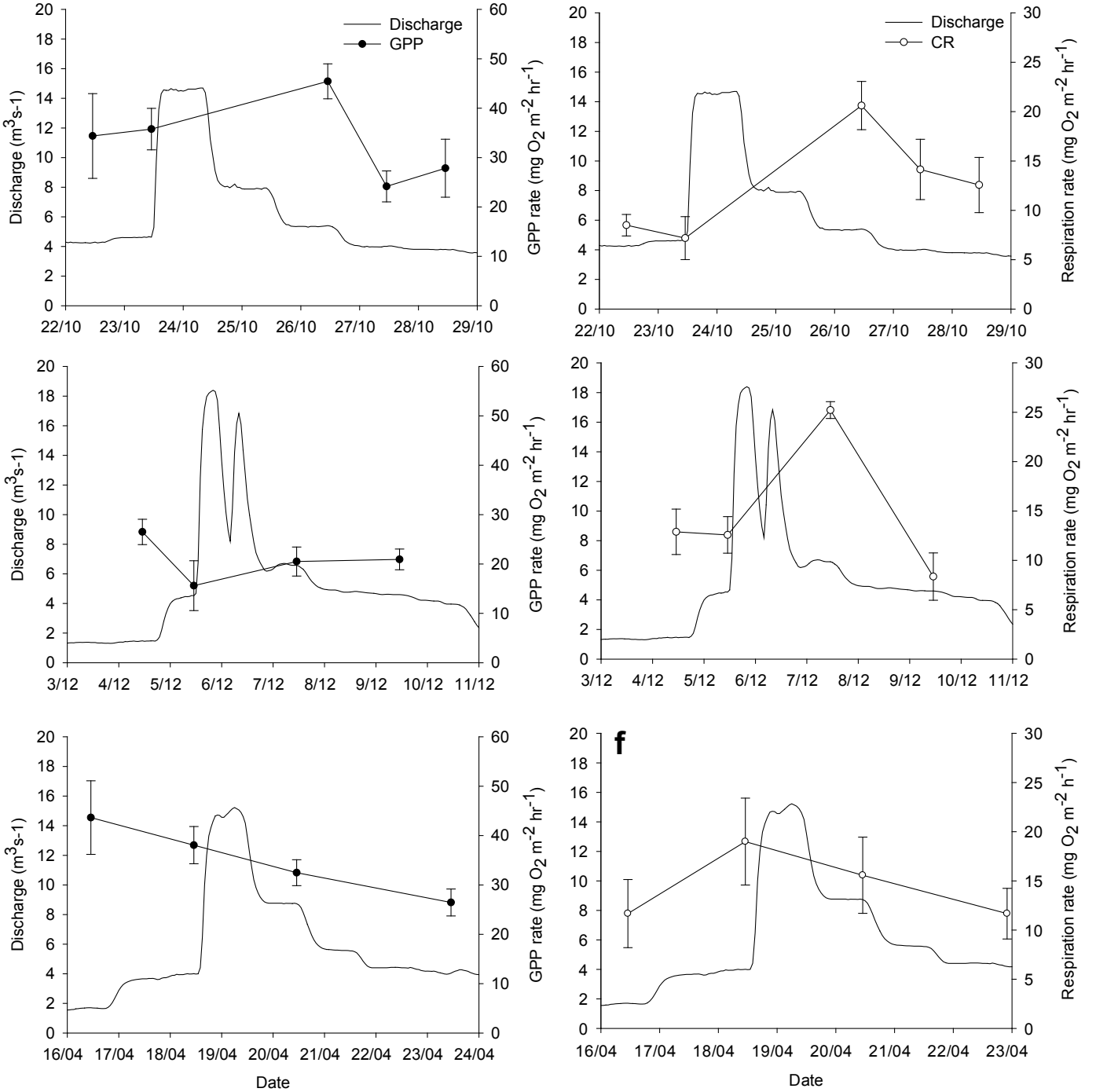


Figure 4.4: Mean biofilm community gross primary production and respiration rates during Event 1 (a,d), Event 2 (b, e) and Event 3 (c,f). Error bars show mean ± SE, n=5. Scale for figures d-e shows oxygen consumption rates.

Table 4.2: Regression parameters for GPP and CR with dissolved resources and environmental variables. * denotes significant regressions.

	GPP				*CR*			
	β_0	β_1	P-value	R^2	B0	B1	P-value	R^2
Dissolved resources								
DOC	33.38	-0.84	0.25	0.11	26.65	-4.41	0.25	0.11
TN	30.87	0.25	0.99	0.00	20.34	-27.14	0.12	0.23
TP	45.60	-691.30	*0.01	0.41	10.77	144.45	0.50	0.04
SRP	48.13	-2005.86	*0.01	0.50	13.97	-15.75	0.97	0.00
Environmental variables								
Water temperature	45.01	-0.73	0.42	0.05	43.20	-0.67	0.60	0.25
Flow rate	31.21	-0.05	0.94	0.00	9.74	0.82	0.08	0.24
Turbidity	46.53	-3.36	*0.02	0.37	12.83	0.21	0.84	0.00
Light	21.73	0.00	0.55	0.05	16.67	0.00	0.72	0.19

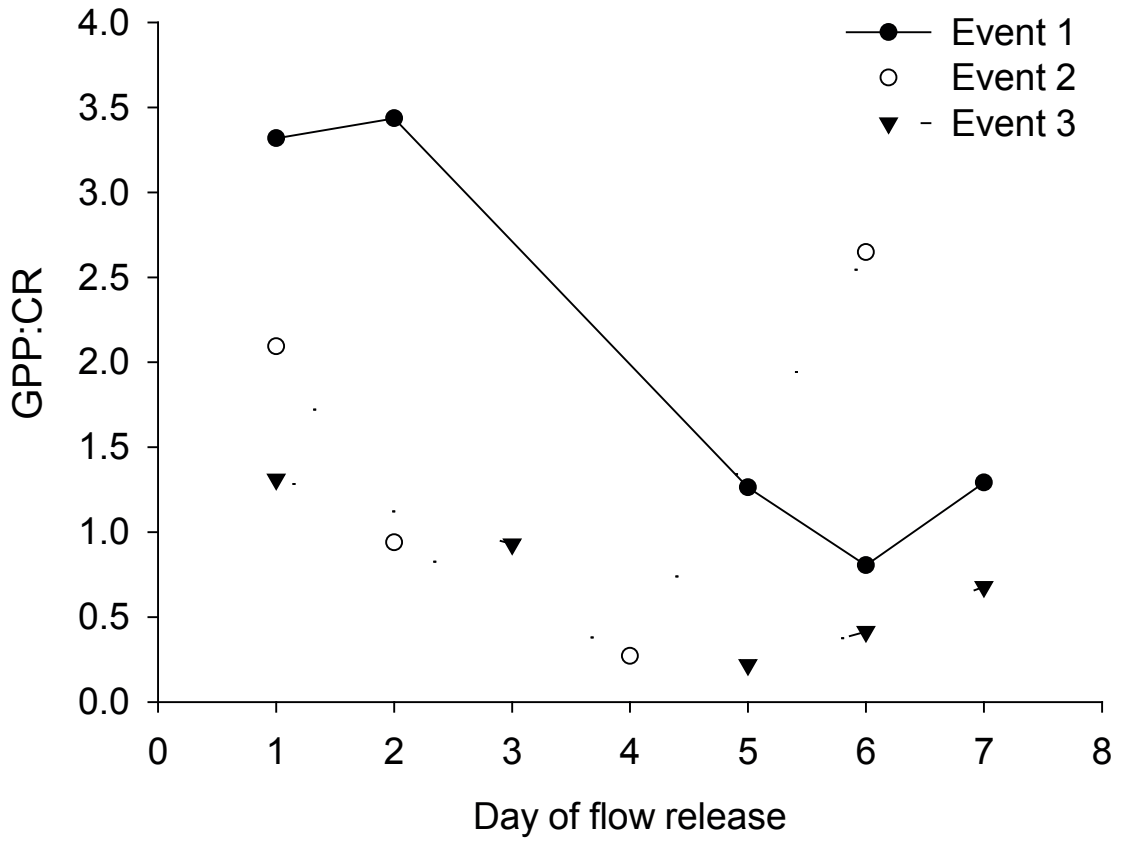


Figure 4.5 – Mean GPP to CR ratio during the three dam release events.

4.4.3 Sediment extracellular enzyme activities

Sediment extracellular enzyme activities in the study stream reach were highly variable both within individual sampling occasions and between the different flow stages of each release event. Mean activity rates of individual enzymes showed an inconsistent response between release events. Relative to initial pre-event levels, mean activity rates of most enzymes increased during the rising, peak or falling stages of release Events 1 and 3 (Table 4.3). In contrast, mean enzyme activity rates decreased or showed negligible change over the course of release Event 2, with the exception of BETA, which increased in the falling flow stage (Table 4.3). PCA analysis yielded two principal components that explained 67% of the variation in the enzyme dataset. All six enzymes loaded highly on PCA axis 1 ($r>0.5$), while

BETA and PHOS loaded highly on PCA axis 2 (r>0.5), with ALPHA and LEU loading negatively. The collective enzyme fingerprint showed a unique response to each of the three experimental releases (Fig 4.6). In release event 1, a clear shift in the enzyme profile occurred during the peak flow stage before returning towards its original location during the falling flow stages. Throughout release event 2, the profile remained closely clustered during the distinct flow stages, and during release event 3, the enzyme profile shifted along PCA axis 1 during the rising flow stage of release event 3 but remained distinct from its original position during the falling flow stage. ALPHA, BETA and PHOS loaded highly on PCA axis 1, which drove most of the separation between different flow stages in release events 1 and 3. BU and LEU loaded highly on PCA axis 2.

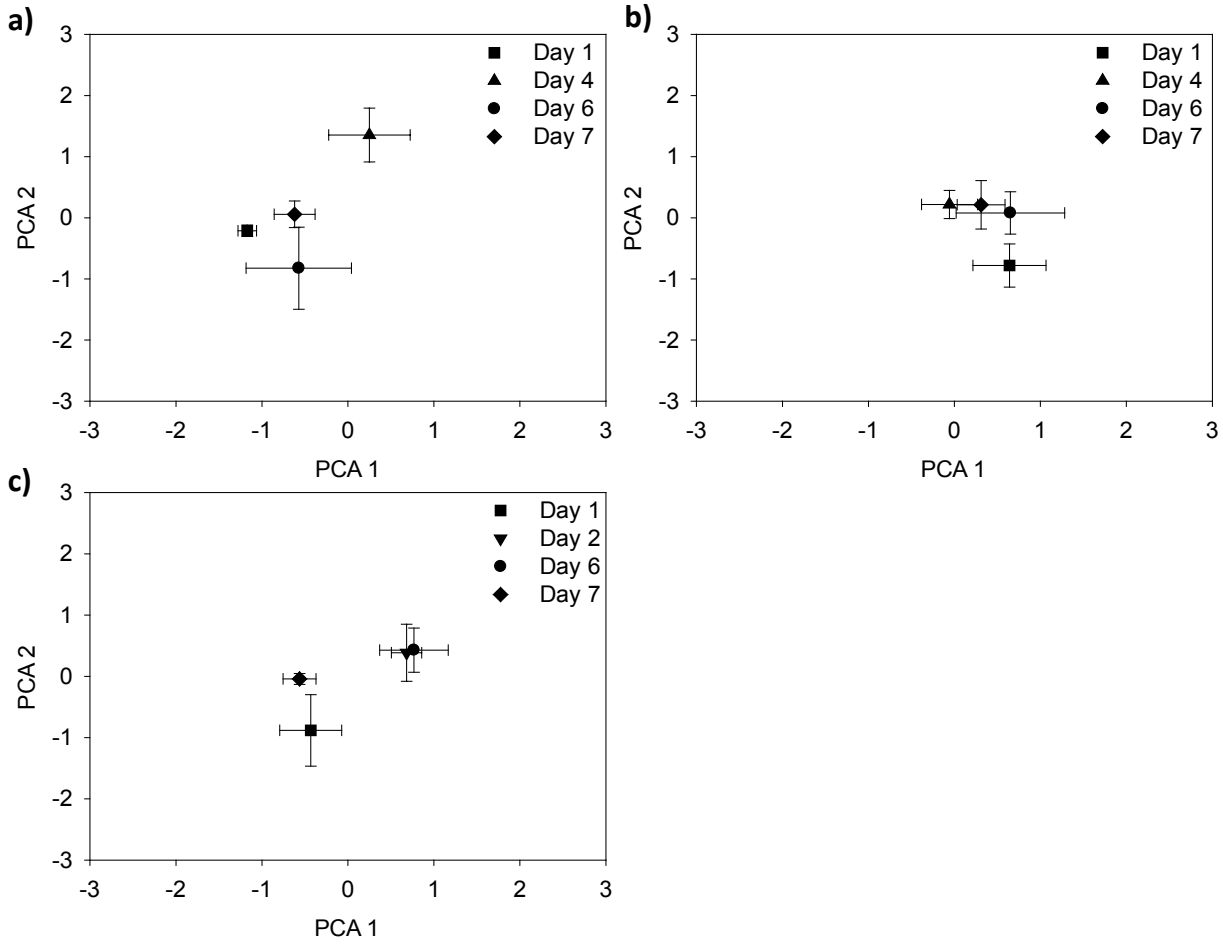


Figure 4.6 – Principal components analysis results for extracellular enzyme activities from a) Event 1, b) Event 2 and c) Event 3. Figures show mean ± SE factor scores shown with flow stage for each of the three events (n=5).

Table 4.3: Mean potential activity rates for sediment bacterial enzymes. EEA rates are in nmol g^{-1} h^{-1} (± SE, n=5)

	BU	PHOS	ALPHA	BETA	LEU	XYL
Event 1						
Day 1	33.27 ± 10.30	1.81 ± 0.52	0.49 ± 0.24	1.31 ± 0.52	64.83 ± 36.32	0.87 ± 0.14
Day 4	67.02 ± 16.48	55.84 ± 16.78	16.41 ± 5.57	63.58 ± 19.17	97.33 ± 40.40	13.78 ± 2.99
Day 6	49.90 ± 28.20	10.11 ± 6.94	31.56 ± 26.83	1.67 ± 0.55	203.96 ± 155.51	3.78 ± 2.42
Day 7	42.20 ± 11.07	3.74 ± 0.80	1.72 ± 0.56	2.33 ± 0.63	136.36 ± 69.14	37.53 ± 11.88
Event 2						
Day 1	37.59 ± 5.81	1.29 ± 0.37	1.03 ± 0.26	7.61 ± 2.24	118.84 ± 29.49	0.91 ± 0.22
Day 4	32.84 ± 8.71	0.99 ± 0.23	0.68 ± 0.19	5.28 ± 0.98	36.48 ± 12.72	1.46 ± 0.41
Day 6	26.21 ± 5.25	1.19 ± 0.35	1.47 ±0.69	14.29 ± 6.11	70.26 ± 19.73	1.81 ± 0.85
Day 7	29.69 ± 7.33	1.75 ± 0.54	1.09 ± 0.17	5.64 ± 1.50	48.22 ± 8.17	1.07 ± 0.33
Event 3						
Day 1	1.67 ± 0.77	1.68 ± 0.63	0.81 ± 0.34	5.69 ± 3.79	18.62 ± 5.68	25.02 ± 15.80
Day 2	2.83 ± 0.20	15.66 ± 2.10	3.50 ± 1.01	79.99 ± 21.67	20.44 ± 4.11	3.47 ± 1.30
Day 7	7.82 ± 1.07	24.87 ± 3.94	1.88 ± 0.77	17.53 ± 3.86	11.99 ± 7.54	4.45 ± 0.47
Day 8	3.83 ± 1.13	9.83 ± 1.95	2.02 ± 0.39	7.57 ± 1.85	5.30 ± 2.30	1.51 ± 0.60

4.5 Discussion

In this study, we investigated the effects of resource delivery from three experimental pulsed dam releases on microbial processes, using epilithic biofilm metabolism and in-situ sediment bacterial extracellular enzyme expression as metrics of microbial functioning, and to infer changes in DOC composition. The increased biofilm respiration rates indicate that pulsed dam release waters can trigger transient shifts in microbial metabolism via a mechanism that operates independently from the direct physical disturbance effects of increased flow velocities. Furthermore, shifts in the sediment bacterial enzyme profiles suggest that high-flow dam releases can trigger transient, compositional changes in the DOC and nutrient supply.

4.5.1 Biofilm metabolism

Exposure to dam release waters significantly increased biofilm community respiration rates in two of the three release events, suggesting that pulsed high flow releases have the capacity to trigger small-scale microbial biogeochemical hot moments. The increased respiration rates may have been resource-driven, as small increases in DOC and nutrient concentrations were recorded during the rising flow stage of each of the release events (Fig 4.3). However, the timing of the DOC, TN and TP pulses was uncoupled from the respiration peaks observed during release events 1 and 2 (Figs 4.3-4.4), occurring later during the falling flow stage. While we were unable to definitively link biofilm metabolism to changes in DOC or nutrient concentrations in this study (Table 4.2), resource variability may still have contributed to the significant respiration response. Water column nutrients may have been adsorbed onto the biofilm structure (Wetzel, 2005), buffering short term variability in the nutrient supply (Freeman & Lock, 1995) and delaying the bacterial respiration response relative to planktonic systems. Additionally, the temporal resolution of the metabolism measurements may not have been high enough to capture rapid changes in biofilm metabolism. Inundation of the lower benches within the former river channel by release waters may also have shifted the composition of the DOC supply (O'Connell *et al.*, 2000), delivering more bioavailable substrates (Hitchcock & Mitrovic, 2015) that would have been undetectable when simply examining DOC concentration.

In addition to dissolved resources (Kreutzweiser & Capell, 2003), benthic community respiration rates vary with several abiotic environmental variables, including water temperature (Phinney & McIntire, 1965; Rees, Bowen & Watson, 2005), flow velocity (Boynton *et al.*) and light availability (Wagner *et al.*, 2015). It is unlikely the increases in respiration observed in the present study resulted from variation in mesocosm flow velocity or water temperature as chamber recirculation rates were controlled and neither GPP nor CR rates varied predictably with chamber temperature (Table 4.2). Additionally, light availability influences the production of highly bioavailable DOC exudates by biofilm algae, which can support increased respiration by heterotrophic microbes within the biofilm community (Wagner *et al.*, 2015). However, light intensity was not a significant predictor of biofilm CR in the present study, and while stream water turbidity was negatively related to GPP, it was not strongly related to CR (Table 4.2). Consequently, a resource-mediated mechanism remains a likely explanation for the observed biofilm response.

Several factors may have impeded the detection of any significant change in respiration rates during the third release event. In release event 3, the tile biofilm community contained a significantly higher chlorophyll-a concentration than the two preceding events, indicating a higher algal density within the biofilm. Algal exudates produced within the biofilm matrix are an easily utilised source of DOC for biofilm bacteria (Neely & Wetzel, 1995), and a greater availability of algal exudates may have reduced the dependence of biofilm heterotrophs on fluctuations in the external DOC supply. Potential differences in respiration between flow stages may also have been obscured by the higher variability in respiration rates measured during release event 3, relative to the previous two release events.

4.5.2 Sediment enzyme profiles

EEA were highly variable both within and between sampling days, indicating a high degree of patchiness in heterotrophic metabolic activity within the study site. The high heterogeneity in bacterial EEA rates concurs with findings from other studies of EEA in sediment habitats (Clinton, Edwards & Findlay, 2010). Although this variability precluded further significance testing of individual enzyme expression rates, clear temporal patterns in heterotrophic metabolic activity could be distinguished through analysis of the collective

enzymatic fingerprint (Fig 4.6). The enzymatic response was inconsistent between release events, and suggested that qualitative shifts in the DOC supply occurred during release Events 1 and 3 but not release Event 2. Where a shift in the enzyme profile was evident, this primarily occurred along PCA axis 1, which was more closely associated with variation in cellulose-degrading enzymes ALPHA and BETA, and PHOS, which cleaves organic phosphate links. Increased activity rates of these enzymes have been recorded in response to wetting of riverbank sediments (Burns & Ryder, 2001). The magnitude of shift in the enzyme fingerprint was smaller in release event 3 than release event 1, and the recovery time was also slower. This difference could be hydrologically-driven, with release event 3 having a slightly slower rate of flow rise than that of release event 1 (Fig. 4.2). The DOC pulse during the rising flow stage of release event 3 was larger and longer in duration than in release event 1 (Fig. 4.3), and this may have prolonged the recovery time of the benthic metabolic response. These results underscore the role of hydrological disturbance in moderating the duration of resource pulses, and therefore the extent to which dissolved resources can be utilised locally before being transported out of the river reach (Newbold *et al.*, 1981).

4.5.3 Effects of pulsed dam releases on ecosystem functioning

By separating out the effects of flow velocity and scouring, we were able to demonstrate that changes in the physicochemistry of release water can stimulate small-scale biogeochemical 'hot moments' in the form of short-duration increases in community respiration rates. If this mechanism also operates in-channel, then a higher frequency of pulsed releases may contribute to increased DOC cycling through the microbial loop (Meyer, 1994), and either increased mineralisation of dissolved carbon or pulses of energy that may support productivity of higher trophic levels in the food web (Cook *et al.*, 2015) on a more regular basis.

Our findings contradict other studies which report the stimulation of autotrophy in biofilm communities during a natural, 3.96 m^3 s^{-1} rainy season storm event in a third-order tropical river (Donato, Abuhatab & Sabater, 2014) and in response to smaller (<80th flow percentile) pulsed dam releases in south-east Australia (Chester & Norris, 2006). Microbial metabolic processes occur nested within a hierarchy of physical processes operating at larger spatial scales (Biggs, Smith & Duncan, 1999). It is possible that, during high-flow conditions,

stimulation of heterotrophic activity is masked by physical disturbance from increased flow velocities, which can enhance the photosynthetic activity of a mature biofilm by thinning the biofilm, sloughing senescent cells (Wetzel, 2005) and increasing diffusion of nutrients (Lock & John, 1979). In contrast, our findings were more consistent with studies of in-stream sediment metabolism, which report significant increases in benthic respiration during and immediately after both natural storms (O'Connor, Harvey & McPhillips, 2012) and pulsed dam releases (Uehlinger, Kawecka & Robinson, 2003). This discrepancy may be due to physical conditions in the experimental flumes, which were operated at a constant flow rate, or may reflect the dominance of heterotrophic organisms in darkened hyporheic habitats, rather than the mixed autotrophic-heterotrophic epilithic biofilm community.

The variations in stream-water DOC and nutrient concentrations that occurred in the present study were smaller in magnitude relative to lowland rivers (Rees, Bowen & Watson, 2005), particularly during larger overbank high-flow events resulting in floodplain inundation (Carney *et al.*, 2015). Our finding of a significant increase in biofilm CR suggests that even these small changes in dissolved resources associated with in-channel dam releases are sufficient to affect microbial processes in montane river systems. Our results are supported by experimental evidence from other upland systems that showed increases of approximately 1 mgL^{-1} of DOC were sufficient to stimulate benthic microbial process rates (Sobczak, Findlay & Dye, 2003).

4.5.4 Microbial metabolic activity as functional indicators

Our results show that significant, short-duration changes in benthic community metabolism and enzyme expression occurred in response to in-channel pulsed dam releases. This sensitivity is linked to the high degree of metabolic plasticity within microbial communities (Comte, Fauteux & del Giorgio, 2013), which can respond rapidly to changing resources phenotypically, without necessarily requiring a shift in community structure (Kreutzweiser & Capell, 2003). The differing response of the enzymatic fingerprint suggests that within a given catchment, the DOC composition delivered by similar-sized pulsed dam releases can vary temporally. Due to their high variability, we suggest that measurements of EEA are most useful as indicators of qualitative shifts in resource availability when coupled with an additional measure of DOC composition or bioavailability.

We found a significant microbial response to dam release waters occurred within the duration of individual release events (Fig 4.4). The few studies that report benthic metabolism during high-flow events tend to include only before and after-event measurements (Uehlinger, Kawecka & Robinson, 2003; Chester & Norris, 2006), most likely due to logistical difficulties involved in accessing the stream benthos during high-flow periods. This underscores the importance of higher frequency, event-based data collection when considering community metabolism as an indicator for assessing ecological responses to environmental water allocation strategies.

4.6 Conclusions

The results of this study contribute to an improved understanding of the potential pathways through which pulsed dam releases may influence ecological processes in regulated montane rivers. Whilst we could demonstrate a physicochemically-mediated effect of dam release water on biofilm respiration rates, we were not able to explicitly link this to variations in dissolved organic carbon or nutrients. Changes to the sediment extracellular enzyme activity profile suggest that qualitative changes in the DOC supply occurred during some of the release events, and further studies that incorporate measurements of DOC composition and bioavailability may potentially resolve the underlying mechanism for the observed biofilm metabolic response. This study also highlights the importance of high-flow disturbance frequency in stimulating biofilm respiration, and in the creation of biogeochemical hot moments. Further investigation is required to clarify the potential effects of microbial hot moments on energy flows to higher trophic levels.

Chapter 5: Effects of terrestrial carbon supply regime on bacterial function and community structure in an epilithic biofilm

5.1 Abstract

Aquatic microbial communities are instrumental in facilitating 'hot moments' of increased biogeochemical processing, which can be triggered by fluctuations in resource availability. Large reservoirs are known to alter the sources, composition and timing of resource inputs to a river network. However, the potential implications of these altered resource flows for downstream microbial processing and biogeochemical hot moments are still poorly understood. This 15-day laboratory mesocosm study simulated the contrasting DOC supply regimes associated with natural storm events and managed dam releases by exposing an epilithic biofilm community to rapid, 'pulse' and more gradual 'press' additions of terrestrial DOC leachate. Microbial responses to the DOC additions were assessed by measuring electron transport system activity and bacterial extracellular enzyme expression. Bacterial community structure was determined using Illumina sequencing of the 16S RNA gene. The pulse DOC amendment produced a rapid, transient shift in bacterial extracellular enzyme expression and taxonomic structure that was not evident in the press or control treatments, implying that DOC input rate may modulate the effect of terrestrial DOC on epilithic bacterial communities. These findings suggest that phototrophic biofilm communities are more sensitive to fluctuations in terrestrial DOC than commonly believed, and illustrate how river regulation impacts on the resource supply regime may influence the occurrence of microbial hot moments.

5.2 Introduction

Biogeochemical 'hot moments' are short time periods where the transient availability of limiting reactants stimulates increased process rates relative to longer intervening time periods (McClain *et al.*, 2003). Aquatic microbial communities are instrumental in facilitating hot moments as they mediate many biogeochemical reactions through processes such as metabolism, biomass accumulation and denitrification (Cotner & Biddanda, 2002). Although microbial hot moments may potentially make substantial contributions to carbon and nutrient fluxes, such periods can be challenging to predict and capture with conventional sampling programs. This is partially due to an incomplete understanding of the mechanisms linking resource variability and microbial functioning (Findlay & Sinsabaugh, 1999).

In freshwater systems, variability in the carbon and nutrient supply exerts a bottom-up influence on microbial community structure and functioning (Hitchcock *et al.*, 2016). In particular, microbial metabolic activity is strongly affected by the chemical composition of primary energy sources such as dissolved organic carbon (DOC) (Findlay *et al.*, 2003), which may range from rapidly degraded, labile DOC to more slowly metabolised, recalcitrant DOC (Sinsabaugh & Foreman, 2003). Episodic inputs of highly bioavailable DOC may contribute to hot moments as microbes rapidly utilise the new DOC source (e.g. Levi *et al.*, 2013). DOC input rate may also be instrumental in triggering hot moments, with a higher bacterial productivity observed in lake mesocosms receiving a rapid DOC pulse compared to those dosed with DOC added at a slower rate (Lennon & Cottingham, 2008). The effects of resource quality may even be contingent upon resource schedule, with refractory DOC pulses producing a higher cumulative bacterial productivity than labile DOC pulses (Lennon & Cottingham, 2008). In rivers and streams, biogeochemical hot moments can be triggered when precipitation events mobilise terrestrial carbon and nutrients into the water column (Marin-Spiotta *et al.*, 2014; Buffam *et al.*, 2001). Enhanced microbial production and respiration during or shortly after large floods (Cook *et al.*, 2015; Farjalla *et al.*, 2006) can be considered examples of precipitation-driven hot moments.

Bacterial communities possess numerous physiological, taxonomic and metabolic capabilities that allow them to exploit a highly dynamic resource base. Firstly, bacteria are able to synthesise a variety of enzymes that cleave smaller functional groups from large,

chemically complex DOC macromolecules and facilitate their assimilation across the cell membrane (Arnosti, 2003). Up-regulation of bacterial enzyme production can occur within hours of exposure to a DOC source (Foreman, Franchini & Sinsabaugh, 1998), allowing bacteria to rapidly respond to terrestrial DOC inputs. Secondly, terrestrial DOC composition may structure the bacterial community by differentially selecting for the growth of specific taxa with the metabolic capacity to process available DOC compounds (Kirchman *et al.*, 2004). Taxonomic shifts in bacterial community structure have been documented following exposure to terrestrial DOC sources (Wagner *et al.*, 2014; Carney *et al.*, 2016), but shifts in community composition occur at longer time-scales than metabolic changes, as they are sometimes contingent on population turnover (Findlay *et al.*, 2003). In a series of cross-inoculation experiments, Judd *et al.* (2006) demonstrated that bacterial taxonomic composition was strongly structured by DOC source, suggesting that changes in bacterial processing are likely to be mediated by shifts in community composition. Despite the current understanding of the mechanisms through which bacteria may utilise terrestrial DOC, consistent relationships between DOC variability and bacterial community structure and functioning have yet to be demonstrated.

Human activities that alter the sources, composition and timing of resource inputs to aquatic systems may modify microbial process rates, and therefore the occurrence of biogeochemical hot moments. In particular, river regulation from large reservoirs can alter DOC and nutrient transport to the downstream river, by interrupting the hydrological connections that transport these resources through the river network (Ward & Stanford, 1983). However, we still have a limited understanding of the potential implications that regulation-induced changes to resource flows would have for riverine microbial processes and biogeochemical hot moments. Many regulated rivers now receive environmental flows, delivered as releases of stored reservoir water, which can ameliorate many of the impacts of river regulation (Gillespie *et al.*, 2015). The effects of dam releases on microbial processes remain poorly studied, and may differ from natural, precipitation-driven events, depending on the factors controlling the quantity, composition, timing and delivery pattern of resources mobilised by dam releases. For example, flood releases to the Snowy River from Jindabyne Dam produced a very low, constant DOC regime lacking the carbon or nutrient pulses characteristic of natural floods (Rohlfs *et al.*, 2016). Conversely, another study in the

same river suggested that benthic microbial communities do respond to even small differences in resources during experimental dam releases, implying that managed, in-channel floods have some capacity to instigate biogeochemical hot moments (Chapter 4). A better understanding of the finer-scale mechanisms of microbial responses to resource variability would help to unravel these apparent contradictions, and improve our ability to predict and manage the consequences of altered resource flows in regulated and managed rivers.

This study simulated the contrasting DOC regimes associated with natural storm events and managed dam releases by exposing an epilithic biofilm community to rapid, 'pulse' and more gradual 'press' additions of terrestrial DOC leachate in a 15-day laboratory mesocosm study. A range of metabolism and diversity metrics were used to distinguish potential mechanisms of bacterial response to the DOC additions. We hypothesised that the addition of a terrestrial DOC source would significantly increase bacterial metabolic activity rates and shift bacterial taxonomic composition within the biofilm. We further hypothesised that a DOC treatment added at a rapid rate would elicit higher rates of bacterial metabolic activities and a faster shift in bacterial community structure than an equivalent DOC treatment added at a slower rate.

5.3 Methods

5.3.1 Biofilm colonisation

We established an experimental biofilm system by colonising rock substrates with a natural biofilm community and exposing them to DOC manipulations in laboratory mesocosm tanks. Commercially available river pebbles (approx. 40 mm) were used as a biofilm colonisation substrate. The pebbles were scrubbed thoroughly, soaked overnight in tap water and rinsed prior to colonisation. The pebbles were then incubated in the Snowy River at a site approximately 22 km downstream of Jindabyne Dam (36°26'38"S, 148° 56'16"E) for a period of 6 weeks during the Austral spring (Oct-Nov). The site is surrounded by predominantly agricultural grazing catchment, and during the incubation period, experienced base-flows of approximately 7 m^3s^{-1}, with the exception of a small storm event that occurred in the second incubation week.

The pebbles were arranged one layer deep in polyethylene crates with a surface area of approximately 3830 cm^2. Each crate was raised approximately 25 cm from the channel substrate to prevent burial from sediments, and was oriented so that water flowed across the upper surface of the pebbles. The crates were placed in an unshaded, well-mixed location that had a mixed benthic substrate of pebbles interspersed with fine sediments. When a mature biofilm had developed, the pebbles were carefully transported to the laboratory, and placed into glass flow-through mesocosm tanks. Each mesocosm was filled with 20 L of copper-free tap water, and consisted of a top tray (500 mm x 140 mm x 350 mm) which held the pebbles and a bottom sump (500 mm x 220 mm x 70 mm) fitted with a pump (Maxi Powerhead 104, Aqua One) that kept the water continuously circulating through the system (Fig 5.1). The pebbles were allowed to equilibrate for 48 h under continuous flow prior to the application of experimental treatments.

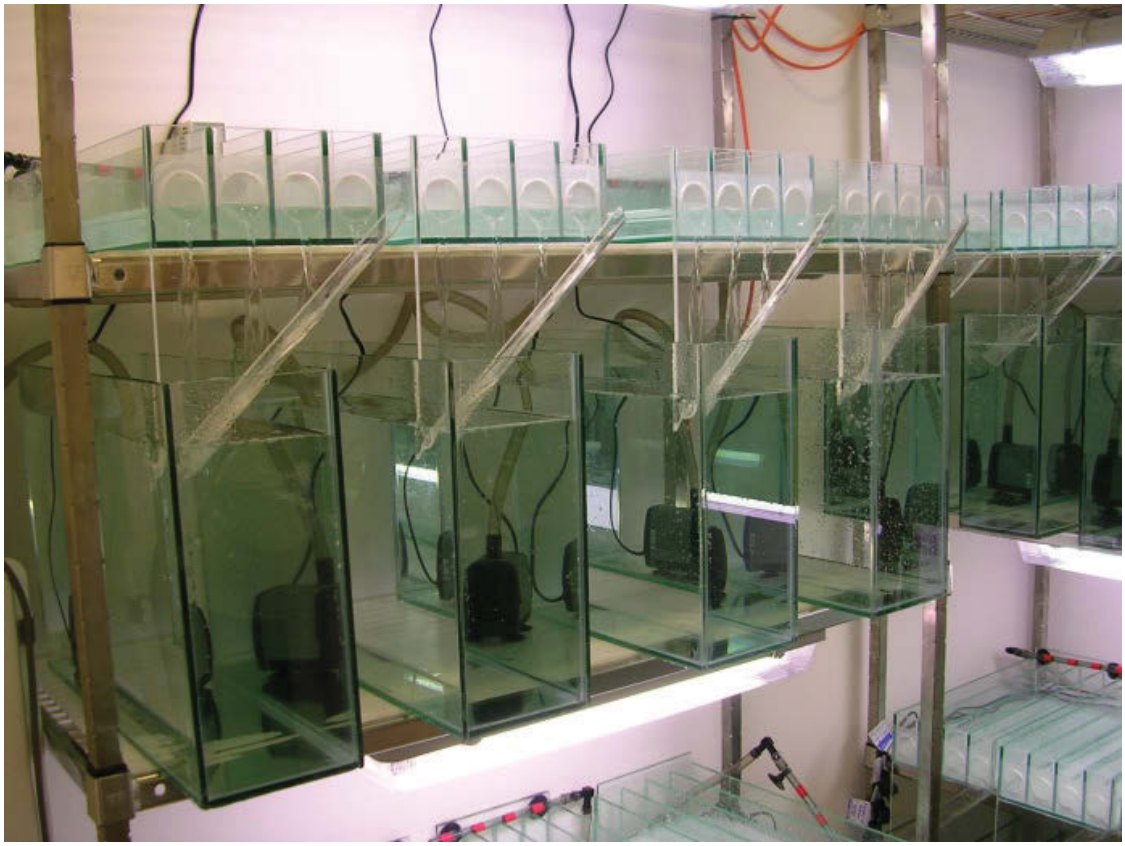


Figure 5.1: Experimental flow-through mesocosm system. Each mesocosm was a closed system consisting of a top tray holding biofilm substrates connected to a sump tank.

5.3.2 Experimental procedure

Leachate was prepared from fresh leaves of dominant riparian species *Melaleuca* spp. and *Poa* spp., collected from the vicinity of the field incubation site. The leaves were soaked at room temperature for 48 h in 5 L of reverse osmosis water, replacing leaves with fresh material twice daily to produce a concentrated leachate. The leachate was filtered through a 0.2 µm pore size cellulose acetate filter, subsampled to determine DOC and nutrient concentrations and stored frozen at -20°C until required.

During the experimental incubation, the mesocosms were maintained at 24°C under fluorescent lights set to a 12 hour light-dark cycle. Evaporative water loss was offset by adding distilled tap water to the sump tanks every 3-4 days, and tank water was kept aerated by passing it through a plexiglass funnel as it flowed back into the sump. Three experimental treatments were used: addition of 200 mL of leaf leachate administered as a single 'pulse' dose, addition of leachate as a series of smaller daily 'press' doses, and a control treatment that received no leachate addition. Pulse dose tanks received leachate added to a total concentration of 10 mg L^{-1} DOC, 0.53 mg L^{-1} TN and 0.13 mg L^{-1} TP, and the initial press leachate dose was added to a concentration of 0.66 mg L^{-1} DOC, 0.03 mg L^{-1} TN and 0.008 mg L^{-1} TP, with daily leachate doses of 13.3 mL added for the remainder of the experiment.

Mesocosm water physicochemistry and biofilm response variables were measured prior to the application of experimental treatments (Day 0), and for a period of 15 days following the initial leachate additions (Days 1-15). Measurements of mesocosm water physicochemistry, biofilm structure and functional assays were taken on experiment Days 0, 1, 2, 3, 5, 7 and 15, and were made prior to addition of any scheduled treatment doses. Biofilm was sampled by scrubbing the surface of 2 pebbles from each tank into a sterile plastic container with a sterile toothbrush in a known volume of autoclaved reverse osmosis water. Aliquots of the resulting slurry were subsampled into centrifuge tubes. For bacterial community structure analysis, separate pairs of pebbles were sampled as described above on Days 0, 1, 7 and 15. The slurry was centrifuged for 10 mins at 3500 g, liquid supernatant was discarded and the remaining pellet snap-frozen in liquid nitrogen and stored at -20°C. The mean surface area of each pair of scrubbed pebbles was 101 ± 1.67 cm^2, as estimated from

surface area models (Netfabb Professional, V6 Netfabb GmBH, Lupburg) compiled using an ARTEC Spider 3D scanner (Artec Studio V9, Artec Group, Luxembourg).

5.3.3 Water chemistry

A multiprobe (Multiline, WTW GmBH) was used to measure mesocosm sump water temperature, pH, conductivity and dissolved oxygen. Water temperature and light intensity in the trays housing the pebbles were recorded every 30 minutes with HOBO loggers. Mesocosm water was collected into pre-rinsed polyethylene bottles and one set of samples was pre-filtered with a 0.45 µm pore size filter for DOC, nitrogen oxides (NO_x), NH_3 and soluble reactive phosphorus (SRP) analysis, while the unfiltered samples were used for total nitrogen (TN) and total phosphorus (TP) analysis. Water samples were stored at -20°C until analysis. DOC was analysed using the high temperature combustion method (APHA, 2005) and TN, TP, NO_x, NH_3 and SRP were analysed according to standard methods (American Public Health Association)using a segmented flow analyser (OI Analytical Model FS3100).

5.3.4 DOC composition

Mesocosm water was sampled on Days 0, 1, 2, 3, 5, 7 and 15, filtered through pre-rinsed 0.2 µm pore size cellulose acetate filters and stored at -20°C in the dark until analysis by fluorescence spectrophotometry. Excitation-emission matrices (EEMs) were generated for each sample by scanning excitation wavelengths 230-465 nm and emission wavelengths 260-465 nm, in ratio mode with a bandpass width of 5 nm and scan speed of 120 nm s^{-1}. Filtered (0.2 µm) reverse osmosis water was used as a blank. The EEMs were corrected for instrument bias (Cory *et al.*, 2010), inner-filter effects (Ohno, 2002) and Raman-normalised (Lawaetz & Stedmon, 2009). A fluorescence index value was calculated from each EEM following the proceedure of McKnight et al. (2001).

5.3.5 Biofilm composition

Bacterial biomass was sampled by fixing 10 mL of biofilm slurry with filter-sterilised formaldehyde (4% final concentration) A 250 µL subsample of fixed slurry was diluted in 10 mL of filter-sterilised reverse osmosis water and sonicated at 10 W for 30 s (Vibrasonic) to detach bacteria from the biofilm matrix. 2 mL of diluted slurry was incubated with 200 µL of DAPI (4'6-diamindion-2-phenylindole) for 10 mins in the dark and then filtered through black 0.2 µm pore size polycarbonate filters. The filters were mounted onto glass microscope slides under non-fluorescing immersion oil. Bacteria were quantified using an Olympus BX41 compound microscope under a mercury lamp. Automated image analysis was used to quantify bacterial abundance for at least 15 fields per slide using CellC software (Selinummi *et al.*, 2005). Bacterial biomass was calculated using the formula of Romanova and Sazhin (2010)

For chlorophyll-a determination, a separate 10 mL aliquot of biofilm slurry was filtered through 0.7 µm pore size GFF filter paper (Microanalytix) and stored at -20°C until analysis by solvent extraction with correction for phaeophytin (APHA, 2005). Chlorophyll-a concentrations were normalised for pebble surface area.

5.3.6 Biofilm respiratory activity

Electron transport system (ETS) activity was used to estimate biofilm community respiratory activity, following the iodonitrotetrazolium (INT) reduction method of Blenkinsopp and Lock (1990). INT assays were conducted using pebbles with a mean surface area of 27.77 ± 0.97 cm^2. Each pebble was incubated in 30 mL 0.02% INT (Sigma Aldrich) solution for 8 hours and the colonising biofilm was killed with formalin and sonicated for 1 min at 40% power. Four replicate pebbles per treatment were used for incubations, while one pebble per treatment was used as a killed control to estimate passive INT reduction (Blenkinsopp & Lock, 1990). Formazan precipitate was extracted into a known volume of 95% methanol for 1 h at -20°C, with additional methanol added where the solution colour approached saturation. The extracts were filtered through 0.45 µm pore size GF/F filters and the absorbance of the filtrate was measured at 480 nm with a Cary 50 UV-Vis spectrophotometer. Absorbance values were converted to INT formazan concentrations using a standard curve prepared

from a 0.30 ug L^{-1} stock solution of INT formazan (Sigma Aldrich). For each incubation, mean rates of ETS activity in killed controls were subtracted from those of the live pebbles, and the final rate estimates were normalised for pebble surface area.

5.3.7 Bacterial extracellular enzyme activities

Methylumelliferyl-linked fluorescent substrates were used to estimate the potential bacterial enzyme activity rates of six different carbon and nutrient-acquiring enzymes; butyl esterase (BU), phosphatase (PHOS), α-glucosidase (ALPHA), β-glucosidase (BETA), leucine aminopeptidase (LEU) and xylosidase (XYL). Biofilm slurry was sonicated at 10% power for 30 s (Vibrasonic) to disperse material more evenly. 1 mM enzyme substrate solutions were prepared in sterile 5 mM bicarbonate (PHOS, ALPHA, BETA, XYL) or phosphate (BU, LEU) buffer and stored at 4°C for no more than 24 hours before use. Biofilm slurry was diluted to 25% in sterile reverse osmosis water and loaded into black 96-well plates and enzyme substrate added to a final concentration of 500 µM, which was predetermined to be saturating. The plates were then incubated for 2 h in the dark at 21°C and fluorescence intensity was measured at an excitation wavelength of 365 nm and emission wavelength of 445 nm at 5 minute intervals (Tecan). A 100 µM stock solution of methylumbelliferone (MUB) prepared in 5 mM $NaPO_4$ buffer was used to generate a standard curve and convert fluorescence readings into extracellular enzyme rates. Correction factors were applied to sample fluorescence rates to account for autofluorescence (Hoppe, 1993) and for quenching due to sample turbidity (Jackson, Tyler & Millar, 2013).

5.3.8 Bacterial community structure

DNA was extracted from the thawed, homogenised biofilm pellet using the MoBio Powersoil extraction kit according to the manufacturer's directions, and DNA yield was quantified using a Nanodrop 2000. Illumina sequencing was used to determine biofilm bacterial taxonomic composition. The V4 variable region of the 16S rRNA gene was amplified with the 515F/806R bacterial primers. PCR was carried out using HotStarTaq Plus Master Mix Kit (Qiagen, USA) with a thermal cycling protocol of 94°C for 3 minutes, 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, followed by a final elongation step at 72°C for 5 minutes. PCR products were checked with 2% agarose gel, and purified using

Ampure XP beads. Samples were sequenced on a MiSeq platform following the Illumina TruSeq DNA protocol at the Molecular Research Labs (Shallowater, Texas, USA).

The resulting DNA sequences were processed with the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso *et al.*, 2010). Sequences were de-multiplexed and those with reads <200 bp, a quality score <25 or containing homopolymers exceeding 6 bp were filtered from the dataset. OTUs were clustered to 97% similarity using UCLUST (Edgar *et al.*, 2011) and assigned taxonomic identities from the Greengenes database (McDonald *et al.*, 2012) using closed-reference picking. Chimeras were identified using ChimeraSlayer (Haas *et al.*, 2011) and excluded.

5.3.9 Statistical analysis

The mean and standard error of mesocosm DOC and nutrient concentrations, fluorescence index, biofilm bacterial biomass, chlorophyll-a density and ETS activity were calculated for each treatment group on each sampling day. Differences between treatments and time were tested using the PRIMER-E and PRIMER + PERMANOVA version 6 software package (Anderson, 2001). Univariate, 2-way PERMANOVA analyses were performed to check for significant effects of time and treatment type on mesocosm DOC, nutrients, fluorescence index, biofilm bacterial biomass, chlorophyll-a density and ETS activity.

The mean potential activity rate of each enzyme was calculated from replicate biofilm samples within each treatment group on each day. Enzyme rate data from Days 2 and 5 were excluded from further analyses due to analytical errors resulting in the loss of several replicate measurements. Shifts in the collective bacterial enzyme profile were visualised using principal co-ordinates ordination (PCO) based on a Euclidean distance matrix generated from EEA rates standardised to maximum values. PERMDISP was used to check the enzyme data for homogeneity of multivariate dispersion. The effects of experimental treatment and day on the bacterial enzyme profile were tested using a multivariate, 2-factor PERMANOVA. Where either the treatment, day or interaction terms were significant, pair-wise comparisons were carried out to determine between-group differences.

Bacterial sequence data were rarefied following removal of chloroplast OTUs, square-root transformed and analysed graphically using nMDS plots generated from a Bray-Curtis

similarity matrix. Bacterial community alpha diversity was estimated using the Shannon Index:

$$H=-\sum_{i=1}^{S}(p_i \log 2 p_i) \quad (5.1)$$

A multivariate 2-factor PERMANOVA was used to test the effects of treatment and time, after first checking homogeneity of dispersion with PERMDISP. Where significant differences were detected, pair wise comparisons were used to identify differences between specific groups. SIMPER analysis was performed to examine bacterial community relative similarity between time and treatment groups, and to identify the specific taxa driving between-group dissimilarity.

5.4 Results

5.4.1 Physicochemical variables

For the duration of the experiment, mesocosm water pH remained at 7.92 ± 0.01, dissolved oxygen was 8.39 ± 0.01 mg L^{-1} and mean daily water temperature was 24.0 ± 0.1°C except for a slight decline to 23.0 ± 0.1 °C on day 15. Mesocosm mean conductivity increased gradually from 238 ± 1 µS cm^{-2} at day 1 to 263 ± 4 µS cm^{-2} at day 5 before declining to 245 ± 3 µS cm^{-2} by day 15.

The mean concentrations of DOC and all measured nutrients varied significantly over time but not between treatments (Fig 5.2, Table 5.1). Mean DOC concentration in the pulse mesocosms increased from 7.73 ± 0.4 mg L^{-1} at Day 0, to a maximum of 13.3 ± 0.6 mg L^{-1} by Day 1, before declining to approximately 12 mg L^{-1} for the remainder of the experiment. In the press mesocosms, DOC concentration increased gradually from an initial value of 6.77 ± 0.4 mg L^{-1} to a maximum of 14.11 ± 0.4 mg L^{-1} on Day 7, before reaching a final concentration of 13.66 ± 0.6 mg L^{-1} by Day 15. DOC concentration in the control mesocosms also increased from 8.21 ± 0.4 mg L^{-1} on Day 1 to 12.92 ± 0.6 mg L^{-1} on Day 7, but declined to a final value of 8.24 ± 0.3 mg L^{-1} on Day 15 (Fig 5.2a).

Mesocosm fluorescence index values remained above 1.6, indicating the dominance of microbial, 'protein-like' DOC in all treatment groups throughout the experiment. Mean fluorescence index values initially decreased in the pulse mesocosms, but remained similar in the press mesocosms and increased in the control mesocosms (Fig 5.2b). However, these variations were insignificant between treatments and sampling days (Table 5.1), and by Day 5, mean fluorescence index in all three treatment groups had returned to initial levels (Fig 5.2b).

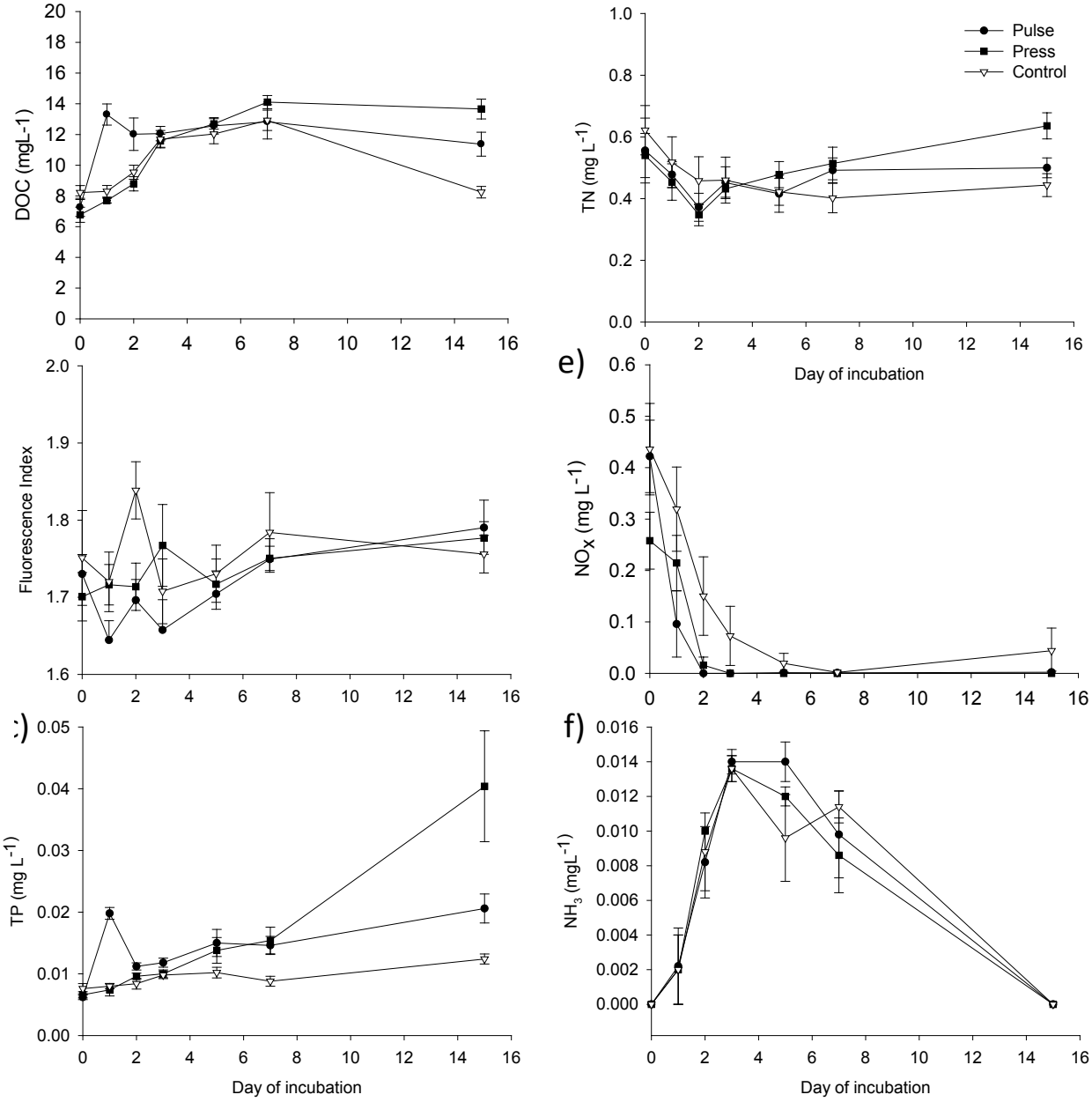


Figure 5.2: Mesocosm water chemistry including a) DOC concentration, b) fluorescence index (measure of DOC composition), and c) total phosphorus, d) total nitrogen, e) nitrogen oxides and f) ammonia concentrations. Figure shows mean ± SE, n=5.

Table 5.1: Pseudo F-values, degrees of freedom and P values from PERMANOVA analysis of water chemistry and biofilm response variables. * Denotes non-homogenous dispersion (p<0.05) and a lowered significance level of 0.01. Bold denotes significant tests.

	Treatment			Day			Treatment*Day		
	Pseudo-F	df	*P*	Pseudo F	df	*P*	Pseudo F	df	*P*
Univariate tests									
DOC	0.976	2	0.397	13.776	6	**0.001**	0.156	12	0.156
Total nitrogen	0.438	2	0.653	2.759	6	**0.01**	0.474	12	0.919
Total phosphorus	0.962	2	0.41	11.393	6	**0.001**	1.6	12	0.095
Nitrogen oxides (NOx)	2.573	2	0.082	27.043	6	**0.001**	1.206	12	0.3
Soluble reactive P	0.143	2	0.87	49.192	6	**0.001**	0.600	12	0.837
Chlorophyll-a	0.484	2	0.626	13.23	6	**0.001**	0.705	12	0.726
NH_3	0.006	2	0.936	52.126	6	**0.001**	1.068	12	0.391
Fluorescence index	0.213	2	0.783	1.902	6	0.093	0.798	12	0.671
Bacterial abundance	2.673	2	0.080	3.966	6	**0.007**	0.502	12	0.895
Bacterial biomass	2.980*	2	0.054*	5.055	6	**0.001***	0.656	12	0.747*
Bacterial diversity	1.746	2	0.192	2.833	2	0.078	2.152	4	0.096
ETS activity	6.537*	2	**0.002***	10.37	6	**0.001**	0.712	12	0.708
Multivariate tests									
Bacterial community structure	1.456	2	**0.003***	2.9994	2	**0.001***	0.92924	4	0.811*
Enzyme activity fingerprint	4.9791	2	**0.003***	7.0771	3	**0.001***	1.7399	6	**0.039***

5.4.2 Biofilm structure

Mean bacterial biomass increased in all three treatments during the experiment and by Day 7, was higher in both pulse and press treatments than in the control (Fig 5.3a). Bacterial biomass differed by day but not experimental treatment, with Days 2 and 3 significantly different from 15 (Table 5.1).

Biofilm chlorophyll-a concentration was not affected by DOC input regime, increasing slightly between Days 1 and 2, and then declining between Days 2 and 15 in all treatment groups (Fig 5.3b, Table 5.1).

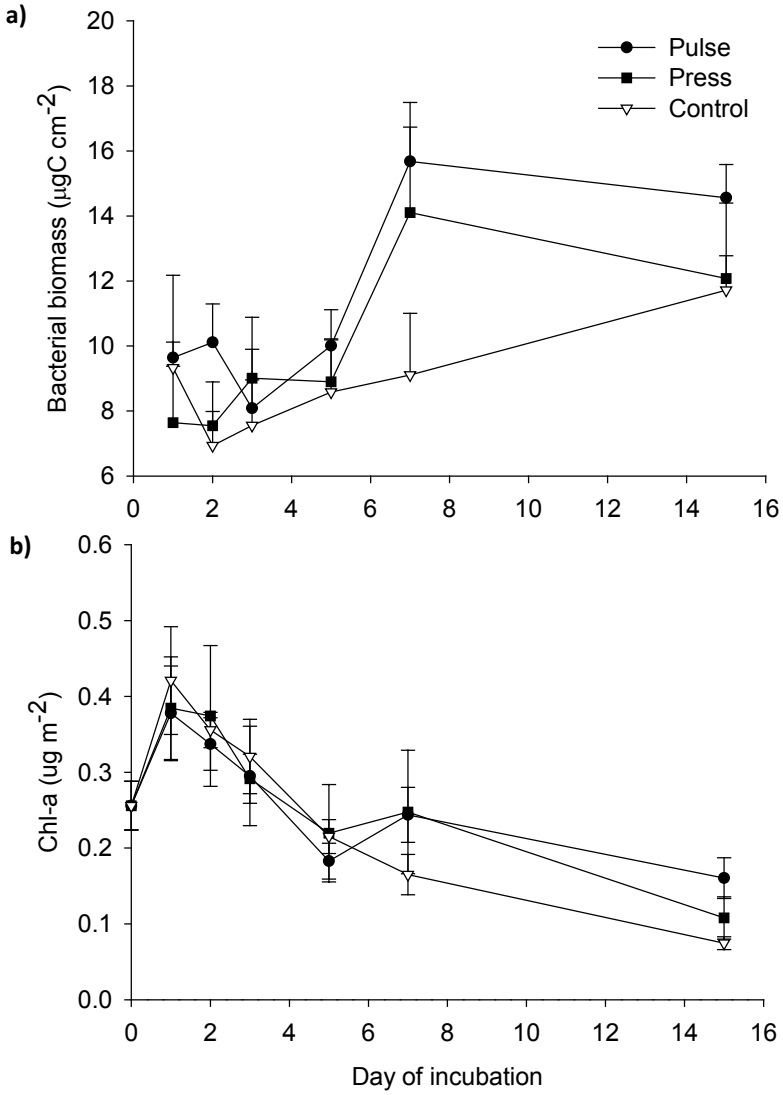


Figure 5.3: Biofilm structural characteristics including mean a) bacterial biomass, b) chlorophyll-a concentration Error bars show ± SE, n=5.

5.4.3 Biofilm bacterial metabolism

Biofilm ETS activity rates were highest in the pulse treatment throughout the experiment, peaking on Day 2 in pulse and control treatments, and on Day 5 in the press treatment (Fig 5.4). ETS activity rates varied significantly between DOC treatments (Table 5.1) with the pulse differing from the press ($t=3.59$, $p<0.05$) and control ($t=2.59$, $p<0.05$) treatments. Further analysis with pair-wise tests revealed significant differences only between pulse and press treatments on Day 7 ($t=3.64$, $p<0.05$) and Day 15 ($t=5.59$, $p<0.05$). ETS activity also varied significantly by day (Table 5.1), with Days 1, 2 and 3 differing from Days 7 ($p<0.05$), and all days differing from Day 15 ($p<0.05$).

Potential extracellular enzyme activity rates ranged between 0 and 107.9 µmol m^{-2} hr^{-1} (Table 5.2). Activity rates of carbohydrate-degrading enzymes peaked within the first 3-5 days of the experiment in all treatments. PCO ordination explained 85.6% of the total variation in the enzyme dataset, and showed a clear separation of the pulse treatment from the press and control treatments on Day 1 (Fig 5.5). The collective bacterial enzyme fingerprint differed significantly by both treatment and day, with a significant treatment by day interaction (Table 5.1). Pair-wise comparisons showed that biofilm enzyme expression in the pulse treatment differed from the control treatment on Day 1 ($t=5.02$, $p<0.01$). When analysed individually, none of the enzymes were influenced by resource supply regime alone, but a significant interaction between treatment and day was present for α-glucosidase and xylosidase (Table 5.3).

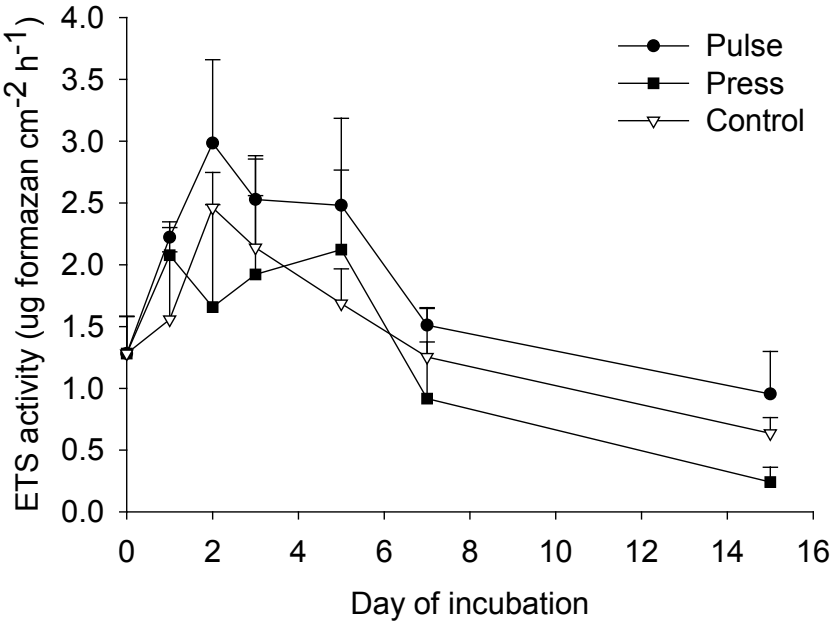


Figure 5.4: Mean biofilm electron transport system activity. Error bars show ± SE, n=5.

Table 5.2: Mean potential activity rates for biofilm bacterial enzymes. EEA rates are in nmol cm^{-1} h^{-1} (± SE, n=5)

	BU	PHOS	ALPHA	BETA	LEU	XYL
Day 0	1.54±0.44	0.82±0.24	2.39±1.18	25.60 ±7.08	1.77 ±1.85	3.53 ±1.26
Pulse						
Day 1	1.30±0.31	27.84± 4.56	4.73 ±0.87	41.08 ±36.47	8.58 ±1.77	4.67 ±1.21
Day 3	2.91±1.06	78.04±14.59	11.78 ±6.62	14.03 ±3.49	16.86± 4.19	11.69 ±4.01
Day 7	5.88± 4.14	69.96±7.88	5.26 ±0.89	46.05±13.46	19.49 ±3.01	1.89 ±0.58
Day 15	8.13± 6.91	107.90±22.07	3.49 ±1.23	15.58 ±4.38	36.27 ±11.90	3.36 ±0.62
Press						
Day 1	0.58± 0.23	26.02±12.64	2.20±0.47	1.98 ±0.45	6.59 ±0.84	2.37± 0.74
Day 3	2.50 ±0.95	89.58±14.88	14.17±5.09	7.24 ±2.02	24.91 ±6.05	9.53 ±4.38
Day 7	0.53± 0.12	35.80±10.11	2.65±0.68	50.64 ±16.35	17.16±6.78	1.66 ±0.47
Day 15	0.39± 0.14	27.58 ±3.55	1.55 ±0.34	10.71 ±3.19	15.92 ±4.33	1.50 ±0.47
Control						
Day 1	1.12 ±0.32	29.02±3.40	3.64± 0.62	7.61 ±2.22	7.28± 1.49	4.99 ±1.68
Day 3	1.44 ±0.23	60.00±6.06	6.41 ±2.10	10.16 ±2.37	23.76 ±8.87	7.89 ±2.51
Day 7	0.66 ±0.25	60.47±15.01	2.80±1.00	22.50 ±8.16	12.39 ±6.31	1.71 ±0.57
Day 15	1.84±0.83	106.97±22.71	4.22± 1.68	9.74 ±1.70	13.59± 3.06	1.89± 0.30

Table 5.3: Pseudo F-values, degrees of freedom and P values from PERMANOVA analysis of bacterial enzyme activity rates. * Denotes non-homogenous dispersion (p<0.05) and a lowered significance level of 0.01. Bold denotes significant tests.

	Treatments			Days			Treatment*Day		
	Pseudo-F	df	*P*	Pseudo F	df	*P*	Pseudo F	df	*P*
Butyl-esterase	1.559	2	0.224	0.53817	4	0.793	0.65081	8	0.816
Phosphatase	2.259	2	0.107	15.11	4	**0.001**	1.8054	8	0.086
α-glucosidase	3.499	2	**0.023**	11.192	4	**0.001**	7.7156	8	**0.001**
β-glucosidase	0.533	2	0.624	3.1505	4	**0.011**	1.0655	8	0.392
Leucine aminopeptidase	0.999	2	0.361	8.1392	4	**0.001**	1.7229	8	0.112
Xylosidase	3.5181	2	**0.039**	24.312	4	**0.001**	5.6824	8	**0.001**

5.4.4 Bacterial community structure

Excluding algal plastids, the biofilm bacterial community was largely dominated by taxa within the *Alphaproteobacteria*, particularly by the orders *Rhizobiales*, *Rhodobacteriales, Sphingomonadales* and *Burkholderiales* (Fig 5.7). Resolved to order level, bacterial taxonomic composition did not show strong differentiation between DOC treatment groups, although an increase in the abundance of *Burkholderiales* was evident in the pulse treatment on Day 1 (Fig 5.7). The initial biofilm bacterial community had an alpha diversity of 6.87 ± 0.1, which did not vary significantly with time or treatment throughout the duration of the experiment (Table 5.1).

Bacterial biofilm community composition was loosely clustered by day, with some separation between pulse and control treatment groups evident on Days 1 and 7 (Fig 5.6). PERMANOVA analysis showed a significant effect of treatment (Table 5.1) on bacterial community composition, with the pulse treatment differing from the control ($t=1.36$, $p<0.01$). However, subsequent pair-wise comparisons failed to detect significant differences between treatment groups on individual sampling days. Average dissimilarity between pulse and control groups was 59.13, and was driven by the taxa in the *Comamonadaceae, Microbacteriaceae*, *Caulobacteraceae, Xanthobacteraceae and Hyphomonadaceae*. Bacterial taxonomic structure also differed significantly between each sampling day (Table 5.1, $p<0.05$).

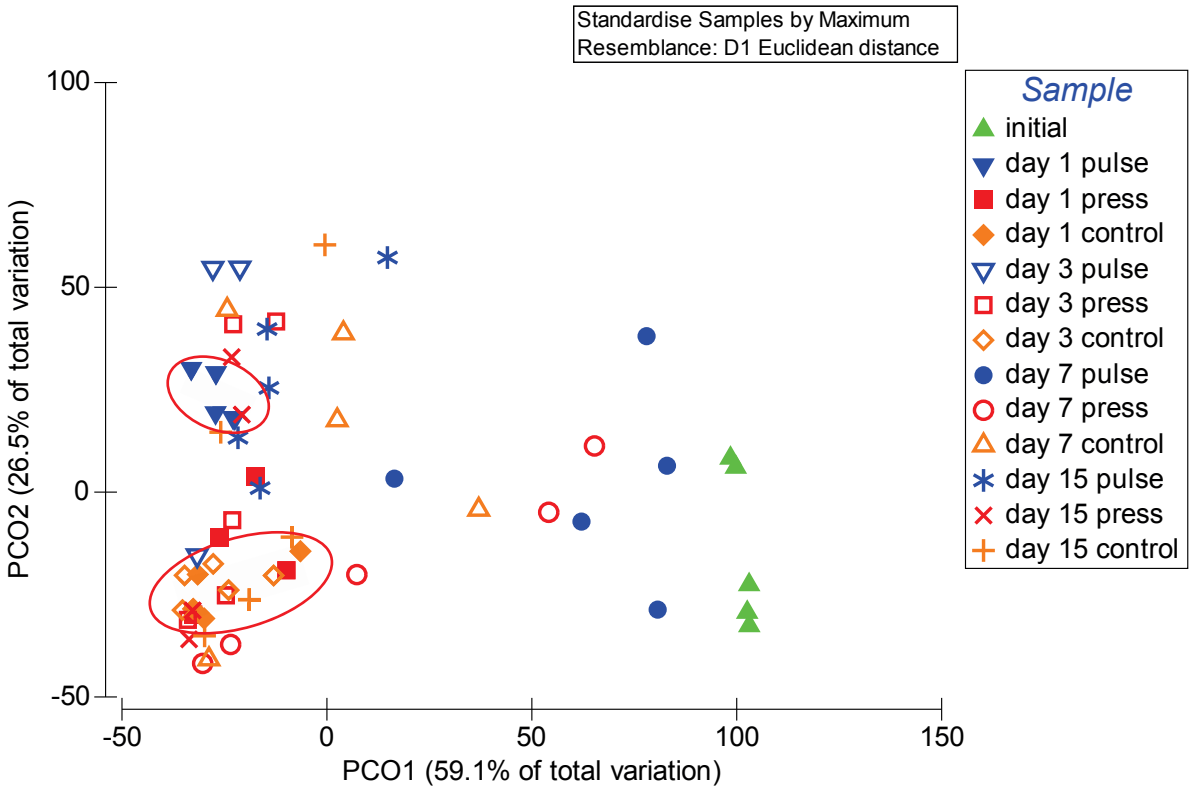


Figure 5.5: PCO ordination of EEA profiles (maximum standardised, Euclidian distance matrix). Ellipses show separation of pulse and control treatment groups on day 1.)

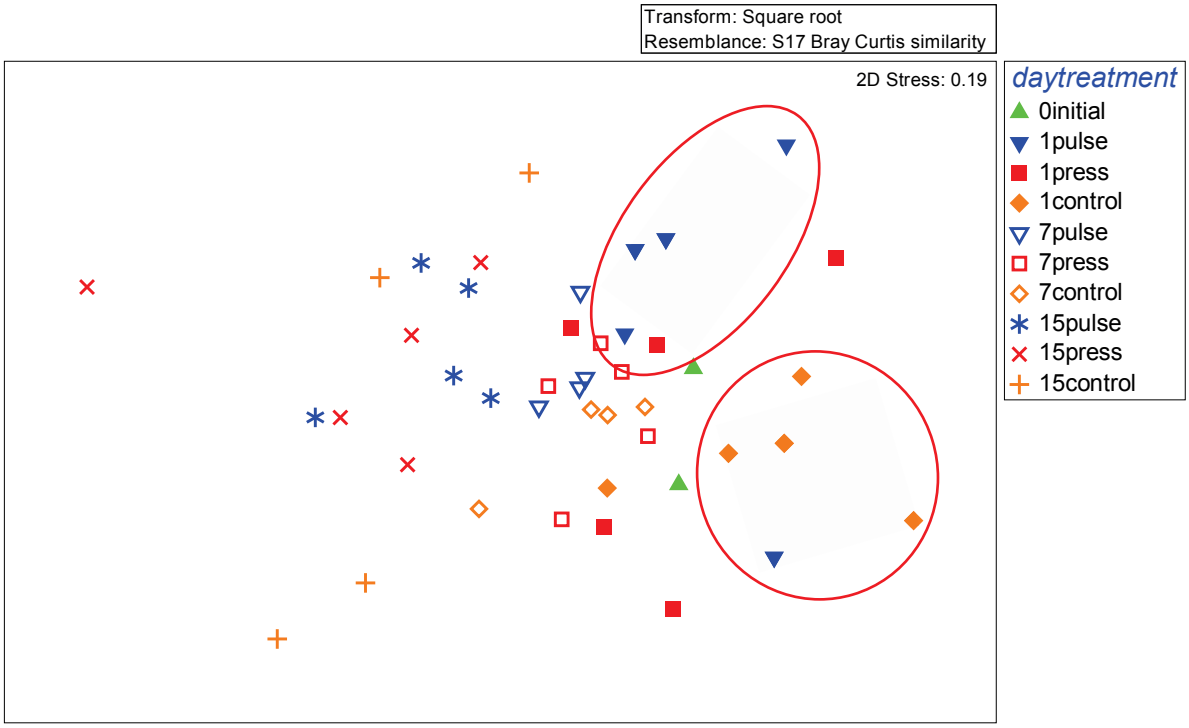


Figure 5.6: nMDS plot of square root transformed bacterial sequencing data, resolved to genus level. Ellipses show separation of pulse and control treatment groups on day 1.

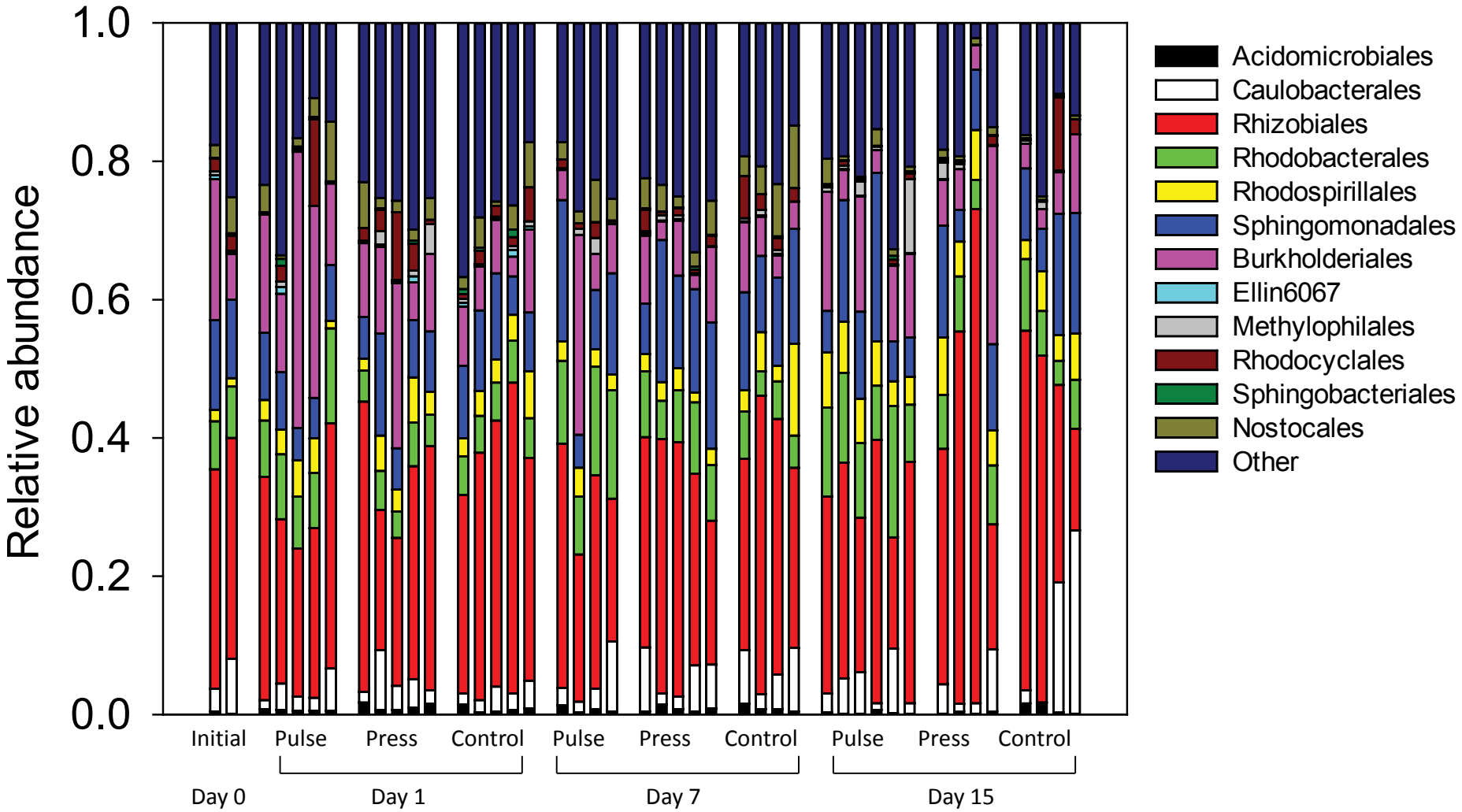


Figure 5.7: Bacterial community taxonomic composition, showing bacterial OTU relative abundances clustered at 97% similarity and identified to order level.

5.5 Discussion

We performed a mesocosm experiment to test whether terrestrial DOC supply regime influenced metabolic activity and community structure in an epilithic biofilm. The results showed an overall effect of DOC treatment type on biofilm ETS activity, bacterial enzyme expression and bacterial taxonomic structure, with the pulse differing from the control treatment group. This effect was most obvious on Day 1, where biofilm bacterial enzyme expression and taxonomic structure separated from press and control groups. However, the effect of the terrestrial DOC pulse on epilithic metabolic activity and community structure was highly transient, with pulse, press and control treatments converging for most response variables for the remainder of the incubation period.

5.5.1 Microbial metabolic activities

The major effect of DOC supply regime on biofilm metabolic activity was a shift in the collective bacterial enzyme fingerprint that occurred in the pulse treatment on Day 1. Separation of pulse treatment enzyme expression from press and control groups was rapid and occurred for only one day following initial leachate additions. This response pattern may have been caused by bacterial metabolism of low molecular weight terrestrial carbon substrates (Berggren *et al.*, 2010), particularly as the leachate was prepared from freshly abscised leaves, which can produce highly bioavailable extracts (Baldwin, 1999). The shift in pulse treatment enzymatic expression coincided with increased β-glucosidase production relative to controls, which may indicate increased bacterial metabolism of cellulose degradation products (Chróst 1996).

Separation of pulse and control enzyme fingerprints coincided with a shift towards a more terrestrial-like DOC signal in the pulse tanks, seen as a decrease in DOC fluorescence index. This pattern supports the notion that the pulsed input of terrestrial leachate shifted the composition of DOC substrates actively supporting bacterial metabolic activity (Arnosti, 2003; McKnight *et al.*, 2001). Moreover, the more pronounced effect of the faster DOC input rate on enzyme expression suggests that resource regime played a role in triggering the overall response. DOC and nutrients from the leachate may also have fuelled the increased bacterial biomass observed in pulse and press treatments on Day 7. However, the

similar bacterial biomass in pulse and press treatments implies that supply regime may be of limited importance to longer-term bacterial growth.

5.5.2 Bacterial community structure

The biofilm bacterial community contained high abundances of bacteria in the class *Alphaproteobacteria*, which have been found to dominate epilithic biofilm communities in other upland streams (Anderson-Glenna, Bakkestuen & Clipson, 2008). Members of the *Alphaproteobacteria* have been associated with the degradation of humic substances (Battin *et al.*, 2016), which are major constituent of terrestrial DOC. The orders *Sphingomonadales, Burkholderiales* and *Rhodobacter* were also present in relatively high abundances, reflecting findings from other stream biofilm communities (Battin *et al.*, 2016; Wagner *et al.*, 2015).

We detected an overall difference between the bacterial community structure of pulse and control treatment groups, which was most evident as a separation between the two groups on Day 1 (Fig 5.6). Further examination of bacterial taxonomic composition on Day 1 revealed an increase in the relative abundance of the order *Burkholderiales* in the pulse treatment group (Fig 5.7). This order has been linked to terrestrial organic matter degradation, with *Burkholderiales* increasing in abundance during the breakdown of terrestrial leaves (Newman, Liles & Feminella, 2015). It is therefore likely that the increase in the abundance of *Burkholderiales* was a response to the pulse leaf leachate addition. Other bacterial orders present in higher abundances, including the *Sphingomonadales* and the *Rhodospirillales* have also been linked to the degradation of terrestrial organic matter (Newman, Liles & Feminella, 2015).

The high variability within treatment groups may have contributed to the insignificant differences in bacterial community structure between treatment groups on individual sampling days. Biofilm bacterial communities often show high spatial variability, even at the scale of individual stream rocks (Lear *et al.*, 2008). Nevertheless, the pulse DOC treatment clearly produced a stronger shift bacterial community structure than the press DOC treatment on Day 1 (Fig 5.6), suggesting that input rate may play a role in modulating the bacterial community structural response to terrestrial DOC.

5.5.3 Timing of biofilm response

These results indicate that bacteria in light-grown biofilms may be more metabolically sensitive to fluctuations in ambient dissolved resources than previously believed (Kamjunke, Herzsprung & Neu, 2015). While planktonic bacteria are directly exposed to water column DOC and nutrients, biofilm bacteria are buffered from external resource fluctuations by an extracellular polysaccharide matrix (Lock *et al.*, 1984; Freeman & Lock, 1995). Externally derived organic matter and locally produced algal photosynthates are stored within the biofilm matrix, giving bacteria access to a more continuous resource supply (Sutherland, 2001; Espeland, Francoeur & Wetzel, 2001). However, the rapid timing of bacterial responses observed in this study suggests that heterotrophs in light-grown biofilms are sensitive to the external resources provided in the leachate. The shift in bacterial enzyme production and community structure in the pulse treatment could have occurred in response to an increased availability and wider diversity of highly bioavailable DOC substrates contained within the leachate (Attermeyer *et al.*, 2014; Sieczko & Peduzzi, 2014).

The availability of algal DOC originating from the biofilm itself may also have contributed to the transiency of the bacterial enzyme and taxonomic responses. The increase in bulk DOC concentrations in the control mesocosms, combined with consistently high fluorescence indices, implies that algal exudates contributed to available DOC supplies throughout the experiment (c.f. McKnight *et al.*, 2001). This DOC may have been actively produced by biofilm algae (Ziegler, Lyon & Townsend, 2009), or passively released from storage within the exopolymer matrix (Allan & Castillo, 2007). It is possible that the biofilm bacteria resumed metabolism of algal DOC after rapidly exhausting a supply of high bioavailability DOC substrates from the leachate, producing a corresponding shift in community structure and enzyme expression. This would also explain the contrast between the results of study and those of dark-grown biofilm bacteria, that showed distinct, permanent shifts in bacterial community structure following exposure to allochthonous DOC (Wagner *et al.*, 2014). It is also possible that the magnitude and duration of bacterial response in the leachate treatments was shortened by nutrient limitation, as tank water NO_x and TP concentrations decreased rapidly after day 1 (Fig. 5.2).

5.5.4 Implications for the management of regulated rivers

The pulse supply regime used in this study is analogous to the rapid increase in stream water solute concentrations that occurs during natural precipitation events (Hinton, Schiff & English, 1997), whereas the press supply regime approximates the more constant resource concentrations observed in managed water releases from large reservoirs (Rohlfs *et al.*, 2016). This study shows that these differing DOC supply regimes do have the potential to influence biofilm bacterial taxonomic structure and enzyme expression. Furthermore, these results illustrate a mechanism through which the reduction or removal of pulsed resource inputs may influence the occurrence of microbial hot moments in rivers regulated by large dams. Further studies are required to determine whether these changes to terrestrial DOC supply regime translate into quantitative differences in bacterial respiration and productivity, and their potential effects on downstream communities (Fasching *et al.*, 2014) or organisms at higher trophic levels (Hitchcock & Mitrovic, 2015). These insights into the role of DOC input rate in driving microbial structure and function may also assist in predicting and avoiding unintended negative outcomes of management actions, such as hypoxic events following floodplain inundation by environmental releases (Whitworth, Baldwin & Kerr, 2012).

The relative influence of resource input rate at environmental scales may also be moderated by broader hydrologic effects on biofilm bacteria, such as disturbance-induced scouring (Blenkinsopp & Lock, 1992), reduced opportunity for DOC uptake at higher flow velocities (Kamjunke *et al.*, 2016) and sediment deposition (Bouletreau *et al.*, 2006). Resource-mediated effects may also be more important during periods when algal photosynthesis is limited by high turbidity (Wagner *et al.*, 2015), or when terrestrial DOC inputs are particularly high (Cook *et al.*, 2015) times of prolonged biofilm stability, such as longer inter-flood periods (Biggs, 1996). Further field-based studies are required to elucidate the interactive effects of resource input rate with these other factors on microbial processing and the occurrence of hot moments.

5.6 Conclusion

This study compared the effects of rapid, 'pulse' and slower 'press' resource supply regimes on bacterial metabolic functioning and community structure within an epilithic biofilm community. Our results show that pulsed inputs of terrestrial DOC exert a detectable but transient effect on microbial enzyme expression and bacterial taxonomic structure relative to press and control treatments. These findings suggest that phototrophic biofilm communities are more sensitive to fluctuations in terrestrial DOC than commonly believed, and illustrate a mechanism through which river regulation impacts on the resource supply regime may influence the occurrence of microbial hot moments.

Chapter 6: General discussion

This thesis examined the influence of dam and tributary environmental water releases on DOC transport and utilisation in the heavily regulated Snowy River. A conceptual model was developed to support the comparison of DOC resource regimes supplied by the different environmental release types, which were further explored and tested in the individual studies within this thesis. Monitoring studies were conducted to characterise patterns of organic carbon mobilisation by environmental water releases from either a large reservoir or a small weir on a tributary. Manipulative field and laboratory mesocosm experiments were then carried out to further examine the implications of these differing carbon supply regimes for microbial community structure and functioning. Collectively, these studies provide new insights into the effects of environmental water delivery on DOC dynamics, and their implications for microbial processes.

6.1 The organic carbon regime in regulated river systems

Models of catchment flushing in unregulated streams stipulate that terrestrial organic material is transported into rivers from the surrounding catchment during high-flow events, causing pulses in water column DOC concentration (Hornberger, Bencala & McKnight, 1994; Boyer *et al.*, 1997). Monitoring of DOC dynamics in the regulated Snowy River revealed a very different pattern of low, stable DOC concentrations during high-flow dam release events (Chapters 2, 3 and 4). Decreased DOC concentrations were documented at some sites during periods of higher discharge rates from Jindabyne Dam, showing that dam water may even dilute dissolved resources in downstream reaches (Chapter 3). These results support the contention that impoundment and diversion of stream flow disrupts the hydrological mechanisms driving the organic carbon regime in the downstream river (Miller, 2012). Therefore, while environmental flow releases from Jindabyne Dam may mimic the hydrology of natural high-flow events, they do not appear to supply the equivalent basal resources as the natural flood and storm events they are intended to simulate (Buffam *et al.*, 2001). This finding has broader significance in the context of emerging paradigms of

process-based rehabilitation (Beechie *et al.*, 2010), which include restoring organic matter flows that may eventually subsidise organisms at higher trophic levels, such as fish (Cross *et al.*, 2011).

The monitoring studies in Chapters 2 and 3 contribute to more comprehensive understanding of DOC supply regimes from in-channel and upland dam releases. The low DOC concentrations found during high-flow releases from Jindabyne Dam contrast with other studies in upland rivers, which showed that dam releases delivered increased DOC concentrations to downstream sites (Henson *et al.*, 2007; Ulseth & Hall Jr, 2015). The differing DOC supply regimes arising from the current study and those of Henson (2007) and Ulseth (2015) are most likely a consequence of reservoir residence time, water quality, and local catchment and riparian conditions in the individual systems (Ulseth & Hall Jr, 2015; Rohlfs *et al.*, 2016). The longitudinal increase in DOC load and DOC compositional shift observed during the Jindabyne Dam releases (Chapter 3) show that the dam releases mobilised DOC originating from the reaches below the dam. Possible sources of this DOC include bed sediments, riparian areas and lower channel benches (Coleman & Williams, 2016).

The insights into Snowy River DOC dynamics gained through this thesis contribute to a broader understanding of organic carbon transport through regulated river networks. Many of the studies examining DOC delivery from environmental releases focus on the role of high-flow dam events in mobilising large DOC loads through floodplain inundation in lowland rivers (Westhorpe & Mitrovic, 2012). Although this approach is relevant in lowland river systems, the dominant DOC delivery mechanisms operating in upland rivers differ from those in larger floodplain rivers (Junk, Bayley & Sparks, 1989; McGlynn & McDonnell, 2003). Additionally, the volume of water available for environmental releases is often restricted (Arthington & Pusey, 2003), and may only be sufficient to generate water levels that cause limited lateral expansion of the wetted area, even in lowland systems.

The diversity of DOC responses highlights the need to consider the hydrological processes driving DOC dynamics in each given system when predicting and managing reservoir impacts on organic carbon transport. In a broader context, the effects of river

management on DOC transport are also important due to mounting evidence that the cumulative effect of river impoundment may significantly modify global scale carbon flux to the atmosphere (Tranvik *et al.*, 2009).

6.2 Role of tributaries in river rehabilitation

Baldwin et al. (2016) argue that the trophic base of many Australian rivers has shifted from terrestrial to algal-derived organic carbon, due to a reduction in terrestrial subsidies caused by river regulation, land use changes and riparian degradation. Environmental releases from regulated tributaries may potentially be used to mobilise additional terrestrial DOC to impounded rivers, but the effectiveness of this approach has not previously been tested. Accordingly, a specific focus of this thesis was to evaluate the role of tributary water in delivering terrestrial organic carbon to regulated rivers. We found that river discharge was strongly linked to DOC concentration in unregulated sections of the tributary (Chapter 2), reflecting a DOC regime driven by lateral connectivity with the riparian zone and surrounding catchment (McGlynn & McDonnell, 2003). Tributary carbon inputs contributed to longitudinal recovery of the DOC regime below Jindabyne Dam, with an increased magnitude and frequency of DOC pulses, and stronger DOC-discharge relationships evident downstream of the tributary junction (Chapter 2). These results suggest that tributary flows can play an important role in supporting the ecological integrity of regulated rivers by maintaining longitudinal carbon transport pathways from headwater streams (Freeman, Pringle & Jackson, 2007).

This thesis contains the first studies that specifically address active DOC regime management through the use of tributary environmental flow releases (Chapters 2 and 3). In Chapter 2, we found that water diversion into the reservoir from a tributary prevented almost 80% of the annual tributary DOC export from reaching the regulated main stem. In this system, diverted tributary water therefore contains a considerable pool of terrestrial carbon that could be redirected downstream by allowing tributary water to flow directly to the Snowy River rather than routing it through Jindabyne Dam. Chapters 2 and 3 showed that active management of tributary flow by providing

environmental water from smaller weirs can increase the total DOC load, and magnitude and frequency of DOC pulses delivered to regulated rivers. The results presented in Chapters 2 and 3 illustrate that tributary DOC delivery can be actively managed, and establish tributary environmental water releases as a legitimate management strategy to increase terrestrial DOC subsidies to highly regulated rivers. These findings have relevance beyond the Snowy River catchment, as many larger impounded river basins such as the Murray River also have regulated tributaries (Walker & Thoms, 1993).

6.3 Benthic microbial responses to high-flow dam releases

Ecological processes form key components of river ecosystems, and are increasingly being adopted in assessments of ecosystem health and in river rehabilitation strategies (Young, Matthaei & Townsend, 2008). The microbial community facilitates many fundamental ecological processes (Fischer *et al.*, 2005; Fellows, Valett & Dahm, 2001), yet measurements of microbial functioning are still rarely incorporated in the monitoring of rehabilitation activities (but see Uehlinger, Kawecka & Robinson, 2003). This is partly because the mechanisms linking environmental variation and microbial responses are numerous and complex (e.g. Rees, Bowen & Watson, 2005), and require further investigation. This knowledge gap was investigated in Chapter 4, where a streamside mesocosm system was used to explore the role of resource supply regime in driving benthic microbial metabolism during experimental pulsed dam releases. Rapid pulses in epilithic biofilm respiration were detected during two of three high-flow dam releases, implying that changes in the dissolved resource supply occurring during the release events were sufficient to stimulate microbial activity. Further studies are required to definitively identify which specific aspects of the resource regime may have been driving enhanced benthic metabolism during the dam releases (section 6.7). However, the significant biofilm responses documented in Chapter 4 are consistent with the contention that resource delivery from high-flow dam releases can influence benthic microbial functioning. This represents an important additional perspective as benthic responses to environmental flows are mainly considered to be a

consequence of physical disturbance induced by high flow velocities (Jakob, Robinson & Uehlinger, 2003).

The study in Chapter 4 was unique in that it examined short-term functional responses taking place within the high-flow release period, rather than the more common approach of measuring at a pre- and post-event scale (Chester & Norris, 2006; Uehlinger, Kawecka & Robinson, 2003). Significant increases in biofilm respiration, and shifts in sediment enzyme activity (Chapter 4) suggest that pulsed dam events can trigger brief but important periods of increased or altered microbial activity. These periods may represent hot moments (McClain *et al.*, 2003) of increased microbial processing, driven by a shift in the basal resource supply during the flow release. Cumulatively, resource-mediated pulses in microbial metabolic activity could be an important contributor to whole-ecosystem processing and alter net fluxes of carbon and major nutrients through river systems (Cole & Caraco, 2001; Fellows, Valett & Dahm, 2001). This study showed that microbial responses can occur at rapid timescales, and are likely to be overlooked if events are monitored only at pre and post-event intervals. Data collection at a daily frequency or shorter should be considered in future investigations of microbial responses to high-flow events.

6.4 Microbial responses to DOC input rate

We performed a laboratory mesocosm experiment to further investigate the potential for terrestrial DOC pulses to contribute to benthic microbial hot moments. Manipulations of terrestrial DOC input rate were carried out, and several aspects of microbial response were explored by measuring biofilm composition, metabolic activities and taxonomic structure. A rapid, transient shift in biofilm bacterial enzyme expression and bacterial community structure was observed in response to the pulse DOC treatment, separating it from press and control treatments (Chapter 5). This experiment was one of the first studies to test DOC input rate effects on epilithic biofilm communities. The epilithic biofilm response to terrestrial DOC differed considerably from that of hyporheic sediments (Wagner *et al.*, 2014) and planktonic communities (Lennon & Cottingham, 2008; Judd, Crump & Kling, 2006) in that it was

highly transient, being detectable for a period of only one day. The results presented in Chapter 5 showed that epilithic biofilm communities may be more sensitive to the external dissolved resource supply regime than previously believed. Further studies are required to unravel if and how the heterotrophic aspects of biofilm functioning may be influenced by terrestrial DOC variability under environmental conditions.

6.5 Evaluation of resource regime conceptual model

Section 1.10 introduced a framework that described the DOC resource regime in terms of three main components; DOC quantity, input rate and composition. A conceptual model of natural and regulated resource regimes was developed based on the anticipated state of each of these components in unmodified and regulated rivers (Figure 6.1). The original conceptual model predicted that a natural DOC regime with rapid increases in DOC concentration would have a greater capacity to trigger microbial hot moments than a regulated, homogenous DOC regime. Dam and tributary environmental flow releases were expected to shift the DOC regime from a regulated state towards a natural state, thereby facilitating increased microbial process rates.

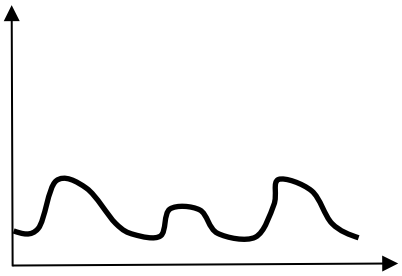
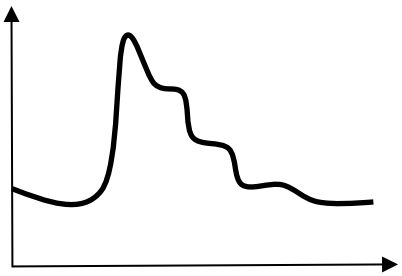
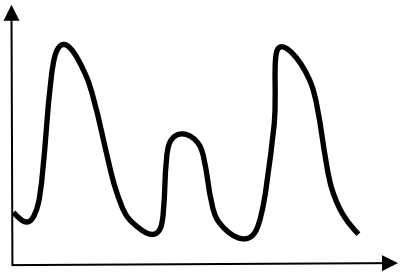
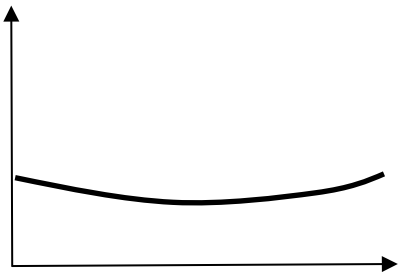
Based on the results of this thesis, the major differences between the DOC regimes associated with tributary and pulsed dam releases are summarised in Table 6.1. As expected, tributary environmental flow releases helped to shift the main stem towards a natural DOC regime by delivering rapid pulses of terrestrial DOC (Chapter 3). However, during pulsed dam releases, DOC concentrations were unexpectedly low and constant, remaining more similar to the DOC pattern predicted for a regulated DOC regime (Chapter 3). DOC composition during the dam releases did not reflect the expected pattern for either natural or regulated DOC regimes. While a terrestrial DOC signal was detected in the main stem, the larger volume dam release also mobilised a weak microbial DOC signature further downstream of the dam wall at higher release rates (Chapter 3). This finding indicates that the effects of high-flow dam releases on DOC composition are more complex than initially conceived in the conceptual model. DOC composition is a multifaceted property, and may be better represented in the

conceptual model by including direct metrics of DOC bioavailability, such as bioassays (Guillemette & del Giorgio, 2011). Inclusion of DOC bioassays would also support stronger conclusions to be drawn when linking compositional shifts with predicted or observed microbial response patterns (Baldwin & Valo, 2015).

The effects of natural and regulated DOC regimes on microbial metabolic activities are more complex to interpret. Some of the microbial responses documented in Chapters 4 and 5 were inconsistent with the patterns expected for either natural or regulated DOC regimes. The original conceptual model predicted that DOC pulses would trigger accelerated microbial processing rates. However, experimental results showed that biofilm respiration increased in response to pulsed dam releases, even in the absence of notable DOC pulses (Chapter 4). Conversely, a simulated DOC pulse triggered transient increases in microbial metabolic activity relative to controls (Chapter 5).

These findings raise several questions regarding potential interactions between resource regime components that may affect their relative importance and duration in influencing microbial processes. Shifts in the bacterial enzyme fingerprint suggested that bacteria were responding to changing DOC composition in both field and lab mesocosm studies (Chapters 4 and 5). An overriding influence of DOC composition over DOC quantity or input rate could have contributed to the unexpected microbial responses to natural and regulated DOC regimes. This notion is supported by studies of planktonic communities that found the effects of resource input rate are contingent upon resource quality (Lennon & Cottingham, 2008). In order to more adequately account for observed microbial metabolic responses, the resource regime conceptual model requires adaptation to allow for hierarchical or interactive relationships between the DOC components.

Table 6.1 Summary of DOC regime characteristics and microbial responses observed in monitoring studies of the Snowy River below Jindabyne Dam and in laboratory studies simulating natural and regulated DOC regimes.

	Tributary Release	Pulsed reservoir release
Hydrology		
DOC concentration		
	Low to high (Chapter 3)	Low (Chapter 2, 3)
DOC input rate	High (Chapter 2, 3)	Low (Chapter 2 and 3)
DOC composition	Terrestrial-like (Chapter 3)	Terrestrial-like (Chapter 3)
		Protein-like DOC at higher discharge volumes (Chapter 3)
Microbial responses (field)	Not tested	Increased biofilm respiration (Chapter 4)
	Not tested	Shift in bacterial enzyme expression (Chapter 4)
Microbial responses (laboratory)	Shift in bacterial enzyme expression (Chapter 5)	No shift in bacterial enzyme expression
	Shift in bacterial community structure, increased abundance of *Burkholderiales*	No shift in bacterial community structure

While conceptually distinct, the separation between natural and regulated resource regime types is less clear in practise. In many rivers, the DOC regime could most realistically be positioned along a gradient between natural and regulated states. Identifying what constitutes a 'high' or 'low' resource concentration or input rate is also highly dependent on the individual characteristics of the river in question. For example, the magnitude of change in DOC and nutrient concentrations during the experimental dam releases (Chapter 4) would be considered small relative to changes observed in lowland systems (Kobayashi *et al.*, 2009). However, these minor pulses were still sufficient to trigger increased microbial respiration. To be of use in guiding adaptive management, the distinction between natural and regulated regimes may ultimately need to be defined in terms of the microbial response patterns they are likely to elicit for each individual system, similar to the approach adopted by Yang et al. (2008) in defining resource pulses.

Despite these challenges, a conceptual framework for characterising natural and regulated DOC regimes is a useful foundation to support incorporation of DOC into rehabilitation objectives and environmental flow strategies. Further conceptual development and additional empirical data are required for the DOC resource regime model to be useful in linking particular DOC regimes to microbial response patterns.

6.6 Recommendations for further studies

6.6.1 Microbial responses to natural high-flow events

While chapter 3 examined microbial responses to a regulated DOC regime delivered by pulsed dam release events, we were unable to conduct a comparative study of a natural DOC resource regime. A field study of microbial responses to natural high-flow events involving a wider gradient of DOC concentrations, input rates and compositions would reveal more about the role of the DOC resource regime in stimulating biogeochemical hot moments. Despite the logistical difficulties inherent in capturing rapid and unpredictable natural events, empirical field data are essential in establishing microbial response patterns to resource variability during natural high-

flow events. These patterns would then form a baseline for comparison with microbial response data from modified systems, and in response to managed environmental flow events. Studies of planktonic communities suggest that changes in DOC quality during catchment runoff-driven storms and snowmelt can stimulate bacterial activity (Buffam *et al.*, 2001), and it would be informative to clarify the extent to which natural DOC variability drives benthic microbial contributions to ecosystem processes and net biogeochemical fluxes.

6.6.2 Interactions between different resource regime components

The experimental components of this thesis included field-scale flow manipulations that delivered a 'regulated' DOC regime (Chapter 4) and a laboratory study simulating 'natural' and 'regulated' resource regimes by exposing biofilms to terrestrial DOC added at rapid and slow rates. These studies tested only a limited subset of the resource supply regime components outlined in the conceptual model presented in Chapter 1 of this thesis (see section 6.1). Testing for interactions between different components of the resource regime was beyond the scope of this thesis. However, manipulative studies varying multiple resource regime components in combination could provide valuable information regarding potential interactive effects of different aspects of the DOC regime. Multifactorial experiments could also be used to show whether the different resource regime components are hierarchical; for instance, whether DOC quality overrides the effect of DOC input rate on benthic microbial processes, as seen in the pelagic community (Lennon & Cottingham, 2008), and as suggested by the findings of this thesis (Chapters 4 and 5).

6.6.3 Field studies at wider spatial scales

The field studies described in this thesis were largely limited in spatial extent to a section of the Snowy River extending 24 km immediately downstream of Jindabyne Dam (Chapters 2-4). As the DOC regime is more homogenous closer to the dam, tributary inputs are most strongly evident in this section (Stanford & Ward, 2001), and presumably lessen with increasing distance from the dam due to inputs from larger tributaries further downstream. Monitoring of DOC dynamics for a longer distance

downstream of Jindabyne Dam would allow further definition of the spatial extent to which particular management actions produce a detectable effect on the DOC regime. The total DOC load mobilised by dam releases increased longitudinally (Chapter 3), representing a net export of carbon to downstream reaches. Studies at greater spatial scales would help to determine the ultimate fate of this carbon, including the extent to which it subsidises river and estuarine productivity (Hitchcock *et al.*, 2016).

Monitoring basal resource regimes and associated microbial responses in a wider range of regulated catchments would also confirm whether the trends observed in the Snowy River could be generalised to other river systems. Various aspects of the local environment including climate, soils, vegetation, catchment land use and geomorphology can all influence resource dynamics as well as microbial metabolic activities. Additionally, the DOC and nutrient load contained within outflowing waters is often reflective of in-lake processes (Larson *et al.*, 2007). While the DOC regime below Lake Jindabyne consisted of low, homogenous DOC concentrations, water released from other reservoirs may produce different DOC regimes depending on factors such as algal productivity and water residence time within the reservoir (Miller, 2012).

6.6.4 Additional DOC and microbial response metrics

A wide variety of methodologies may be used to quantify various aspects of microbial metabolic activity. The microbial functional metrics used in this thesis included direct measurements of oxygen consumption and production (Chapter 4), extracellular enzyme activities (Chapters 4 and 5), and electron transport system activity (Chapter 5). Another common metric of microbial carbon uptake is bacterial productivity, estimated as the rate of ^{3}H-leucine incorporation into bacterial biomass (Kirchman, 1993). Unfortunately ^{3}H-leucine assays could not be included in these studies, but measurements of bacterial productivity would be useful in future studies assessing the effects of varying DOC resource regimes on microbial activity. This would allow clearer conclusions to be drawn regarding the effects of basal resource variability on broader ecosystem processes.

Shifts in bacterial enzyme expression occurred during resource supply manipulations in field (Chapter 4) and laboratory (Chapter 5) settings, indicating that bacterial metabolic responses may have been related to qualitative shifts in the DOC supply. The studies in this thesis utilised fluorescence spectra as a measure of DOC composition (Chapters 3, 5), which distinguish between microbial and terrestrial-derived DOC fluorophores. However, this method has recently been critiqued as DOC optical properties do not necessarily predict actual susceptibility to microbial degradation (Baldwin & Valo, 2015). Other studies have also shown that the terrestrial DOC pool can include both labile and recalcitrant DOC fractions (McDowell *et al.*, 2006), which cannot be resolved using optical methods (Fellman, Hood & Spencer, 2010). It is recommended that future studies of DOC regime variability incorporate bioassays of DOC consumption as a metric of DOC bioavailability (Guillemette & del Giorgio, 2011).

6.6.6 Linkages to organisms at higher trophic levels.

Further studies are required to establish the extent to which organic carbon metabolised by the microbial community subsidises the energetic requirements of organisms at higher trophic levels. DOC assimilated by bacteria may be incorporated into the metazoan food web through ingestion of detrital particles by invertebrates (Hall & Meyer, 1998), which may then be consumed by predator organisms such as fish (Cross *et al.*, 2013) and platypus (Klamt *et al.*, 2015). Tracing of stable isotope signatures (Anderson & Cabana, 2007), in combination with co-ordinated measurements of DOC, microbial and consumer biomass and process rates would be a useful approach for further investigation into the effects of specific environmental release strategies on terrestrial carbon utilisation and transfer to consumer organisms.

6.7 Management recommendations

The water allocation available for environmental flows is set by the *Snowy Water Inquiry Outcomes Implementation Deed* (SWIOID) 2002, which stipulates a water recovery target of 21% of mean annual natural flow, or 212 GL per year. The Snowy River environmental water allocation is delivered according to release strategies prescribed in the Snowy River Increased Flows (SRIF) adaptive management program.

Although the total environmental flow volume is fixed, there is scope to vary the hydrological characteristics (i.e. timing, magnitude, frequency, rate of change and duration), and the source of water releases within each annual water allocation period (Williams, 2015a).

This study occurred during the third release strategy in the SRIF, which prioritised the delivery of a single, large annual flushing flow designed to simulate a snowmelt peak. In 2013, the release strategy was revised to better reflect the hydrology of a Snowy montane river by providing multiple high-flow events, and by increasing daily, seasonal and annual flow variability (Reinfelds *et al.*, 2013). These changes provide several opportunities to further incorporate active monitoring and management of the DOC regime into rehabilitation activities in the Snowy River. Based on the findings of the studies in this thesis, the following recommendations are made regarding the future environmental water strategies for the Snowy River and in New South Wales more broadly.

- Environmental water releases from the Mowamba Weir should be incorporated into the SRIF. This may be achieved either through temporarily suspending water diversions or through permanently decommissioning the Mowamba Weir. Higher tributary inflows to the Snowy River would help maintain a more natural DOC regime in the Snowy River, including more frequent, larger magnitude DOC concentration pulses below Jindabyne Dam.
- Tributary releases should be used in preference to dam releases to achieve increased DOC delivery. Although the pulsed dam releases transported larger total DOC load than tributary releases, they had a much lower DOC export per megalitre of environmental water used. Tributary releases are therefore more efficient at mobilising terrestrial DOC than pulsed releases from Jindabyne Dam.
- Tributary releases should be employed in combination with larger, pulsed releases from Jindabyne Dam. The higher flow velocities required to achieve other rehabilitation objectives such as sediment flushing cannot be delivered via the Mowamba River. These larger flushing releases are therefore still

required to facilitate recovery of key ecosystem processes below Jindabyne Dam.

- Other aspects of tributary water quality should be considered when evaluating the suitability of a tributary as a source for environmental flow releases. The benefits of tributary contributions to the downstream resource regime may be outweighed by inputs of less desirable solutes such as nutrient or sediment inputs from agricultural activities. In such cases, tributary flow releases may be most effective when implemented alongside rehabilitation efforts targeting the condition of riparian areas upstream pollution sources.
- Functional metrics such as community metabolism should be considered in the monitoring of future environmental release events. This would provide useful information in evaluating the ecosystem response to resources mobilised by environmental release events. Functional metrics may better capture rapid or event-specific biotic responses in comparison to more conventional assessments of biotic community structure.

6.8 Conclusions

Environmental water releases to the Snowy River produced markedly different DOC regimes depending on whether they were derived from a tributary or a large dam. DOC concentration was strongly related to stream discharge in the tributary, reflecting a relationship commonly found in unregulated rivers with strong hydrological connections to the surrounding catchment. In contrast, DOC concentration showed no significant relationship with discharge directly below the dam, and remained relatively low and constant over time. These differing relationships between DOC concentration and discharge drove the DOC regimes seen in the Snowy River during active environmental water release events.

Tributary environmental water releases increased the magnitude and frequency of DOC pulses delivered to the regulated Snowy River, and increased the DOC load exported from the tributary. Conversely, DOC concentration remained low and constant during the high-flow dam releases, and lacked the rapid pulses characteristic of natural high-flow events. A longitudinal increase in DOC load and a shift in

downstream DOC composition suggest that DOC sources mobilised from the reaches below the dam contribute to the DOC regime generated by pulsed dam releases. Water diversions dampened the tributary DOC signal by preventing almost 80% of the annual DOC export from reaching the main stem, and by reducing the frequency of storm-associated DOC pulses supplied to the Snowy River.

Exposure of epilithic biofilm communities to dam release waters in a field mesocosm study revealed rapid pulses in epilithic biofilm respiration during two of three high-flow dam releases. Although biofilm metabolism could not be directly related to resource variability during the releases, shifts in sediment bacterial enzyme expression suggested changes in DOC composition occurred during the releases. Subsequent manipulations of terrestrial DOC input rate in a laboratory mesocosm experiment revealed that DOC supply regime may modulate microbial responses, with pulsed DOC inputs triggering a transient shift in biofilm bacterial enzyme expression and bacterial community composition. Collectively these results show that benthic microbial communities can respond rapidly to hydrologically-driven variations in resource regime, providing a potential mechanism through which environmental flows may trigger hot moments of increased microbial processing.

The concepts and knowledge developed in this thesis contribute to a more comprehensive understanding of organic carbon supply and utilisation in regulated rivers, and the ecological mechanisms linking environmental water delivery and biotic processes. This research reveals the considerable scope for organic matter flows to be actively managed through environmental water delivery. In particular, these results establish tributary flow releases as a legitimate strategy to actively manage the DOC regime, and support their wider implementation in river rehabilitation programs.

Bibliography

Abboudi M., Jeffrey W.H., Ghiglione J.-F., Pujo-Pay M., Oriol L., Sempéré R., Charrière B. & Joux F. (2008) Effects of Photochemical Transformations of Dissolved Organic Matter on Bacterial Metabolism and Diversity in Three Contrasting Coastal Sites in the Northwestern Mediterranean Sea during Summer. *Microbial Ecology,* **55,** 344-357.

Acuna V. & Tockner K. (2010) The effects of alterations in temperature and flow regime on organic carbon dynamics in Mediterranean river networks. *Global Change Biology,* **16,** 2638-2650.

Agren A., Buffam I., Jansson M. & Laudon H. (2007) Importance of seasonality and small streams for the landscape regulation of dissolved organic carbon export. *Journal of Geophysical Research-Biogeosciences,* **112**.

Ahearn D.S., Sheibley R.W. & Dahlgren R.A. (2005) Effects of river regulation on water quality in the lower Mokelumne River, California. *River Research and Applications,* **21,** 651-670.

Aiken G.R., Hsu-Kim H. & Ryan J.N. (2011) Influence of Dissolved Organic Matter on the Environmental Fate of Metals, Nanoparticles, and Colloids. *Environmental Science & Technology,* **45,** 3196-3201.

Aitkenhead-Peterson J.A., Mcdowell W.H. & Neff J.C. (2003) Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters. In: *Interactivity of Dissolved Organic Matter.* (Eds S. Findlay & R.L. Sinsabaugh), pp. 26-70. Academic Press, California.

Algesten G., Sobek S., Bergstrom A.K., Agren A., Tranvik L.J. & Jansson M. (2004) Role of lakes for organic carbon cycling in the boreal zone. *Global Change Biology,* **10,** 141-147.

Allan J.D. & Castillo M.M. (2007) *Stream Ecology: Structure and Function of Running Waters,* Springer, The Netherlands.

Amon R.M.W. & Benner R. (1996) Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography,* **41,** 41-51.

Anderson-Glenna M.J., Bakkestuen V. & Clipson N.J. (2008) Spatial and temporal variability in epilithic biofilm bacterial communities along an upland river gradient. *Fems Microbiology Ecology,* **64,** 407-418.

Anderson C. & Cabana G. (2007) Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. *Journal of the North American Benthological Society,* **26,** 273-285.

Anderson M.J. (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology,* **26,** 32-46.

Apha. (2005) *Standard Methods for the Examination of Water and Wastewater,* APHA: Washington, DC.

Arnosti C. (2003) Microbial Extracellular Enzymes and Their Role in Dissolved Organic Matter Cycling. In: *Interactivity of Dissolved Organic Matter.* (Eds S. Findlay & R.L. Sinsabaugh), pp. 316-342. Academic Press, California.

Arthington A.H. & Pusey B.J. (2003) Flow restoration and protection in Australian rivers. *River Research and Applications,* **19,** 377-395.

Attermeyer K., Hornick T., Kayler Z.E., Bahr A., Zwirnmann E., Grossart H.P. & Premke K. (2014) Enhanced bacterial decomposition with increasing addition of autochthonous to allochthonous carbon without any effect on bacterial community composition. *Biogeosciences,* **11,** 1479-1489.

Augspurger C. & Kusel K. (2010) Flow velocity and primary production influences carbon utilization in nascent epilithic stream biofilms. *Aquatic Sciences,* **72,** 237-243.

Autio I., Soinne H., Helin J., Asmala E. & Hoikkala L. (2016) Effect of catchment land use and soil type on the concentration, quality, and bacterial degradation of riverine dissolved organic matter. *Ambio,* **45,** 331-349.

Baker A. & Inverarity R. (2004) Protein-like fluorescence intensity as a possible tool for determining river water quality. *Hydrological Processes,* **18,** 2927-2945.

Baldwin D.S. (1999) Dissolved organic matter and phosphorus leached from fresh and 'terrestrially' aged river red gum leaves: implications for assessing river-floodplain interactions. *Freshwater Biology,* **41,** 675-685.

Baldwin D.S., Colloff M.J., Mitrovic S.M., Bond N.R. & Wolfenden B. (2016) Restoring dissolved organic carbon subsidies from floodplains to lowland river food webs: a role for environmental flows? *Marine and Freshwater Research***,** -.

Baldwin D.S. & Valo W. (2015) Exploring the relationship between the optical properties of water and the quality and quantity of dissolved organic carbon in aquatic ecosystems: strong correlations do not always mean strong predictive power. *Environmental Science-Processes & Impacts,* **17,** 619-630.

Battin T.J., Besemer K., Bengtsson M.M., Romani A.M. & Packmann A.I. (2016) The ecology and biogeochemistry of stream biofilms. *Nat Rev Micro,* **14,** 251-263.

Beechie T.J., Sear D.A., Olden J.D., Pess G.R., Buffington J.M., Moir H., Roni P. & Pollock M.M. (2010) Process-based Principles for Restoring River Ecosystems. *Bioscience,* **60,** 209-222.

Benda L., Poff N.L., Miller D., Dunne T., Reeves G., Pess G. & Pollock M. (2004) The network dynamics hypothesis: How channel networks structure riverine habitats. *Bioscience,* **54,** 413-427.

Berggren M., Laudon H., Haei M., Strom L. & Jansson M. (2010) Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources. *Isme Journal,* **4,** 408-416.

Bernhardt E.S. & Likens G.E. (2002) Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. *Ecology,* **83,** 1689-1700.

Biggs B.J.F. (1996) Patterns in Benthic Algae of Streams. In: *Algal ecology : freshwater benthic ecosystems.* (Eds R.J. Stevenson & M.L. Bothwell & R.L. Lowe). Academic Press, San Diego.

Biggs B.J.F., Smith R.A. & Duncan M.J. (1999) Velocity and Sediment Disturbance of Periphyton in Headwater Streams: Biomass and Metabolism. *Journal of the North American Benthological Society,* **18,** 222-241.

Blenkinsopp S. & Lock M. (1990) The measurement of electron transport system activity in river biofilms. *Water Research,* **24,** 441-445.

Blenkinsopp S.A. & Lock M.A. (1992) Impact of storm-flow on electron transport system activity in river biofilms. *Freshwater Biology,* **27,** 397-404.

Bott T.L. (2006) Primary Productivity and Community Respiration. In: *Methods in Stream Ecology.* (Eds F.R. Hauer & G.A. Lamberti). Elsevier, Amsterdam.

Bott T.L., Brock J.T., Baattruppedersen A., Chambers P.A., Dodds W.K., Himbeault K.T., Lawrence J.R., Planas D., Snyder E. & Wolfaardt G.M. (1997) An evaluation of techniques for measuring periphyton metabolism in chambers. *Canadian Journal of Fisheries and Aquatic Sciences,* **54,** 715-725.

Bouletreau S., Garabetian F., Sauvage S. & Sanchez-Perez J.M. (2006) Assessing the importance of a self-generated detachment process in river biofilm models. *Freshwater Biology,* **51,** 901-912.

Boyer E.W., Hornberger G.M., Bencala K.E. & Mcknight D.M. (1997) Response characteristics of DOC flushing in an alpine catchment. *Hydrological Processes,* **11,** 1635-1647.

Boyer E.W., Hornberger G.M., Bencala K.E. & Mcknight D.M. (2000) Effects of asynchronous snowmelt on flushing of dissolved organic carbon: a mixing model approach. *Hydrological Processes,* **14,** 3291-3308.

Boynton W.R., Kemp W.M., Osborne C.G., Kaumeyer K.R. & Jenkins M.C. (1981) Influence of water circulation rate on in situ measurements of benthic community respiration. *Marine Biology,* **65,** 185-190.

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

Bunn S.E. & Arthington A.H. (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management,* **30,** 492-507.

Burns A. & Ryder D.S. (2001) Response of bacterial extracellular enzymes to inundation of floodplain sediments. *Freshwater Biology,* **46,** 1299-1307.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Pena A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., Mcdonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Tumbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J. & Knight R. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature Methods,* **7,** 335-336.

Carney R.L., Mitrovic S.M., Jeffries T., Westhorpe D., Curlevski N. & Seymour J.R. (2015) River bacterioplankton community responses to a high inflow event. *Aquatic Microbial Ecology,* **75,** 187-205.

Carney R.L., Seymour J.R., Westhorpe D. & Mitrovic S.M. (2016) Lotic bacterioplankton and phytoplankton community changes under dissolved organic-carbon amendment: evidence for competition for nutrients. *Marine and Freshwater Research***,** -.

Chester H. & Norris R. (2006) Dams and Flow in the Cotter River, Australia: Effects on Instream Trophic Structure and Benthic Metabolism. *Hydrobiologia,* **572,** 275-286.

Clinton S.M., Edwards R.T. & Findlay S.E.G. (2010) Exoenzyme activities as indicators of dissolved organic matter composition in the hyporheic zone of a floodplain river. *Freshwater Biology,* **55,** 1603-1615.

Coble P.G. (1996) Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry,* **51,** 325-346.

Colangelo D.J. (2007) Response of river metabolism to restoration of flow in the Kissimmee River, Florida, USA. *Freshwater Biology,* **52,** 459-470.

Cole J.J. & Caraco N.F. (2001) Carbon in catchments: connecting terrestrial carbon losses with aquatic metabolism. *Marine and Freshwater Research,* **52,** 101-110.

Cole J.J., Pace M.L., Carpenter S.R. & Kitchell J.F. (2000) Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. *Limnology and Oceanography,* **45,** 1718-1730.

Cole J.J., Prairie Y.T., Caraco N.F., Mcdowell W.H., Tranvik L.J., Striegl R.G., Duarte C.M., Kortelainen P., Downing J.A., Middelburg J.J. & Melack J. (2007) Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems,* **10,** 171-184.

Coleman D. & Williams S. (2016) Mobilising fine sediment in a highly regulated upland snowmelt river using hydrological scaled experimental floods. *Marine and Freshwater Research***,** -.

Comte J., Fauteux L. & Del Giorgio P.A. (2013) Links between metabolic plasticity and functional redundancy in freshwater bacterioplankton communities. *Frontiers in Microbiology,* **4**.

Cook R.A., Gawne B., Petrie R., Baldwin D.S., Rees G.N., Nielsen D.L. & Ning N.S.P. (2015) River metabolism and carbon dynamics in response to flooding in a lowland river. *Marine and Freshwater Research,* **66,** 919-927.

Cooper D.M. & Watts C.D. (2002) A comparison of river load estimation techniques: application to dissolved organic carbon. *Environmetrics,* **13,** 733-750.

Cory R.M. & Kaplan L.A. (2012) Biological lability of streamwater fluorescent dissolved organic matter. *Limnology and Oceanography,* **57,** 1347-1360.

Cory R.M., Miller M.P., Mcknight D.M., Guerard J.J. & Miller P.L. (2010) Effect of instrument-specific response on the analysis of fulvic acid fluorescence spectra. *Limnology and Oceanography-Methods,* **8,** 67-78.

Cotner J.B. & Biddanda B.A. (2002) Small Players, Large Role: Microbial Influence on Biogeochemical Processes in Pelagic Aquatic Ecosystems. *Ecosystems,* **5,** 105-121.

Covert J.S. & Moran M.A. (2001) Molecular characterization of estuarine bacterial communities that use high- and low-molecular weight fractions of dissolved organic carbon. *Aquatic Microbial Ecology,* **25,** 127-139.

Cross W.F., Baxter C.V., Donner K.C., Rosi-Marshall E.J., Kennedy T.A., Hall R.O., Kelly H.a.W. & Rogers R.S. (2011) Ecosystem ecology meets adaptive management: food web response to a controlled flood on the Colorado River, Glen Canyon. *Ecological Applications,* **21,** 2016-2033.

Cross W.F., Baxter C.V., Rosi-Marshall E.J., Hall R.O., Kennedy T.A., Donner K.C., Wellard Kelly H.A., Seegert S.E.Z., Behn K.E. & Yard M.D. (2013) Food-web dynamics in a large river discontinuum. *Ecological Monographs,* **83,** 311-337.

Cross W.F., Wallace J.B. & Rosemond A.D. (2007) Nutrient enrichment reduces constraints on material flows in a detritus-based food web. *Ecology,* **88,** 2563-2575.

Davie A.W. & Mitrovic S.M. (2014) Benthic algal biomass and assemblage changes following environmental flow releases and unregulated tributary flows downstream of a major storage. *Marine and Freshwater Research,* **65,** 1059-1071.

Dawson J.J.C., Billett M.F., Neal C. & Hill S. (2002) A comparison of particulate, dissolved and gaseous carbon in two contrasting upland streams in the UK. *Journal of Hydrology,* **257,** 226-246.

Doi H., Chang K.H., Ando T., Imai H., Nakano S.I., Kajimoto A. & Katano I. (2008) Drifting plankton from a reservoir subsidize downstream food webs and alter community structure. *Oecologia,* **156,** 363-371.

Donato J., Abuhatab Y. & Sabater S. (2014) Epilithic biofilm metabolism during the high water flow period in an Andean neotropical stream. *Hydrobiologia,* **728,** 41-50.

Eaton A.D. & Franson M.H. (2005) *Standard methods for the examination of water & wastewater,* American Public Health Association.

Edgar R.C., Haas B.J., Clemente J.C., Quince C. & Knight R. (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics,* **27,** 2194-2200.

Elser J.J. & Hessen D.O. (2005) Biosimplicity via stoichiometry: the evolution of food-web structure and processes. In: *Aquatic Food Webs: an ecosystem approach.* (Eds A. Belgrano & U.M. Scharler & J. Dunne & R.E. Ulanowicz). Oxford Univeristy Press, New York.

Elwood J.W., Newbold J.D., O'neill R.V. & Van Winkle W. (1983) Resource spiralling: An operational paradigm for analyzing lotic ecosystems. In: *Dynamics of Lotic Ecosystems.* (Eds T.D. Fontaine & S.M. Bartell), pp. 3-28. Ann Arbour Science Publishers, Michigan.

Erskine W., Terrazzolo N. & Warner R.F. (1999) River rehablitation from the hydrogeomorphic impacts of a large hydro-electric power project: Snowy River, Australia. *Regulated Rivers: Research & Management,* **15,** 3-14.

Erskine W.a.W., A. . (2000) Fluvial Geomorphology of the Snowy River below Jindabyne Dam. In: *Snowy Flow Response Monitoring and Modelling program*. NSW Department of Land and Water Conservation.

Espeland E.M., Francoeur S.N. & Wetzel R.G. (2001) Influence of Algal Photosynthesis on Biofilm Bacterial Production and Associated Glucosidase and Xylosidase Activities. *Microbial Ecology,* **42,** 524-530.

Essington T.E. & Carpenter S.R. (2000) Nutrient cycling in lakes and streams: Insights from a comparative analysis. *Ecosystems,* **3,** 131-143.

Farjalla V.F., Azevedo D.A., Esteves F.A., Bozelli R.L., Roland F. & Enrich-Prast A. (2006) Influence of hydrological pulse on bacterial growth and DOC uptake in a clear-water Amazonian lake. *Microbial Ecology,* **52,** 334-344.

Fasching C., Behounek B., Singer G.A. & Battin T.J. (2014) Microbial degradation of terrigenous dissolved organic matter and potential consequences for carbon cycling in brown-water streams. *Scientific Reports,* **4**.

Fasching C., Ulseth A.J., Schelker J., Steniczka G. & Battin T.J. (2016) Hydrology controls dissolved organic matter export and composition in an Alpine stream and its hyporheic zone. *Limnology and Oceanography,* **61,** 558-571.

Fellman J.B., Hood E. & Spencer R.G.M. (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. *Limnology and Oceanography,* **55,** 2452-2462.

Fellman J.B., Petrone K.C. & Grierson P.F. (2011) Source, biogeochemical cycling, and fluorescence characteristics of dissolved organic matter in an agro-urban estuary. *Limnology and Oceanography,* **56,** 243-256.

Fellman J.B., Spencer R.G.M., Raymond P.A., Pettit N.E., Skrzypek G., Hernes P.J. & Grierson P.F. (2014) Dissolved organic carbon biolability decreases along with its modernization in fluvial networks in an ancient landscape. *Ecology,* **95,** 2622-2632.

Fellows C.S., Valett H.M. & Dahm C.N. (2001) Whole-stream metabolism in two montane streams: Contribution of the hyporheic zone. *Limnology and Oceanography,* **46,** 523-531.

Filella M. (2010) Quantifying 'humics' in freshwaters: purpose and methods. *Chemistry and Ecology,* **26,** 177-186.

Findlay S., Quinn J.M., Hickey C.W., Burrell G. & Downes M. (2001) Effects of land use and riparian flowpath on delivery of dissolved organic carbon to streams. *Limnology and Oceanography,* **46**.

Findlay S. & Sinsabaugh R.L. (1999) Unravelling the sources and bioavailability of dissolved organic matter in lotic aquatic ecosystems. *Marine and Freshwater Research,* **50,** 781-790.

Findlay S. & Sinsabaugh R.L. (2003) Interactivity of Dissolved Organic Matter. In: *Aquatic Ecology Series.* (Ed J.H. Thorp). Academic Press, USA.

Findlay S., Strayer D., Goumbala C. & Gould K. (1993) Metabolism of streamwater dissolved organic carbon in the shallow hyporheic zone. *Limnology and Oceanography,* **38,** 1493-1499.

Findlay S.E.G., Sinsabaugh R.L., Sobczak W.V. & Hoostal M. (2003) Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnology and Oceanography,* **48,** 1608-1617.

Fischer H., Kloep F., Wilzcek S. & Pusch M. (2005) A River's Liver – Microbial Processes within the Hyporheic Zone of a Large Lowland River. *Biogeochemistry,* **76,** 349-371.

Fisher S.G., Grimm N.B., Marti E., Holmes R.M. & Jones J.B. (1998) Material spiraling in stream corridors: A telescoping ecosystem model. *Ecosystems,* **1,** 19-34.

Fisher S.G., Sponseller R.A. & Heffernan J.B. (2004) Horizons in stream biogeochemistry: Flowpaths to progress. *Ecology,* **85,** 2369-2379.

Foreman C.M., Franchini P. & Sinsabaugh R.L. (1998) The trophic dynamics of riverine bacterioplankton: Relationships among substrate availability, ectoenzyme kinetics, and growth. *Limnology and Oceanography,* **43,** 1344-1352.

Franks S.W. & Kuczera G. (2002) Flood frequency analysis: Evidence and implications of secular climate variability, New South Wales. *Water Resources Research,* **38**.

Freeman C. & Lock M.A. (1995) The biofilm polysaccharide matrix: A buffer against changing organic substrate supply? *Limnology and Oceanography,* **40,** 273-278.

Freeman M.C., Pringle C.M. & Jackson C.R. (2007) Hydrologic connectivity and the contribution of stream headwaters to ecological integrity at regional scales. *Journal of the American Water Resources Association,* **43,** 5-14.

Friedl G. & Wuest A. (2002) Disrupting biogeochemical cycles - Consequences of damming. *Aquatic Sciences,* **64,** 55-65.

Gabor R., Baker A., Mcknight D. & Miller M. (2014) Fluorescence Indices and Their Interpretation. In: *Aquatic Organic Matter Fluorescence.* (Eds C. Pg & L. J & B. A & R. Dm & S. Rgm), pp. 303 - 338. Cambridge University Press.

Giles C.D., Lee L.G., Cade-Menun B.J., Hill J.E., Isles P.D., Schroth A.W. & Druschel G.K. (2015) Characterization of Organic Phosphorus Form and Bioavailability in Lake Sediments using P Nuclear Magnetic Resonance and Enzymatic Hydrolysis. *J Environ Qual,* **44,** 882-894.

Giling D.P., Mac Nally R. & Thompson R.M. (2015) How Might Cross-System Subsidies in Riverine Networks be Affected by Altered Flow Variability? *Ecosystems,* **18,** 1151-1164.

Gillespie B.R., Desmet S., Kay P., Tillotson M.R. & Brown L.E. (2015) A critical analysis of regulated river ecosystem responses to managed environmental flows from reservoirs. *Freshwater Biology,* **60,** 1365-2427.

Gilligan D. & Williams S. (2008) Changes in fish assemblages after the first flow releases to the Snowy River downstream of Jindabyne Dam. In: *Snowy River Flow Response Monitoring*. Department of Water and Energy, Sydney.

Goodman K.J., Baker M.A. & Wurtsbaugh W.A. (2011) Lakes as buffers of stream dissolved organic matter (DOM) variability: Temporal patterns of DOM characteristics in mountain stream-lake systems. *Journal of Geophysical Research-Biogeosciences,* **116**.

Growns I. (2016) The implementation of an environmental flow regime results in ecological recovery of regulated rivers. *Restoration Ecology,* **24,** 406-414.

Growns I. & Reinfelds I. (2014) Environmental flow management using transparency and translucency rules. *Marine and Freshwater Research,* **65,** 667-673.

Growns I., Reinfelds I., Williams S. & Coade G. (2009) Longitudinal effects of a water supply reservoir (Tallowa Dam) on downstream water quality, substrate and riffle macroinvertebrate assemblages in the Shoalhaven River, Australia. *Marine and Freshwater Research,* **60,** 594-606.

Guillemette F. & Del Giorgio P.A. (2011) Reconstructing the various facets of dissolved organic carbon bioavailability in freshwater ecosystems. *Limnology and Oceanography,* **56,** 734-748.

Haas B.J., Gevers D., Earl A.M., Feldgarden M., Ward D.V., Giannoukos G., Ciulla D., Tabbaa D., Highlander S.K. & Sodergren E. (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome research,* **21,** 494-504.

Hadwen W.L., Fellows C.S., Westhorpe D.P., Rees G.N., Mitrovic S.M., Taylor B., Baldwin D.S., Silvester E. & Croome R. (2010) Longitudinal trends in river functioning: patterns of nutrient and carbon processing in three Australian rivers. *River Research and Applications,* **26,** 1129-1152.

Hall E.K., Besemer K., Kohl L., Preiler C., Riedel K., Schneider T., Wanek W. & Battin T.J. (2012) Effects of resource chemistry on the composition and function of stream hyporheic biofilms. *Frontiers in Microbiology,* **3,** 14.

Hall R.O. & Meyer J.L. (1998) The trophic significance of bacteria in a detritus-based stream food web. *Ecology,* **79,** 1995-2012.

Henson S.S., Ahearn D.S., Dahlgren R.A., Van Nieuwenhuyse E., Tate K.W. & Fleenor W.E. (2007) Water quality response to a pulsed-flow event on the Mokelumne River, California. *River Research and Applications,* **23,** 185-200.

Hinton M.J., Schiff S.L. & English M.C. (1997) The significance of storms for the concentration and export of dissolved organic carbon from two Precambrian Shield catchments. *Biogeochemistry,* **36,** 67-88.

Hitchcock J.N. & Mitrovic S.M. (2013) Different resource limitation by carbon, nitrogen and phosphorus between base flow and high flow conditions for estuarine bacteria and phytoplankton. *Estuarine, Coastal and Shelf Science,* **135,** 106-115.

Hitchcock J.N. & Mitrovic S.M. (2015) After the flood: changing dissolved organic carbon bioavailability and bacterial growth following inflows to estuaries. *Biogeochemistry,* **124,** 219-233.

Hitchcock J.N., Mitrovic S.M., Hadwen W.L., Roelke D.L., Growns I.O. & Rohlfs A.-M. (2016) Terrestrial dissolved organic carbon subsidizes estuarine zooplankton: An in situ mesocosm study. *Limnology and Oceanography,* **61,** 254-267.

Hood E., Mcknight D.M. & Williams M.W. (2003) Sources and chemical character of dissolved organic carbon across an alpine/subalpine ecotone, Green Lakes Valley, Colorado Front Range, United States. *Water Resources Research,* **39**.

Hood E., Williams M.W. & Mcknight D.M. (2005) Sources of dissolved organic matter (DOM) in a Rocky Mountain stream using chemical fractionation and stable isotopes. *Biogeochemistry,* **74,** 231-255.

Hope D., Billett M.F. & Cresser M.S. (1994) A review of the export of carbon in river water - fluxes and processes. *Environmental Pollution,* **84,** 301-324.

Hoppe G.H. (1993) Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurements of bacteria. In: *Handbook of methods in Aquatic Microbial Ecology.* (Eds P.F. Kemp & E.F. Sherr & E.B. Sherr & J.J. Cole), pp. 423-432. CRC Press, LLC., Boca Raton.

Hornberger G.M., Bencala K.E. & Mcknight D.M. (1994) Hydrological controls on dissolved organic carbon during snowmelt in the Snake River near Montezuma, Colorado. *Biogeochemistry,* **25,** 147-165.

Howitt J.A., Baldwin D.S., Rees G.N. & Hart B.T. (2008) Photodegradation, interaction with iron oxides and bioavailability of dissolved organic matter from forested floodplain sources. *Marine and Freshwater Research,* **59,** 780-791.

Inamdar S. (2011) The use of geochemical mixing models to derive runoff sources and hydrologic flow paths. In: *Forest Hydrology and Biogeochemistry: Synthesis of Research and Future Directions.* (Eds D.F. Levia & D. Carlyle-Moses & T. Tanaka), pp. 163-. Ecological Studies 216. Springer, New York.

Inamdar S., Singh S., Dutta S., Levia D., Mitchell M., Scott D., Bais H. & Mchale P. (2011) Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. *Journal of Geophysical Research-Biogeosciences,* **116,** 23.

Jackson C.R., Tyler H.L. & Millar J.J. (2013) Determination of Microbial Extracellular Enzyme Activity in Waters, Soils, and Sediments using High Throughput Microplate Assays. *Journal of Visualized Experiments : JoVE***,** 50399.

Jaffe R., Mcknight D., Maie N., Cory R., Mcdowell W.H. & Campbell J.L. (2008) Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. *Journal of Geophysical Research-Biogeosciences,* **113**.
Jakob C., Robinson C.T. & Uehlinger U. (2003) Longitudinal effects of experimental floods on stream benthos downstream from a large dam. *Aquatic Sciences,* **65,** 223-231.
Johnson L., Royer T., Edgerton J. & Leff L. (2012) Manipulation of the Dissolved Organic Carbon Pool in an Agricultural Stream: Responses in Microbial Community Structure, Denitrification, and Assimilatory Nitrogen Uptake. *Ecosystems,* **15,** 1027-1038.
Jonsson M. & Stenroth K. (2016) True autochthony and allochthony in aquatic–terrestrial resource fluxes along a landuse gradient. *Freshwater Science,* **35,** 882-894.
Jordan T.E., Correll D.L. & Weller D.E. (1997) Relating nutrient discharges from watersheds to land use and streamflow variability. *Water Resources Research,* **33,** 2579-2590.
Judd K.E., Crump B.C. & Kling G.W. (2006) Variation in dissolved organic matter controls bacterial production and community composition. *Ecology,* **87,** 2068-2079.
Junk J.W., Bayley P.B. & Sparks R.E. (1989) The flood pulse concept in river–floodplain systems. In: *Proceedings of the International Large River Symposium.* (Ed D.P. Dodge), p. 106. Canadian Journal of Fisheries and Aquatic Sciencecs Special Publication.
Kaartokallio H., Asmala E., Autio R. & Thomas D.N. (2016) Bacterial production, abundance and cell properties in boreal estuaries: relation to dissolved organic matter quantity and quality. *Aquatic Sciences,* **78,** 525-540.
Kamjunke N., Herzsprung P. & Neu T.R. (2015) Quality of dissolved organic matter affects planktonic but not biofilm bacterial production in streams. *Science of the Total Environment,* **506,** 353-360.
Kamjunke N., Oosterwoud M.R., Herzsprung P. & Tittel J. (2016) Bacterial production and their role in the removal of dissolved organic matter from tributaries of drinking water reservoirs. *Science of the Total Environment,* **548,** 51-59.
Kelly H.a.W., Rosi-Marshall E.J., Kennedy T.A., Hall R.O., Jr., Cross W.F. & Baxter C.V. (2013) Macroinvertebrate diets reflect tributary inputs and turbidity-driven changes in food availability in the Colorado River downstream of Glen Canyon Dam. *Freshwater Science,* **32,** 397-410.
Kiffney P.M., Greene C.M., Hall J.E. & Davies J.R. (2006) Tributary streams create spatial discontinuities in habitat, biological productivity, and diversity in mainstem rivers. *Canadian Journal of Fisheries and Aquatic Sciences,* **63,** 2518-2530.
King A.J., Ward K.A., O'connor P., Green D., Tonkin Z. & Mahoney J. (2010) Adaptive management of an environmental watering event to enhance native fish spawning and recruitment. *Freshwater Biology,* **55,** 17-31.
King J. & Louw D. (1998) Instream flow assessments for regulated rivers in South Africa using the Building Block Methodology. *Aquatic Ecosystem Health and Management,* **1,** 109-124.
Kirchman D.L. (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: *Handbook of Methods in Aquatic Microbial Ecology.* (Eds Kemp P.F. & Sherr B.F. & Sherr E.B. & Cole J. J.), pp. 509-512. Lewis Publishers, Florida.
Kirchman D.L., Dittel A.I., Findlay S.E.G. & Fischer D. (2004) Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. *Aquatic Microbial Ecology,* **35,** 243-257.
Klamt M., Davis J.A., Thompson R.M., Marchant R. & Grant T.R. (2015) Trophic relationships of the platypus: insights from stable isotope and cheek pouch dietary analyses. *Marine and Freshwater Research***,** -.
Kobayashi Y., Kim C., Yoshimizu C., Kohzu A., Tayasu I. & Nagata T. (2009) Longitudinal changes in bacterial community composition in river epilithic biofilms: influence of nutrients and organic matter. *Aquatic Microbial Ecology,* **54,** 135-152.

Konrad C.P., Olden J.D., Lytle D.A., Melis T.S., Schmidt J.C., Bray E.N., Freeman M.C., Gido K.B., Hemphill N.P., Kennard M.J., Mcmullen L.E., Mims M.C., Pyron M., Robinson C.T. & Williams J.G. (2011) Large-scale Flow Experiments for Managing River Systems. *Bioscience,* **61,** 948-959.

Kothawala D.N., Murphy K.R., Stedmon C.A., Weyhenmeyer G.A. & Tranvik L.J. (2013) Inner filter correction of dissolved organic matter fluorescence. *Limnology and Oceanography: Methods,* **11,** 616-630.

Kreutzweiser D.P. & Capell S.S. (2003) Benthic microbial utilization of differential dissolved organic matter sources in a forest headwater stream. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere,* **33,** 1444-1451.

Larson J.H., Frost P.C., Zheng Z.Y., Johnston C.A., Bridgham S.D., Lodge D.M. & Lamberti G.A. (2007) Effects of upstream lakes on dissolved organic matter in streams. *Limnology and Oceanography,* **52,** 60-69.

Lawaetz A.J. & Stedmon C.A. (2009) Fluorescence Intensity Calibration Using the Raman Scatter Peak of Water. *Applied Spectroscopy,* **63,** 936-940.

Lean D. (1998) Attenuation of Solar Radiation in Humic Waters. In: *Aquatic Humic Substances: Ecology and Biogeochemistry.* (Eds D.O. Hessen & L.J. Tranvik), pp. 109-124. Springer Berlin Heidelberg, Berlin, Heidelberg.

Lear G., Anderson M.J., Smith J.P., Boxen K. & Lewis G.D. (2008) Spatial and temporal heterogeneity of the bacterial communities in stream epilithic biofilms. *Fems Microbiology Ecology,* **65,** 463-473.

Lennon J.T. & Cottingham K.L. (2008) Microbial productivity in variable resource environments. *Ecology,* **89,** 1001-1014.

Lennon J.T. & Pfaff L.E. (2005) Source and supply of terrestrial organic matter affects aquatic microbial metabolism. *Aquatic Microbial Ecology,* **39,** 107-119.

Levi P.S., Tank J.L., Tiegs S.D., Chaloner D.T. & Lamberti G.A. (2013) Biogeochemical transformation of a nutrient subsidy: salmon, streams, and nitrification. *Biogeochemistry,* **113,** 643-655.

Lindeman R.L. (1942) The trophic-dynamic aspect of ecology. *Ecology,* **23,** 399-418.

Lock M.A. & John P.H. (1979) The effect of flow patterns on uptake of phosphorus by river periphyton. *Limnology and Oceanography,* **24,** 376-383.

Lock M.A., Wallace R.R., Costerton J.W., Ventullo R.M. & Charlton S.E. (1984) River Epilithon: Toward a Structural-Functional Model. *Oikos,* **42,** 10-22.

Ludwig W., Probst J.L. & Kempe S. (1996) Predicting the oceanic input of organic carbon by continental erosion. *Global Biogeochemical Cycles,* **10,** 23-41.

Lutz B.D., Bernhardt E.S., Roberts B.J. & Mulholland P.J. (2011) Examining the coupling of carbon and nitrogen cycles in Appalachian streams: the role of dissolved organic nitrogen. *Ecology,* **92,** 720-732.

Marcarelli A.M., Baxter C.V., Mineau M.M. & Hall R.O. (2011) Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology,* **92,** 1215-1225.

Marin-Spiotta E., Gruley K.E., Crawford J., Atkinson E.E., Miesel J.R., Greene S., Cardona-Correa C. & Spencer R.G.M. (2014) Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries. *Biogeochemistry,* **117,** 279-297.

Mcclain M.E., Boyer E.W., Dent C.L., Gergel S.E., Grimm N.B., Groffman P.M., Hart S.C., Harvey J.W., Johnston C.A., Mayorga E., Mcdowell W.H. & Pinay G. (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems,* **6,** 301-312.

Mcdonald D., Price M.N., Goodrich J., Nawrocki E.P., Desantis T.Z., Probst A., Andersen G.L., Knight R. & Hugenholtz P. (2012) An improved Greengenes taxonomy with explicit

ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J,* **6,** 610-618.
Mcdonald S., Bishop A.G., Prenzler P.D. & Robards K. (2004) Analytical chemistry of freshwater humic substances. *Analytica Chimica Acta,* **527,** 105-124.
Mcdonald S., Pringle J.M., Prenzler P.D., Bishop A.G. & Robards K. (2007) Bioavailability of dissolved organic carbon and fulvic acid from an Australian floodplain river and billabong. *Marine and Freshwater Research,* **58,** 222-231.
Mcdowell W.H., Zsolnay A., Aitkenhead-Peterson J.A., Gregorich E.G., Jones D.L., Jodemann D., Kalbitz K., Marschner B. & Schwesig D. (2006) A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. *Soil Biology & Biochemistry,* **38,** 1933-1942.
Mcglynn B.L. & Mcdonnell J.J. (2003) Role of discrete landscape units in controlling catchment dissolved organic carbon dynamics. *Water Resources Research,* **39,** 18.
Mcknight D.M., Boyer E.W., Westerhoff P.K., Doran P.T., Kulbe T. & Andersen D.T. (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography,* **46,** 38-48.
Mcknight D.M., Smith R.L., Harnish R.A., Miller C.L. & Bencala K.E. (1993) Seasonal relationships between planktonic micro-organisms and dissolved organic material in an alpine stream. *Biogeochemistry,* **21,** 39-59.
Meyer J.L. (1994) The microbial loop in flowing waters. *Microbial Ecology,* **28,** 195-199.
Miller M.P. (2012) The influence of reservoirs, climate, land use and hydrologic conditions on loads and chemical quality of dissolved organic carbon in the Colorado River. *Water Resources Research,* **48,** 15.
Minor E. & Stephens B. (2008) Dissolved organic matter characteristics within the Lake Superior watershed. *Organic Geochemistry,* **39,** 1489-1501.
Moran M.A. & Covert J.S. (2003) Photochemically Mediated Linkages between Dissolved Organic Matter and Bacterioplankton. In: *Interactivity of Dissolved Organic Matter.* (Eds S. Findlay & R.L. Sinsabaugh), pp. 244-259. Elsevier Science, USA.
Moran N.P., Ganf G.G., Wallace T.A. & Brookes J.D. (2014) Flow variability and longitudinal characteristics of organic carbon in the Lachlan River, Australia. *Marine and Freshwater Research,* **65,** 50-58.
Moreira-Turcq P., Seyler P., Guyot J.L. & Etcheber H. (2003) Exportation of organic carbon from the Amazon River and its main tributaries. *Hydrological Processes,* **17,** 1329-1344.
Mulholland P.J. (1997) Dissolved organic matter concentration and flux in streams. *Journal of the North American Benthological Society,* **16,** 131-141.
Neely R.K. & Wetzel R.G. (1995) Simultaneous use of 14C and 3H to determine autotrophic production and bacterial protein production in periphyton. *Microbial Ecology,* **30,** 227-237.
Nelson R.W., Dwyer J.R. & Greenberg W.E. (1987) Regulated flushing in a gravel-bed river for channel habitat maintenance - a Trinity River fisheries case-study. *Environmental Management,* **11,** 479-493.
Newbold J.D., Elwood J.W., Oneill R.V. & Vanwinkle W. (1981) Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences,* **38,** 860-863.
Newman M.M., Liles M.R. & Feminella J.W. (2015) Litter Breakdown and Microbial Succession on Two Submerged Leaf Species in a Small Forested Stream. *PLoS ONE,* **10,** e0130801.
Nguyen H.V.M., Hur J. & Shin H.S. (2010) Changes in Spectroscopic and Molecular Weight Characteristics of Dissolved Organic Matter in a River During a Storm Event. *Water Air and Soil Pollution,* **212,** 395-406.
Nguyen M.L., Westerhoff P., Baker L., Hu Q., Esparza-Soto M. & Sommerfeld M. (2005) Characteristics and reactivity of algae-produced dissolved organic carbon. *Journal of Environmental Engineering-Asce,* **131,** 1574-1582.

Nilsson C., Reidy C.A., Dynesius M. & Revenga C. (2005) Fragmentation and flow regulation of the world's large river systems. *Science,* **308,** 405-408.
O'connell M., Baldwin D.S., Robertson A.I. & Rees G. (2000) Release and bioavailability of dissolved organic matter from floodplain litter: influence of origin and oxygen levels. *Freshwater Biology,* **45,** 333-342.
O'connor B.L., Harvey J.W. & Mcphillips L.E. (2012) Thresholds of flow-induced bed disturbances and their effects on stream metabolism in an agricultural river. *Water Resources Research,* **48**.
Ohno T. (2002) Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science & Technology,* **36,** 742-746.
Palmer M.A., Ambrose R.F. & Poff N.L. (1997) Ecological theory and community restoration ecology. *Restoration Ecology,* **5,** 291-300.
Patten D.T., Harpman D.A., Voita M.I. & Randle T.J. (2001) A managed flood on the Colorado River: Background, objectives, design, and implementation. *Ecological Applications,* **11,** 635-643.
Petrone K.C., Richards J.S. & Grierson P.F. (2009) Bioavailability and composition of dissolved organic carbon and nitrogen in a near coastal catchment of south-western Australia. *Biogeochemistry,* **92,** 27-40.
Phinney H.K. & Mcintire C.D. (1965) Effect of temperature on metabolism of periphyton communities developed in laboratory streams. *Limnology and Oceanography,* **10,** 341-345.
Platikanov S., Tauler R., Rodrigues P., Antunes M.C.G., Pereira D. & Da Silva J. (2010) Factorial analysis of the trihalomethane formation in the reaction of colloidal, hydrophobic, and transphilic fractions of DOM with free chlorine. *Environmental Science and Pollution Research,* **17,** 1389-1400.
Poff N.L., Allan J.D., Bain M.B., Karr J.R., Prestegaard K.L., Richter B.D., Sparks R.E. & Stromberg J.C. (1997) The natural flow regime. *Bioscience,* **47,** 769-784.
Poff N.L., Olden J.D., Merritt D.M. & Pepin D.M. (2007) Homogenization of regional river dynamics by dams and global biodiversity implications. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 5732-5737.
Poff N.L. & Zimmerman J.K.H. (2010) Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. *Freshwater Biology,* **55,** 194-205.
Pringle C. (2003) What is hydrologic connectivity and why is it ecologically important? *Hydrological Processes,* **17,** 2685-2689.
Quilbe R., Rousseau A.N., Duchemin M., Poulin A., Gangbazo G. & Villeneuve J.P. (2006) Selecting a calculation method to estimate sediment and nutrient loads in streams: Application to the Beaurivage River (Quebec, Canada). *Journal of Hydrology,* **326,** 295-310.
Raymond P.A. & Saiers J.E. (2010) Event controlled DOC export from forested watersheds. *Biogeochemistry,* **100,** 197-209.
Rees G.N., Beattie G., Bowen P.M. & Hart B.T. (2005) Heterotrophic bacterial production in the lower Murray River, south-eastern Australia. *Marine and Freshwater Research,* **56,** 835-841.
Rees G.N., Bowen P.M. & Watson G.O. (2005) Variability in benthic respiration in three southeastern Australian lowland rivers. *River Research and Applications,* **21,** 1147-1156.
Regnier P., Friedlingstein P., Ciais P., Mackenzie F.T., Gruber N., Janssens I.A., Laruelle G.G., Lauerwald R., Luyssaert S., Andersson A.J., Arndt S., Arnosti C., Borges A.V., Dale A.W., Gallego-Sala A., Godderis Y., Goossens N., Hartmann J., Heinze C., Ilyina T., Joos F., Larowe D.E., Leifeld J., Meysman F.J.R., Munhoven G., Raymond P.A., Spahni R.,

Suntharalingam P. & Thullner M. (2013) Anthropogenic perturbation of the carbon fluxes from land to ocean. *Nature Geoscience,* **6,** 597-607.
Reich P. & Lake P.S. (2015) Extreme hydrological events and the ecological restoration of flowing waters. *Freshwater Biology,* **60,** 2639-2652.
Reid M.A., Thoms M.C. & Dyer F.J. (2006) Effects of spatial and temporal variation in hydraulic conditions on metabolism in cobble biofilm communities in an Australian upland stream. *Journal of the North American Benthological Society,* **25,** 756-767.
Reinfelds I., Williams S., Russell M. & Haeusler T. (2013) Scaling environmental flow releases in the Snowy River to unregulated snowmelt rivers of the Snowy Mountains. In: *Snowy Flow Response Monitoring and Modelling Program*. NSW Office of Water, Sydney.
Rice S.P., Kiffney P., Greene C. & Pess G.R. (2008) The ecological importance of tributaries and confluences. In: *River Confluences, Tributaries and the Fluvial Network.* (Ed R.a.G.R.B.L. Rice S.P.), pp. 209-242. John Wiley & Sons.
Richter B.D. & Thomas G.A. (2007) Restoring environmental flows by modifying dam operations. *Ecology and Society,* **12**.
Roberts B., Mulholland P. & Hill W. (2007) Multiple Scales of Temporal Variability in Ecosystem Metabolism Rates: Results from 2 Years of Continuous Monitoring in a Forested Headwater Stream. *Ecosystems,* **10,** 588-606.
Robinson C.T. & Uehlinger U. (2003) Using artificial floods for restoring river integrity. *Aquatic Sciences,* **65,** 181-182.
Robinson C.T. & Uehlinger U. (2008) Experimental floods cause ecosystem regime shift in a regulated river. *Ecological Applications,* **18,** 511-526.
Rohlfs A.-M., Mitrovic S.M., Williams S., Hitchcock J.N. & Rees G.N. (2016) Dissolved organic carbon delivery from managed flow releases in a montane snowmelt river. *Aquatic Sciences***,** 1-15.
Rohlfs A., Mitrovic S.M., Williams S. & Coleman D. (2015) Can tributary in-flows improve DOC regime recovery in a snowmelt river regulated by a large reservoir? *Marine and Freshwater Research,* **In publication**.
Romani A.M. & Sabater S. (1999) Effect of primary producers on the heterotrophic metabolism of a stream biofilm. *Freshwater Biology,* **41,** 729-736.
Romani A.M. & Sabater S. (2001) Structure and activity of rock and sand biofilms in a Mediterranean stream. *Ecology,* **82,** 3232-3245.
Sadiq R. & Rodriguez M.J. (2004) Disinfection by-products (DBPs) in drinking water and predictive models for their occurrence: a review. *Science of the Total Environment,* **321,** 21-46.
Sanderman J., Lohse K.A., Baldock J.A. & Amundson R. (2009) Linking soils and streams: Sources and chemistry of dissolved organic matter in a small coastal watershed. *Water Resources Research,* **45**.
Schindler J. & Krabbenhoft D. (1998) The hyporheic zone as a source of dissolved organic carbon and carbon gases to a temperate forested stream. *Biogeochemistry,* **43,** 157-174.
Selinummi J., Seppala J., Yli-Harja O. & Puhakka J.A. (2005) Software for quantification of labeled bacteria from digital microscope images by automated image analysis. *Biotechniques,* **39,** 859-863.
Sertic Peric M., Jolidon C., Uehlinger U. & Robinson C.T. (2015) Long-term ecological patterns of alpine streams: An imprint of glacial legacies. *Limnology and Oceanography,* **60,** 992-1007.
Sieczko A. & Peduzzi P. (2014) Origin, enzymatic response and fate of dissolved organic matter during flood and non-flood conditions in a river-floodplain system of the Danube (Austria). *Aquatic Sciences,* **76,** 115-129.

Sinsabaugh R.L. & Foreman C.M. (2003) Integrating Dissolved Organic Matter Metabolism and Microbial Diversity: An Overview of Conceptual Models. In: *Interactivity of Dissolved Organic Matter.* (Eds S. Findlay & R.L. Sinsabaugh), pp. 426-454. Academic Press, California.

Sobczak W.V., Findlay S. & Dye S. (2003) Relationships between DOC Bioavailability and Nitrate Removal in an Upland Stream: An Experimental Approach. *Biogeochemistry,* **62,** 309-327.

Stanford J.A. & Ward J.V. (2001) Revisiting the serial discontinuity concept. *Regulated Rivers-Research & Management,* **17,** 303-310.

Stanley E.H., Powers S.M., Lottig N.R., Buffam I. & Crawford J.T. (2012) Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: is there a role for DOC management? *Freshwater Biology,* **57,** 26-42.

Stedmon C.A. & Cory R.M. (2014) Biological origins and fate of fluorescent dissolved organic matter in aquatic environments. In: *Aquatic Organic Matter Fluorescence.* (Eds P.G. Coble & J. Lead & A. Baker). Cambridge University Press, New York.

Stedmon C.A. & Markager S. (2005) Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. *Limnology and Oceanography,* **50,** 1415-1426.

Sun L., Perdue E.M., Meyer J.L. & Weis J. (1997) Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. *Limnology and Oceanography,* **42,** 714-721.

Sutherland I.W. (2001) The biofilm matrix - an immobilized but dynamic microbial environment. *Trends in Microbiology,* **9,** 222-227.

Tank J.L., Rosi-Marshall E.J., Griffiths N.A., Entrekin S.A. & Stephen M.L. (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society,* **29,** 118-146.

Terry J.A., Benskin C., Eastoe E.F. & Haygarth P.M. (2014) Temporal dynamics between cattle in-stream presence and suspended solids in a headwater catchment. *Environmental Science: Processes & Impacts,* **16,** 1570-1577.

Thurman E.M. (1985) *Organic Geochemistry of Natural Water,* Nijhoff, Dordrecht.

Tranvik L.J., Downing J.A., Cotner J.B., Loiselle S.A., Striegl R.G., Ballatore T.J., Dillon P., Finlay K., Fortino K., Knoll L.B., Kortelainen P.L., Kutser T., Larsen S., Laurion I., Leech D.M., Mccallister S.L., Mcknight D.M., Melack J.M., Overholt E., Porter J.A., Prairie Y., Renwick W.H., Roland F., Sherman B.S., Schindler D.W., Sobek S., Tremblay A., Vanni M.J., Verschoor A.M., Von Wachenfeldt E. & Weyhenmeyer G.A. (2009) Lakes and reservoirs as regulators of carbon cycling and climate. *Limnology and Oceanography,* **54,** 2298-2314.

Uehlinger U., Kawecka B. & Robinson C.T. (2003) Effects of experimental floods on periphyton and stream metabolism below a high dam in the Swiss Alps (River Spol). *Aquatic Sciences,* **65,** 199-209.

Ulseth A.J. & Hall Jr R.O. (2015) Dam tailwaters compound the effects of reservoirs on the longitudinal transport of organic carbon in an arid river. *Biogeosciences,* **12,** 4345-4359.

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980) River continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences,* **37,** 130-137.

Verdon D.C., Wyatt A.M., Kiem A.S. & Franks S.W. (2004) Multidecadal variability of rainfall and streamflow: Eastern Australia. *Water Resources Research,* **40**.

Wagner K., Bengtsson M.M., Besemer K., Sieczko A., Burns N.R., Herberg E.R. & Battin T.J. (2014) Functional and Structural Responses of Hyporheic Biofilms to Varying Sources of Dissolved Organic Matter. *Applied and Environmental Microbiology,* **80,** 6004-6012.

Wagner K., Besemer K., Burns N.R., Battin T.J. & Bengtsson M.M. (2015) Light availability affects stream biofilm bacterial community composition and function, but not diversity. *Environmental Microbiology,* **17,** 5036-5047.

Walker K.F. & Thoms M.C. (1993) Environmental effects of flow regulation on the lower River Murray, Australia. *Regulated Rivers-Research & Management,* **8,** 103-119.

Wallis E., Mac Nally R. & Lake S. (2009) Do tributaries affect loads and fluxes of particulate organic matter, inorganic sediment and wood? Patterns in an upland river basin in south-eastern Australia. *Hydrobiologia,* **636,** 307-317.

Ward J.V. & Stanford J.A. (1983) The Serial Discontinuity concept of Lotic Ecosystems. In: *Dynamics of Lotic Ecosystems.* (Eds T.D. Fontaine & S.M. Bartell), pp. 29-42. Ann Arbor Scienec Publishers, Michigan.

Ward J.V. & Stanford J.A. (1995) ECOLOGICAL CONNECTIVITY IN ALLUVIAL RIVER ECOSYSTEMS AND ITS DISRUPTION BY FLOW REGULATION. *Regulated Rivers-Research & Management,* **11,** 105-119.

Webster J.R., Waide J.B. & Patten B.C. (1975) Nutrient Cycling and the Stability of Ecosystems. In: *Mineral Cycling in Southeastern Ecosystems.* (Eds F.G. Howell & J.B. Gentry & M.H. Smith), pp. 1-27. ERDA Symposium Series CONF-740513. NTIS, Springfield, VA.

Westhorpe D.P. & Mitrovic S.M. (2012) Dissolved organic carbon mobilisation in relation to variable discharges and environmental flows in a highly regulated lowland river. *Marine and Freshwater Research,* **63,** 1218-1230.

Wetzel R.G. (1995) Death, detritus and energy-flow in aquatic ecosystems. *Freshwater Biology,* **33,** 83-89.

Wetzel R.G. (2005) Periphyton in the Aquatic Ecosystem and Food Webs. In: *Periphyton Ecology Exploitation and Management.* (Ed V.M.C.J. Azim M.E., Van Dam A.A. And Beveridge M.C.M.). CABI, Oxfordshire.

Whitworth K.L., Baldwin D.S. & Kerr J.L. (2012) Drought, floods and water quality: Drivers of a severe hypoxic blackwater event in a major river system (the southern Murray–Darling Basin, Australia). *Journal of Hydrology,* **450–451,** 190-198.

Wilcox H.S., Wallace J.B., Meyer J.L. & Benstead J.P. (2005) Effects of labile carbon addition on a headwater stream food web. *Limnology and Oceanography,* **50,** 1300-1312.

Williams S. (2015a) Revised strategy for the Snowy River Increased Flows, 2015-16. In: *Snowy Flow Response Modelling and Modelling program*. NSW Office of Water, Sydney.

Williams S. (2015b) Strategy for the Snowy River Increased Flows, 2015-16. In: *Snowy Flow Response Modelling and Modelling program*, Sydney.

Wilson H., Saiers J., Raymond P. & Sobczak W. (2013) Hydrologic Drivers and Seasonality of Dissolved Organic Carbon Concentration, Nitrogen Content, Bioavailability, and Export in a Forested New England Stream. *Ecosystems,* **16,** 604-616.

Wilson H.F., Raymond P.A., Saiers J.E., Sobczak W.V. & Xu N. (2016) Increases in humic and bioavailable dissolved organic matter in a forested New England headwater stream with increasing discharge. *Marine and Freshwater Research,* **67,** 1279-1292.

Wipfli M.S., Richardson J.S. & Naiman R.J. (2007) Ecological linkages between headwaters and downstream ecosystems: Transport of organic matter, invertebrates, and wood down headwater channels. *Journal of the American Water Resources Association,* **43,** 72-85.

Wong J.C.Y. & Williams D.D. (2010) Sources and seasonal patterns of dissolved organic matter (DOM) in the hyporheic zone. *Hydrobiologia,* **647,** 99-111.

Yang L.H., Bastow J.L., Spence K.O. & Wright A.N. (2008) What can we learn from resource pulses? *Ecology,* **89,** 621-634.

Yang L.Y., Guo W.D., Chen N.W., Hong H.S., Huang J.L., Xu J. & Huang S.Y. (2013) Influence of a summer storm event on the flux and composition of dissolved organic matter in a subtropical river, China. *Applied Geochemistry,* **28,** 164-171.

Young R.G., Matthaei C.D. & Townsend C.R. (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society,* **27,** 605-625.

Ziegler S.E., Lyon D.R. & Townsend S.L. (2009) Carbon release and cycling within epilithic biofilms in two contrasting headwater streams. *Aquatic Microbial Ecology,* **55,** 285-300.

Zoppini A., Amalfitano S., Fazi S. & Puddu A. (2010) Dynamics of a benthic microbial community in a riverine environment subject to hydrological fluctuations (Mulargia River, Italy). *Hydrobiologia,* **657,** 37-51.