Chapter 5: Sources of dissolved organic matter and extracellular enzyme activity in vegetated and non-vegetated reaches.

5.1 Introduction

Dissolved organic matter (DOM) is the largest pool of organic carbon within most aquatic ecosystems (Findlay & Sinsabaugh 1999). DOM is comprised of a complex mixture of dissolved organic carbon (DOC) substrates and other nutrients, and provides a major source of energy to heterotrophic communities (Bott et al. 1984, Findlay & Sinsabaugh 1999). A number of other ecosystem processes such as nutrient retention and autotrophic production can also be facilitated and regulated by the quantity and quality of DOC (see Chapters 3 and 4, Findlay & Sinsabaugh 1999). The quality of DOC describes its' chemical composition and has been related to the susceptibility of DOC to enzymatic degradation by heterotrophs (Strauss & Lamberti 2002). DOC quality is largely controlled through the sources of DOC within the system (Findlay & Sinsabaugh 1999) and environmental conditions (Wetzel et al. 1995, Howitt et al. 2008). Within most aquatic systems, the majority of DOC is derived from allochthonous sources which yield high-molecular weight compounds compared to autochthonous sources (McDonald et al. 2004). Coarse, fine and particulate fractions of organic matter undergo physical fragmentation and mineralisation to produce DOC. However, because DOC compounds can be transported large distances (e.g. from floodplains to the main channel, Robertson et al. 1999), the visual and quantitative analysis of the coarse, fine and particulate organic matter fractions present within reaches may not be indicative of the sources or quality of DOC within the overlying water column.

Extracellular enzymes

The uptake of DOC and other energy sources by heterotrophic microbes is generally restricted to low-molecular compounds (i.e. less than 600 daltons) and controlled by the pore-size of the cellular membrane of the consumer (Weiss et al. 1991). Heterotrophic microbes are able to assimilate previously unavailable compounds through the release of extracellular enzymes (proteins predominantly made of carbon and nitrogen) that catalyse specific hydrolytic reactions. During hydrolysis, larger compounds are chemically cleaved into more labile forms

allowing the compounds to be transported through the cellular membrane (Chrost 1991). The extracellular enzymes can be passively or actively released from the microbial cellular membrane. However, the release of most extracellular enzymes is energetically expensive and subsequently most enzymes are withheld until the particular compound is required or becomes available within the system (Arnosti 2003). To acquire carbon and nutrients in the most optimal energetically efficient way, heterotrophic microbes will release suites of enzymes that reflect their specific resource requirements, and the composition and quality of resources available within the environment (Sinsabaugh & Moorehead 1994, Sinsabaugh et al. 2002).

The composition of extracellular enzymes that are attached to benthic substrates and other surfaces, as well as within the water column, have been used to identify the composition and utilisation of DOC in a wide variety of aquatic environments including wetlands (Freeman et al. 1995, Burns & Ryder 2001), floodplain soils (Wilson et al. 2011), freshwater (Chappell & Goulder 1995, Harbott & Grace 2001, Findlay et al. 2003, Ylla et al. 2010) and estuarine systems (Sinsabaugh & Findlay 1995). Patterns of extracellular enzyme activity (EEA) have also been used to model relationships between heterotrophs and autotrophs within biofilms and the water column (Foreman et al. 1998, Artigas et al. 2009).

Analysis of the rate of EEA that are specific to nitrogen and phosphorous uptake/acquisition have also been used to determine heterotrophic nutrient limitation within aquatic systems (Hill et al. 2006, Hill et al. 2010a, Hill et al. 2010b, Rier et al. 2011). The method primarily uses the balance of C-hydrolyisng/acquiring enzymes relative to nutrient (N and P) acquiring enzymes to determine the limiting nutrients, and has recently been termed ‘ecoenzymatic stoichiometry’ (Sinsabaugh et al. 2010).

Anthropogenic disturbances

The removal of riparian vegetation can alter the main sources of DOC through a variety of mechanisms which may lead to a reduction in DOC quality (i.e. higher concentrations of low-molecular weight DOC compounds). For example, the quantity of allochthonous organic matter directly imported to streams may be reduced (Campbell et al. 1992, Sabater et al. 2000, Reid et al. 2008), while an increase in the amount of photosynthetic active radiation (PAR) reaching

the stream may increase the rate of autochthonous-derived low-molecular weight DOC (McTammany et al. 2007, Julian et al. 2008). An increase in PAR can also facilitate the photodegradation of high-molecular weight DOC compounds to low-molecular weight compounds or alternatively photochemically transform autochthonous produced DOC to recalcitrant products (Wetzel et al. 1995, Tranvik & Bertilsson 2001, Howitt et al. 2008). Fine sediment can also increase water column turbidity, especially when surface water is connected (Prosser et al. 2001), which can reduce light penetration and the functioning of some microbial enzymes (Tietjen & Wetzel 2003, Tietjen et al. 2005). The availability of DOC for hydrolysis by microbial enzymes can decrease when DOC aggregate with inorganic particles in the water column or adsorbs onto some clay surfaces (Tietjen & Wetzel 2003), while enzyme production can also be altered (Tietjen et al. 2005). Under particular redox conditions, DOC can also bind with heavy metals (iron) complexes that reduces its' availability to microbes (Jones et al. 1993).

Extensive fertiliser application and other land-use practices can lead to increases the availability of nutrients, predominantly N and P (Townsend & Riley 1999, Harris 2001), which can increase rates of heterotrophic organic matter decomposition of within aquatic environments (Young et al. 1994, Stelzer et al. 2003, Bärlocher et al. 2010), as well as the increased production of autochthonous carbon by primary producers (Rier & Stevenson 2002, Stevenson et al. 2006). It has been suggested that the combination of nutrient availability and increases in temperature and light may lead to an increase in the production and consumption of carbon within aquatic ecosystems (Stanley et al. 2010b, Rier et al. 2011). If there is an increase in nutrient availability, then this may be reflected in higher C-hydrolysing EEA compared to nutrient-acquiring EEA (Rier et al. 2011).

Additionally, stream banks can become destabilised by the removal of riparian vegetation leading to large volumes of sediment transported through the system. The accumulation of sediment within the channel can lower the heterogeneity of instream morphology and geomorphic features, which may lead to a reduction in the retention and availability of allochthonous organic matter for microbial consumers (Wood & Armitage 1997, Pusey & Arthington 2003). Organic matter retained within reaches can become smothered in sediment

(see Chapter 4); a process that can produce anoxic conditions and limit the heterotrophic processing of organic matter (Bunn 1988, Benfield et al. 2001).

Hydrology

The quantity of both allochthonous and autochthonous sources of organic matter within aquatic systems is also dependent on seasonal and hydrological processes, and has the potential to alter the concentration and quality of in-stream DOC (Findlay & Sinsabaugh 1999). Studies in the northern hemisphere have shown that increases in in-stream DOC concentration is aligned with the timing of peak litterfall from deciduous riparian and floodplain trees (Romaní et al. 2006). However, in intermittent streams in southeastern Australia, peak litterfall in summer generally coincides with the low and no-flow periods (Bunn 1988, Boulton & Lake 1992). During these periods, the seasonal increase in PAR and surface water temperatures can also increase the rates of autotrophic production (Mulholland et al. 2001). As such, the higher quality carbon produced by autotrophs that has a low-molecular weight (e.g., peptides providing N and C) would be preferentially used by heterotrophs, switching to lower quality allochthonous sources of carbon (polysaccharides, providing only C) as disconnected pools dry over time (Ylla et al. 2010).

Rates of discharge can vary greatly within intermittent streams, which can cause fluctuations in the quantity of coarse and fine benthic organic matter within reaches (see Chapter 4, Treadwell et al. 1997, Acuña et al. 2007). During precipitation and storm events, large quantities of DOC can be deposited directly from the rainwater into the stream (Willey et al. 2000) or mobilised from terrestrial soils (Kaplan & Newbold 1993, Boyer et al. 1997). The increase in wetted area and alteration of flowpaths through the soil profile can facilitate leaching of DOC from previously dry organic matter and soils. Typically, water transported through the upper soil horizon will contain high concentrations of DOC compared to water flowing through lower soil layers (Kaplan & Newbold 1993, Boyer et al. 1997), and studies have shown that the presence of riparian vegetation can also affect the quantity and quality of DOC in soil (Hinton et al. 1998, Findlay et al. 2001). Therefore, the fluctuation in allochthonous organic matter or the different

flowpaths of DOC to the streams may vary between vegetated and non-vegetated reaches during high-flow periods and be reflected in patterns of EEA.

Following temporary-river ecology and the pattern of DOC concentrations between reaches, it would be expected that the largest variation in DOC sources would occur during the low and no-flow periods when surface water is fragmented (see Chapter 3, Larned et al. 2010). It also suggests that the variation in DOC sources between vegetated and non-vegetated reaches would occur during these periods of fragmentation, however, the evidence from previous studies on flowpaths suggests that differences can still occur during periods of connectivity (Hinton et al. 1998, Findlay et al. 2001). Additionally, as the largest variation in other nutrient concentrations between reaches occurred during the no-flow period, this suggests that variation in the activity of the nutrient acquiring enzymes would also be highest during this period.

Aims

The aim for this chapter is to use EEA to determine if there were significant differences in the sources of DOC between vegetated and non-vegetated reaches, and during different hydrological conditions.

1) To identify the sources of DOC within vegetated and non-vegetated reaches, I aim to investigate the activity and patterns of three C-hydrolysing enzymes: α-glucosidase, β-glucoside and β-xyloside. I expect that there will β-xylosidase activity will be higher at vegetated reaches and higher α-glucosidase activity at non-vegetated reaches.
2) I also aim to examine the activity of the nutrient acquiring enzymes, leucine aminopeptidase, β-N-Acetyl glucosaminidase and alkaline phosphatase. I predict that during low flow conditions, there will be a large variation in enzyme activity within and between vegetated and non-vegetated reaches.
3) A further objective of this chapter is to explore the major biophysical drivers in patterns of EEA within vegetated and non-vegetated reaches. The variables used to explore these relationships included; surface water nutrient stoichiometry and nutrient concentration, organic matter sources, and hydro-geomorphic variables.

5.2 Methods

Water column samples were collected at each reach across four different discharges. At each sampling time, triplicate unfiltered water samples were collected for EEA measurements and frozen until analysis. On each sampling occasion, pH, temperature, conductivity (mS cm^{-1}) were measured using a WP-81 meter (TPS, Brisbane, Australia), and dissolved oxygen (mg L^{-1}) measured using an Orion Star Plus meter (Thermo Fisher Scientific, USA).

Analysis of extracellular enzyme activity

The activity of extracellular enzymes can be analysed through the use of fluorescent 4-methylum-belliferone-derived artificial substrates. The technique was primarily derived for the analysis of enzyme activity in soil substrates, but was later adapted for aquatic ecosystems (Hoppe 1983, Somville 1984). During hydrolysis, the extracellular enzymes that are present within the particular sample will facilitate the release of flourophores, which are part of the chemical bonds of the artificial substrates (Hoppe 1993). Six different substrates (listed in Table 5.1) were selected for their frequent use in the literature for DOC source and nutrient limitation analyses (Jones & Lock 1989, Burns & Ryder 2001, Harbott & Grace 2005, Hill et al. 2006). The three different C-hydrolysing enzymes were also selected as each enzyme targets a different bond, therefore allowing for a broad range of C-compounds to be explored (i.e. allochthonous vs. autochthonous sources of C).

Thawed EEA samples were firstly filtered through a glass fibre filters (0.7 µm pore size Whatman GF/F) and then through a 0.45 µm membrane filters (Millipore). I prepared the six substrates similar to the method described by Harbott and Grace (2005), to optimise substrate saturation concentrations in the filtered water samples. The substrates were dissolved in 5 mM pH 8.2 autoclaved biocarbonate buffer, except for L-leucine 7-amido-4-methyl-coumarin, which was dissolved in autoclaved Milli-Q water, as it is known to be unstable in buffer. Aliquots of 100 µL substrate solution were combined with 100 µL of the filtered water samples, in black 96 well plates (Nunc brand).

Table 5.1: List of substrates used to examine the activity of different extracellular enzymes.

	Enzyme	Substrate	Abbreviation	Action
Carbon specific enzymes	α-1, 4-glucosidase (exocellulase)	4-MUB-α-glucoside	α-glu	Starch degradation: cleaves maltose and starch linkages (Rulík & Spáčil 1999).
	β-1, 4-glucosidase (exocellulase)	4-MUB-β-glucoside	β-glu	Cellulose degradation: cleaves cellobiose into two glucose monomers (Mansfield et al. 1999).
	β-xylosidase (endocellulase)	4-MUB-β-xyloside	Xylo	Cellulose degradation: targets glucosyl bonds along the linear polymer (Sinsabaugh 1981). Produces free-chain ends on the glucose surface for cellobiohydrolases to act upon (Mansfield et al. 1999).
Nutrient specific enzymes	β-N-acetyl glucosaminidase	4-MUB-N-acetyl-β-D-glucosaminide	NAG	Hydrolyses glycosyl-N bond in chitin, releasing a smaller molecule of C-N (Olander & Vitousek 2000). Acquisition of organic N (Sinsabaugh & Moorhead 1994).
	leucine aminopeptidase	L-leucine 7-amido-4-methyl-coumarin	LAP	Acquisition of organic N from protein (Sinsabaugh et al. 1997).
	alkaline phosphatase	MUB-phosphate	AP	Acquisition of organic P (Olander & Vitousek 2000)

Immediately following preparation, fluorescence was read at room temperature (approximately 20°C) on a ISS K2 Multifrequency Phase fluorometer (model 95150) attached to an ISS K428 Microwell plate reader (96-well, model 90340) under optimised conditions of a 365 nm wavelength/5 nm slit width excitation filter and a 450 nm wavelength/2.5 nm slit width emission filter. Fluorescence readings were recorded from time zero, every 30 minutes for nine hours for each well plate, using the ISS Vinci 1.7 software (www.iss.com). The well plates were kept in the dark for the duration of the measurements. The final substrate concentration in each well was 500 µM, corresponding to the concentration at saturation as determined from previous kinetic assays (Harbott & Grace 2005). The increase in fluorescence over time is proportional to the enzyme present in the sample, which then provides a measure of the potential microbial EEA (Findlay et al. 1997, Moorhead & Sinsabaugh 2000). The concentrations of liberated fluorophores were determined from a standard curve of 4-methylumbelliferone (4-MUB) in buffer and milli-Q. The final concentrations used for the standard curve were 0.1, 1.0, 2.5, and 5 µM. Aliquots of 100 µL standard curve solution were combined with 100 µL of autoclaved milli-Q water in the well plates. The same 1:1 ratio of substrate/sample was kept to account for any scattering caused by fine particles (< 0.45 µm) in the filtered water samples. A combination of 100 µL aliquots of substrate solution and 100 µL of autoclaved milli-Q water were used as blanks. The coefficient determined by regression analysis of the 4-MUB standard curve was used to determine the rate of enzyme-released 4-MUB for each sample and substrate combination, and were expressed as µM 4-MUB L^{-1} h^{-1}. Unfortunately, the activity of NAG could not be detected in the fluorescence analyses and so were left out of any statistical analyses.

Statistical analyses

To test for significant differences in the activity of the five extracellular enzymes between vegetated and non-vegetated reaches, and across time, I used a three-factor ANOVA outlined in Chapters 3 and 4. The model consisted of three factors: i) Treatment – vegetated and non-vegetated (a=2; fixed), ii) Stream – Moredun Creek, Gwydir River, Roumalla Creek, Booralong Creek and Laura Creek (b=5; random), iii) Time – four sampling occasions (c=4; random). Individual analyses were run for each enzyme using PERMANOVA. The enzyme data were $\log_{(x+1)}$ transformed prior to analysis to improve normality. PERMDISP analyses were used to test for significant differences in dispersions. MDS plots in PERMANOVA were also used to explore differences in EEA.

The statistical power of the main test was increased through replication at the treatment level, whereby the data from both the control and treatment streams were combined so that the analyses included five vegetated and five non-vegetated reaches. This meant that the statistical design became unbalanced (an unequal number of replicate samples within each factor level) and as such the formulae used for a balanced design (Table 5.2) were invalid. The correct formulae used for the unbalanced design as determined by the PERMANOVA program are listed in Appendix 5 and will be referred to throughout the results section.

To discriminate between the effects of the main treatment (vegetation) and the natural longitudinal change in enzyme activity, single one-way ANOVAs were used to test for significant differences between locations (Lo) on each of the control streams. Three individual tests were performed within PERMANOVA for each enzyme – two were for testing the difference between locations on each control stream separately, and a third test combined the data from both control streams. Data were pooled across time within each test, and Monte-Carlo *p*-values were used as only 35 unique permutations could be generated (tables of formulae are listed in Appendix 3, tables of results are listed in Appendix 4).

Linear regression analyses were conducted to explore the relationships between the activity of individual enzymes and various hydro-geomorphic and biogeochemical variables. The enzyme activity dataset was $\log_{(x+1)}$ transformed prior to linear regression analyses. The predictor variables included: surface water nutrient concentration and nutrient stoichiometry, benthic organic matter composition and hydro-geomorphic variables which are listed with their respective transformations in Table 5.3. Before conducting the analyses, residual plots were examined for outliers, and datasets were checked for samples with large leverage. If identified, these values were removed and the new sample size (n) reported within the results table. As the risk of making Type I errors increases with the number of single linear regressions (Quinn & Keough 2002), the regressions were only accepted as significant if $p \leq 0.025$, following Bonferroni's principal (Quinn & Keough 2002). All analyses were conducted using SYSTAT (Version 12.0).

Following the single linear regressions, all predictor variables that were significantly correlated to an individual enzyme were combined into a dataset, which was specific to each enzyme. The datasets were then used to perform conditional tests within the DISTLM routine in PERMANOVA (outlined in Chapter 4). This was in order to determine which of the significant predictor variable(s) were most correlated with the variation in EEA.

Table 5.2: Table of formulae for a balanced statistical design. Treatment is a fixed factor and has two levels (a=2), stream is a random factor and has five levels (b=5), and time is a random factor with four levels (c=4). Tables of correct formulae for the unbalanced design are listed in Appendix 5 and will be referred to in the results section.

	Source of variation	Multipliers				Degrees of freedom	Expected mean square	Variance component
		i	j	k	r			
1	**Treatment = Tr**	0	b	c	N	a-1	$\sigma^2_e + n\sigma^2_{TrStTi} + bn\sigma^2_{TrTi} + cn\sigma^2_{TrSt} + bcn\sigma^2_{Tr}$	$(MS_{Tr} - MS_e)/bcn$
2	**Stream = St**	a	1	c	N	b-1	$\sigma^2_e + an\sigma^2_{StTi} + acn\sigma^2_{St}$	$(MS_{St} - MS_e)/acn$
3	**Time = Ti**	a	b	1	n	c-1	$\sigma^2_e + an\sigma^2_{StTi} + abn\sigma^2_{Ti}$	$(MS_{Ti} - MS_e)/abn$
4	**Tr x St**	0	1	c	n	(a-1)(b-1)	$\sigma^2_e + n\sigma^2_{TrStTi} + cn\sigma^2_{TrSt}$	$(MS_{TrSt} - MS_e)/cn$
5	**Tr x Ti**	0	b	1	n	(a-1)(c-1)	$\sigma^2_e + n\sigma^2_{TrStTi} + bn\sigma^2_{TrTi}$	$(MS_{TrTi} - MS_e)/bn$
6	**St x Ti**	a	1	1	n	(b-1)(c-1)	$\sigma^2_e + an\sigma^2_{StTi}$	$(MS_{StTi} - MS_e)/an$
7	**Tr x St x Ti**	0	1	1	n	(a-1)(b-1)(c-1)	$\sigma^2_e + n\sigma^2_{TrStTi}$	$(MS_{TrStTi} - MS_e)/n$
8	**Residual = e**	1	1	1	1	abc(n-1)	σ^2_e	MS_e

Table 5.3: List of predictor variables and their respective transformations used in the regression analyses. SA:V = surface area:volume ratio, C-repro = *C. cunninghamiana* reproductive structures, UI = unidentified fraction.

Variable	Transformation	Variable	Transformation
No. of features	-	DOC (mg L^{-1})	-
Flow percentile	$Log_{(x+1)}$	NOx (mg L^{-1})	Box-Cox
Velocity	$Log_{(x+0.1)}$	SRP (mg L^{-1})	1/(x+1)
SA:V	-	TN (mg L^{-1})	$Log_{(x)}$
Wetted area	-	TP (mg L^{-1})	1/(x+1)
CBOM (g m^{-2})	$Log_{(x+1)}$	DOC:NOx	-
FBOM (g m^{-2})	$Log_{(x+1)}$	NOx:SRP	-
Needle (g m^{-2})	$Log_{(x+1)}$	DOC:SRP	-
Wood (g m^{-2})	$Log_{(x+1)}$	DOC:TN	-
C-repro (g m^{-2})	$Log_{(x+1)}$	TN:TP	-
Leaf (g m^{-2})	$Log_{(x+1)}$	DOC:TP	-
Macrophyte (g m^{-2})	$Log_{(x+1)}$	DOC (g day^{-1})	√x with sign reinstated
UI (g m^{-2})	$Log_{(x+1)}$	NOx (g day^{-1})	√x with sign reinstated
CBOM (g)	$Log_{(x+1)}$	SRP (g day^{-1})	√x with sign reinstated
FBOM (g)	$Log_{(x+1)}$	TN (g day^{-1})	$x^{.33}$ with sign reinstated
Needle (g)	$Log_{(x+1)}$	TP (g day^{-1})	√x with sign reinstated
Wood (g)	$Log_{(x+1)}$	CBOM (g day^{-1})	√x with sign reinstated
C-repro (g)	$Log_{(x+1)}$	FBOM (g day^{-1})	√x with sign reinstated
Leaf (g)	$Log_{(x+1)}$	Needle (g day^{-1})	√x with sign reinstated
Macrophyte (g)	$Log_{(x+1)}$	Wood (g day^{-1})	√x with sign reinstated
UI (g)	$Log_{(x+1)}$	C-repro (g day^{-1})	√x with sign reinstated
		Leaf (g day^{-1})	√x with sign reinstated
		Macrophyte (g day^{-1})	√x with sign reinstated
		UI (g day^{-1})	√x with sign reinstated

5.3 Results

The results of the multivariate PERMANOVA test using the activities of all five enzymes showed that there was no overall significant effect of vegetation (Table 5.4). However, the MDS showed that there was variation in mean rates of EEA between the vegetated and non-vegetated reaches during February and May, the low and no-flow periods, as well as during September, the first high-flow sampling period (Figure 5.1). The tests of the effect of location within control streams showed there were no significant differences for each individual enzyme (Appendix 4, Tables A4.16 – A4.20). The result was consistent for each test when data were combined between the two control streams and on each individual control stream (Appendix 4, Tables A4.16 – A4.20).

There was a significant effect of hydrology (time) on enzyme activity as determined by the PERMANOVA test (p = 0.001, Table 5.4). A significant interaction existed between streams and time, which indicates that there was variation in the effect of hydrology on enzyme activity across streams (p = 0.003, Table 5.4). The PERMDISP analyses showed that there was a significant difference in dispersions between sampling times (p = 0.012). Taking into consideration the results of the PERMDISP and PERMANOVA tests, and the MDS, this shows that the activities of extracellular enzymes are sensitive to hydrology, and that there is large variation in EEA between all reaches during no-flow conditions and also during the initial high-discharge events that reconnects pools.

Table 5.4: Results of PERMANOVA main test for significant differences in mean EEA activity using all five substrates (µM 4-MUB L^{-1} h^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions (n=3). All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	4.436	4.436	2.870	0.082
St	4	25.678	6.420	1.601	0.091
Ti	3	74.783	24.928	5.678	**0.001**
TrxSt**	2	3.067	1.533	0.578	0.692
TrxTi	3	2.851	0.951	0.358	0.915
StxTi	12	49.403	4.117	2.701	**0.003**
TrxStxTi**	5	13.267	2.653	1.741	0.099
Res	7	10.669	1.524		
Total	37	185.000			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.11 for correct formulae.

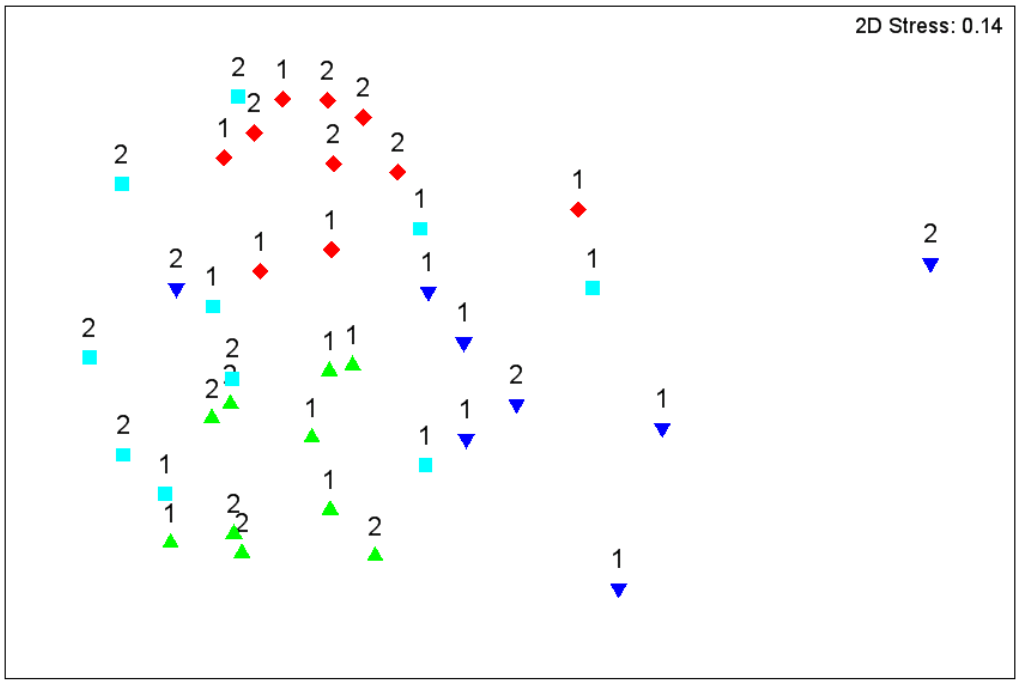

Figure 5.1: MDS of mean (n=3) EEA of all five enzymes (µM 4-MUB L^{-1} h^{-1}). Samples were from all non-vegetated (labeled 1) and vegetated (labeled 2) reaches during the four sampling periods (February – triangles, May – inverted triangles, September – squares, November – diamonds).

Activity of C-hydrolysing enzymes

β-glu activity

There was no significant difference in β-glu activity between vegetated and non-vegetated reaches, however, there was a significant effect of hydrology (p = 0.01, Table 5.5). The β-glu enzyme showed a large amount of variation in mean rates of EEA within vegetated (0.6 to 28.2 µM 4-MUB L^{-1} h^{-1}) and non-vegetated (0.6 to 39.3 µM 4-MUB L^{-1} h^{-1}) reaches across all sampling times and the PERDISP test confirmed that the dispersions were not significantly different (Figure 5.2). Rates of β-glu activity were highest at the non-vegetated downstream control

reach, Laura Bridge, during May which was the no-flow period (39.3 µM 4-MUB L^{-1} h^{-1}, Figure 5.2). During September, the first high-flow period, β-glu activity was highest within the non-vegetated upstream control reach, Laura Homestead (24.1 4-MUB L^{-1} h^{-1}) and at the Moredun non-vegetated reach (18.7 4-MUB L^{-1} h^{-1}). With the exception of the Gwydir vegetated and Booralong Bridge reaches during February and May, β-glu activity across all sampling times was generally lower at the vegetated reaches in comparison to the non-vegetated reaches. During November, the second high-flow period, β-glu activity decreased within both the vegetated and non-vegetated reaches (Figure 5.2). The results of the PERMANOVA test showed that there was a significant interaction between treatment, stream and time, and a significant effect of time (p = 0.021, Table 5.5). The PERMDISP test showed that there was a significant difference in dispersions between sampling times for this enzyme which means that the variation in mean rates of β-glu EEA between reaches is sensitive to changes in hydrology (p = 0.013).

Table 5.5: Results of PERMANOVA main test for significant differences in mean β-glu activity (µM 4-MUB L^{-1} h^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions (n=3). All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	1.282	1.282	3.900	0.092
St	4	6.433	1.608	3.065	0.058
Ti	3	10.644	3.548	6.120	**0.010**
TrxSt**	2	0.552	0.279	0.325	0.737
TrxTi	3	0.822	0.274	0.323	0.809
StxTi	12	6.511	0.543	4.774	**0.021**
TrxStxTi**	5	4.246	0.849	7.472	**0.005**
Res	7	0.796	0.114		
Total	37	32.542			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.11 for correct formulae.

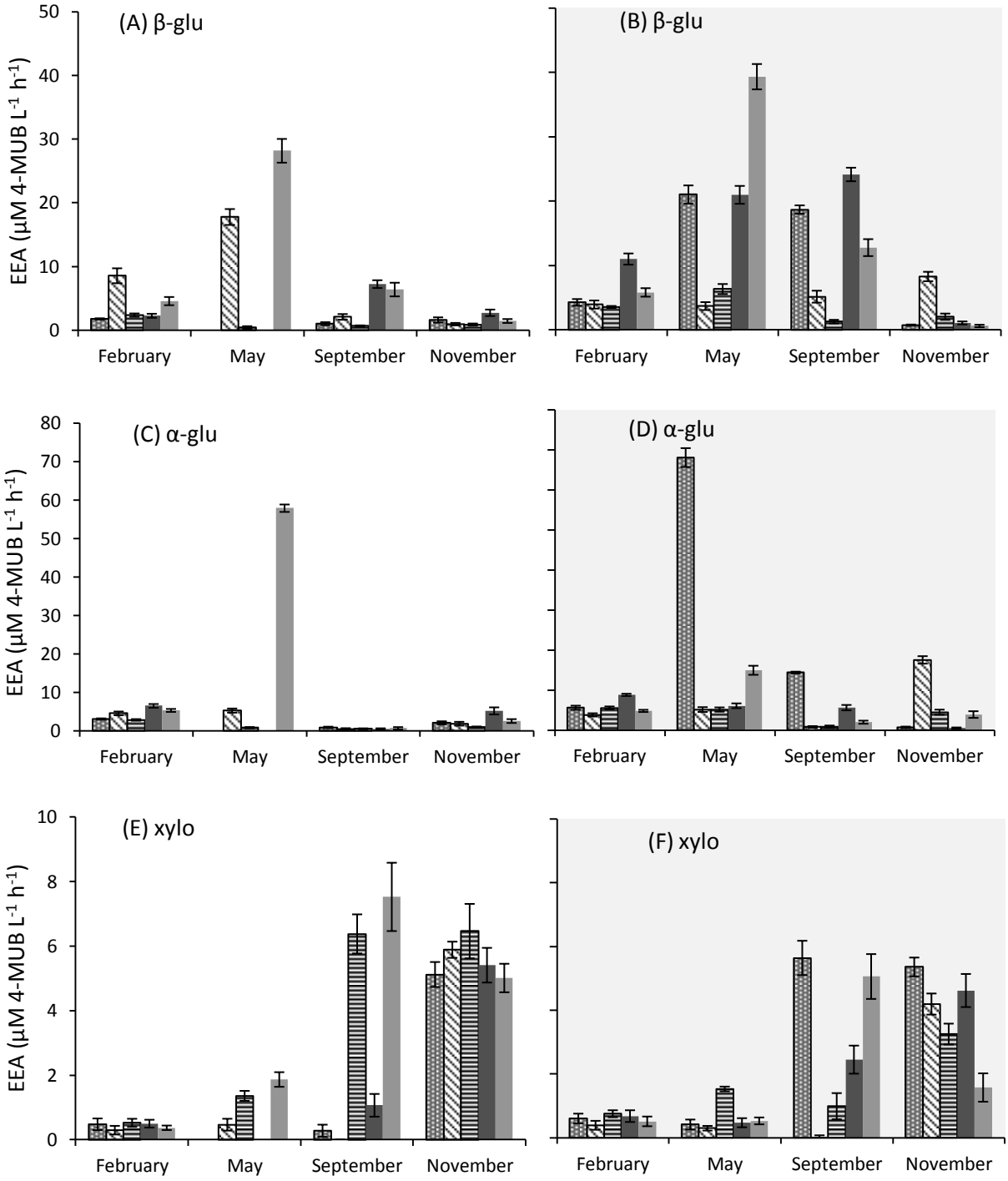

Figure 5.2: Mean surface water extracellular enzyme activity of the carbon specific enzymes (µM 4-MUB L^{-1} h^{-1}), β-glu (A-B), α-glu (C-D), and xylo (E-F) sampled from vegetated (white background) and non-vegetated (grey background) reaches. Error bars represent the standard error of the sample mean (n=3). Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura Homestead, while the light grey bars represent the downstream reaches, Booralong Bridge and Laura Bridge, on each of the control streams.

α-glu activity

The results of the PERMANOVA test showed that there was a significant difference in α-glu enzyme activity between vegetated and non-vegetated reaches (p = 0.003, Table 5.6). During February, the no-flow period, α-glu activity ranged from 2.8 to 6.5 µM 4-MUB L^{-1} h^{-1} within the vegetated reaches, and 3.8 to 8.8 µM 4-MUB L^{-1} h^{-1} within the non-vegetated reaches (Figure 5.2). With the exception of the downstream vegetated control reach, Booralong Bridge, the activity of α-glu activity was consistently lower during the three remaining sampling times. The highest rates of α-glu activity occurred during the low and no-flow periods and then decreased during the high-flow periods September and November, across all non-vegetated reaches and vegetated reaches (Figure 5.2). The results of the PERMANOVA test also showed there was a significant difference in α-glu activity between sampling times (p = 0.014) and a significant interaction between streams and times (p = 0.046, Table 5.6).

Table 5.6: Results of PERMANOVA main test for significant differences in mean α-glu activity (µM 4-MUB L^{-1} h^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions (n=3). All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	2.002	2.002	21.901	**0.003**
St	4	5.655	1.413	1.749	0.189
Ti	3	12.965	4.322	4.865	**0.014**
TrxSt**	2	$7.357e^{-2}$	$3.679e^{-2}$	$5.644e^{-2}$	0.947
TrxTi	3	0.260	$8.657e^{-2}$	0.133	0.942
StxTi	12	9.994	0.833	3.479	**0.046**
TrxStxTi**	5	3.259	0.652	2.722	0.105
Res	7	1.676	0.239		
Total	37	32.783			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.11 for correct formulae.

Xylo activity

There was no significant effect of vegetation on xylo activity (Table 5.7). The PERMANOVA test showed that the effect of time was significant on xylo activity (p = 0.001, Table 5.7). Within both vegetated and non-vegetated reaches, xylo activity was less than 2 µM 4-MUB L^{-1} h^{-1} during February and May, the low and no-flow sampling periods (Figure 5.2). During September, the first high-flow period, xylo activity increased to approximately 6 µM 4-MUB L^{-1} h^{-1} at the Roumalla vegetated, Booralong Bridge, Moredun non-vegetated and Laura Creek reaches. Xylo activity was below detection limits at both the Gwydir River reaches during September. However, during second high-flow sampling period, November, the xylo activity increased to approximately 6 4-MUB L^{-1} h^{-1} and approximately 5 4-MUB L^{-1} h^{-1} at the vegetated and non-vegetated Gwydir river reaches, respectively (Figure 5.2). The rate of xylo activity also increased across all other vegetated and non-vegetated reaches, and was slightly higher at the vegetated reaches compared to the non-vegetated reaches in this period (Figure 5.2).

Table 5.7: Results of PERMANOVA main test for significant differences in mean β-xylo activity (µM 4-MUB L^{-1} h^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions (n=3). All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	$1.560e^{-2}$	$1.560e^{-2}$	0.515	0.728
St	4	1.531	0.383	1.710	0.210
Ti	3	9.910	3.303	13.736	**0.001**
TrxSt**	2	0.941	0.471	1.736	0.245
TrxTi	3	0.207	$6.891e^{-2}$	0.254	0.855
StxTi	12	2.707	0.226	1.068	0.503
TrxStxTi**	5	1.356	0.271	1.284	0.377
Res	7	1.479	0.211		
Total	37	17.898			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.11 for correct formulae.

Sources of DOC

Within the three C-hydrolysing enzymes, most of the activity across all sampling times within the non-vegetated reaches was driven by β-glu enzymes (Figure 5.2). Generally, this β-glu activity was supported by α-glu enzyme activity during February and May, the low and no-flow periods. The high rates of β-glu activity were then supported by xylo enzyme activity during September, the first high-flow sampling period. The vegetated reaches showed a similar pattern of enzyme activity in comparison to the non-vegetated reaches, during February and May, the low and no-flow periods. However, the Roumalla vegetated reach showed only xylo activity (of the three C-hydrolysing enzymes) during May, the no-flow period. Xylo enzyme activity was the main source of activity (> 5 4-MUB L^{-1} h^{-1}) at both vegetated and non-vegetated reaches during November, the second high-flow sampling period (Figure 5.2). The activity of β-glu and α-glu enzymes decreased to less than 5 4-MUB L^{-1} h^{-1} at all reaches, with the exception of the Gwydir non-vegetated reach (approximately 17 4-MUB L^{-1} h^{-1}), during November (Figure 5.2).

Activity of AP and LAP nutrient acquiring enzymes

AP activity

There was no significant difference in mean AP activity between vegetated and non-vegetated reaches, however, there were significant differences in mean AP activity between sampling times (p = 0.025, Table 5.8). The activity of AP was low (< 5.5 µM 4-MUB L^{-1} h^{-1}) during February, the low-flow period, at all vegetated reaches and most non-vegetated reaches (Figure 5.3). With the exception of the Roumalla vegetated reach, AP activity increased at most non-vegetated and vegetated reaches during May (> 12.8 µM 4-MUB L^{-1} h^{-1}), the no-flow period, and the highest AP activity occurred during this period at the downstream vegetated control reach, Booralong Bridge (217 µM 4-MUB L^{-1} h^{-1}). Although there was a general decrease in AP activity during the high-flow periods, there was variability in whether AP activity decreased or increased between September and November, the first and second high-flow periods (Figure 5.3). This variation in mean AP activity between streams and across time was supported by the stream and time significant interaction determined by the PERMANOVA test (p = 0.024, Table 5.8). The PERMDISP analyses showed a significant difference in dispersions between times (p = 0.006) for this enzyme.

Table 5.8: Results of PERMANOVA main test for significant differences in mean AP activity (µM 4-MUB L^{-1} h^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions (n=3). All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	0.701	0.701	1.515	0.315
St	4	3.615	0.904	0.639	0.647
Ti	3	18.345	6.115	3.930	**0.025**
TrxSt**	2	0.381	0.190	0.618	0.559
TrxTi	3	1.456	0.485	1.576	0.281
StxTi	12	17.512	1.459	3.907	**0.024**
TrxStxTi**	5	1.540	0.308	0.824	0.569
Res	7	2.615	0.374		
Total	37	49.697			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.11 for correct formulae.

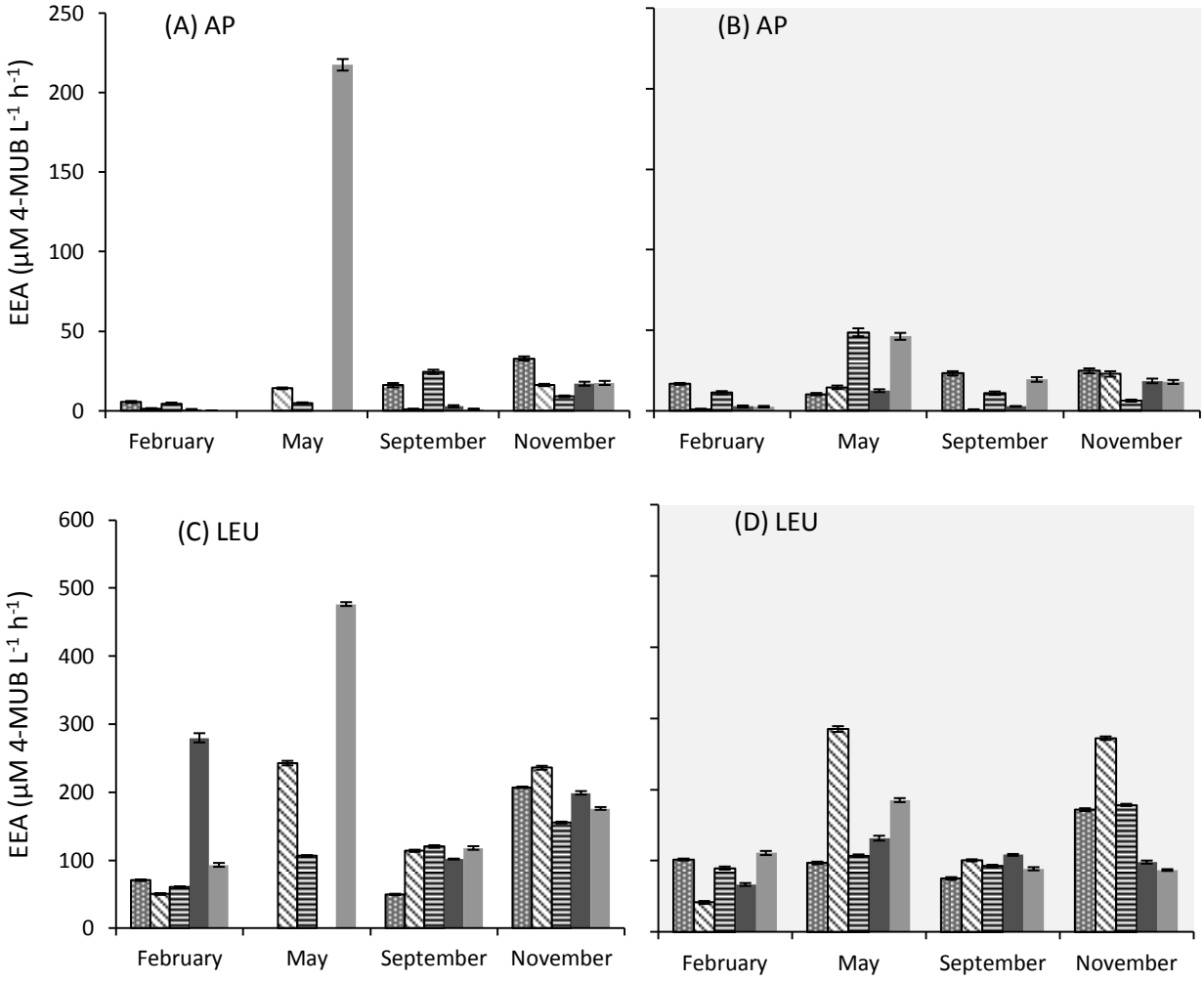

Figure 5.3: Mean surface water extracellular enzyme activity of the nutrient acquiring enzymes (µM 4-MUB L^{-1} h^{-1}), AP (A-B) and LEU (C-D), sampled from vegetated (white background) and non-vegetated (grey background) reaches. Error bars represent the standard error of the sample mean (n=3). Patterning on bars correspond to the reaches on the treatment streams – Moredun Creek (dots), Gwydir River (diagonal stripes), Roumalla Creek (horizontal stripes). Dark grey bars represent the upstream reaches, Booralong CS and Laura Homestead, while the light grey bars represent the downstream reaches, Booralong Bridge and Laura Bridge, on each of the control streams.

LAP activity

There were no significant differences in LAP activity between vegetated and non-vegetated reaches (Table 5.9). The results of the PERMANOVA test showed that the difference in activity across time was significant (p = 0.027, Table 5.9) and the non-significant PERMDISP test confirmed that this was not due to differences in dispersions. The rate of LAP activity was the fastest of the five enzymes, with the highest rate again occurring at the Booralong Bridge reach during May, the no-flow sampling period (476 µM 4-MUB L^{-1} h^{-1}, Figure 5.3). There was a large amount of variation in mean LAP activity within vegetated and non-vegetated reaches during February and May, the low and no-flow periods (Figure 5.3). LAP activity generally decreased across all reaches during September, the first high-flow sampling period, and then increased again during November, the second high-flow sampling period (Figure 5.3).

Table 5.9: Results of PERMANOVA main test for significant differences in mean LAP activity (µM 4-MUB L^{-1} h^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions (n=3). All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	$4.749e^{-2}$	$4.749e^{-2}$	3.240	0.114
St	4	1.765	0.441	1.559	0.240
Ti	3	4.006	1.335	4.323	**0.027**
TrxSt**	2	$3.656e^{-2}$	$1.828e^{-2}$	0.391	0.693
TrxTi	3	$3.228e^{-2}$	$1.076e^{-2}$	0.230	0.889
StxTi	12	3.483	0.290	2.457	0.129
TrxStxTi**	5	0.234	$4.676e^{-2}$	0.396	0.794
Res	7	0.827	0.118		
Total	37	10.660			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.11 for correct formulae.

Relationship between C-hydrolysing EEA with AP and LAP EEA

The regression analyses showed a significant correlation between the activity of AP and LAP within the non-vegetated reaches (r^2 = 0.21, Table 5.10). Within the vegetated reaches, the activity of all C-hydrolysing enzymes (all C) was significantly correlated with AP activity (r^2 = 0.38). The significant relationship between xylo and AP activity within the vegetated reaches yielded the highest coefficient of determination (r^2 = 0.50, Table 5.10).

Table 5.10: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}). All-C is the sum of β-glu, α-glu and xylo activity. Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, = 18, and ## indicates n=16.

	Vegetated		Non-vegetated	
	AP	LAP	AP	LAP
AP				m = 0.08 *r* = 0.21*
LAP				
β-glu				
α -glu				
xylo	m = 0.99## *r* = 0.50**			
All C	m = 0.29 *r* = 0.38*			

Correlation of hydro-geomrophic and biochemical variables to variation in enzyme activity

Within the vegetated reaches, α-glu activity was positively correlated with flow percentile (r^2 = 0.42), and negatively correlated with velocity (r^2 = 0.33) and wetted area (r^2 = 0.26, Table 5.11). Xylo was negatively correlated with flow percentile (r^2 = 0.63) and β-glu was also negatively correlated with wetted area (r^2 = 0.26). Xylo activity in the non-vegetated reaches was negatively correlated with flow percentile (r^2 = 0.75) and positively correlated with velocity (r^2 = 0.55), while the activity of both α-glu (r^2 = 0.50) and β-glu (r^2 = 0.24) were positively correlated with flow percentile (Table 5.11).

Xylo activities within both vegetated and non-vegetated reaches were positively correlated with SRP (vegetated r^2 = 0.48, non-vegetated r^2 = 0.44) and TP concentrations (vegetated r^2 = 0.53, non-vegetated r^2 = 0.67, Table 5.12). TN concentration was significantly negatively correlated with xylo activity within both vegetated (r^2 = 0.25) and non-vegetated (r^2 = 0.32) reaches (Table 5.12). Only a single significant relationship existed between EEA and the retained nutrient loads which was between α-glu activity and TP (r^2 = 0.28) within the non-vegetated reaches (Table 5.13).

Within the non-vegetated reaches, xylo activity was positively correlated with NOx:SRP (r^2 = 0.27), DOC:SRP (r^2 = 0.40) and DOC:TN (r^2 = 0.43) ratios (Table 5.14). The activity of β-glu was negatively correlated with DOC:TN ratio within both the vegetated (r^2 = 0.38) and non-vegetated reaches (r^2 = 0.35). The TN:TP ratios were positively correlated with β-glu activity within the vegetated reaches (r^2 = 0.37). There were significant positive relationships between DOC:SRP and DOC:TN ratios with xylo activity within the vegetated reaches. The activity of α-glu was positively correlated with DOC:SRP ratios (r^2 = 0.38) and negatively correlated with DOC:TN ratios (r^2 = 0.44) within vegetated reaches (Table 5.14).

Correlations between the mean benthic organic matter fractions estimated at a unit area scale (g m^{-2}) and enzyme activity within the non-vegetated reaches showed that CBOM (r^2 = 0.27) and FBOM (r^2 = 0.24) mass were positively correlated with β-glu activity (Table 5.15). However, these correlations were not evident when the organic matter fractions were estimated at whole-reach scales (Table 5.16). Within the vegetated reaches, xylo activity was positively

correlated with *Casuarina* reproductive material at the unit area scale (r^2 = 0.27) and whole-reach scale (r^2 = 0.38, Tables 5.15 and 5.16). Also within the vegetated reaches, xylo activity was significantly positively correlated with the wood fraction (r^2 = 0.23) and β-glu activity was negatively correlated with wood (r^2 = 0.26) at the whole-reach scale (Table 5.16).

There were significant correlations between retained organic matter loads and EEA, however, these were between different organic matter fractions to those identified in the benthic organic matter estimates. Within the vegetated reaches β-glu activity was negatively correlated with the retained leaf fraction (r^2 = 0.33) and α-glu activity was also negatively correlated with the retained macrophyte fraction (r^2 = 0.32, Table 5.17). The activity of LAP within the non-vegetated reaches was positively correlated with retained loads of CBOM (r^2 = 0.33), leaf (r^2 = 0.44) and unidentified fractions (r^2 = 0.41, Table 5.17).

Table 5.11: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}) and hydro-geomorphic variables. The dataset did not include May EEA values (n=15). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, is 18. # indicates n=17 and ## indicates n=16.

	Vegetated					Non-vegetated				
	AP	LAP	β-glu	α-glu	xylo	AP	LAP	β-glu	α-glu	xylo
No. feature										
Flow percentile				m = 1.1 *r* = 0.42**	m = -1.0## *r* = 0.63***			m = 0.57# *r* = 0.24*	m = 1.2### *r* = 0.50**	m = -1.6# *r* = 0.75***
Velocity				m = -0.95# *r* = 0.33*						m = 1.17 *r* = 0.55***
SA:V										
Wetted area			m = -0.85# *r* = 0.26**	m = -0.57# *r* = 0.26*						

Table 5.12: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}) and mean nutrient concentrations (mg L^{-1}). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, = 18.

	Vegetated					Non-vegetated				
	AP	LAP	β-glu	α-glu	xylo	AP	LAP	β-glu	α-glu	xylo
DOC										
NOx										
SRP					m = 0.54 *r* = 0.48***					m = 0.38 *r* = 0.44**
TN					m = -0.39 *r* = 0.25*			m = 0.52 *r* = 0.29*	m = 0.43 *r* = 0.24*	m = -0.33 *r* = 0.32*
TP					m = 0.57 *r* = 0.53***					m = 0.45 *r* = 0.67***

Table 5.13: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}) and retained nutrient loads (g day^{-1}). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, = 18.

	Vegetated					Non-vegetated				
	AP	LAP	β-glu	α-glu	xylo	AP	LAP	β-glu	α-glu	xylo
DOC										
NOx										
SRP										
TN										
TP									m = -0.01 *r* = 0.28*	

Table 5.14: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}) and nutrient ratios. Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, = 18, and # n=17.

	Vegetated					Non-vegetated				
	AP	LAP	β-glu	α-glu	xylo	AP	LAP	β-glu	α-glu	xylo
DOC : NOx										
NOx : SRP										m = 1.05 *r* = 0.27*
DOC : SRP				m < 0.01# *r* = 0.38**	m < 0.01 *r* = 0.47**					m < 0.01# *r* = 0.40**
DOC : TN			m = -0.09 *r* = 0.38**	m = -0.07# *r* = 0.44**	m = 0.08 *r* = 0.36**			m = -0.09 *r* = 0.35**		m = 0.06 *r* = 0.43**
TN : TP			m = 0.04# *r* = 0.37*							
DOC : TP										

Table 5.15: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}) and organic matter variables (g m^{-2}). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, is 18, # indicates n=17, and ## indicates n=16.

	Vegetated					Non-vegetated				
	AP	LAP	β-glu	α-glu	xylo	AP	LAP	β-glu	α-glu	xylo
CBOM								m = 0.61 *r* = 0.27*		
FBOM								m = 1.02## *r* = 0.24*		
Needle										
Wood										
C – repro					m = 1.62 *r* = 0.27*					
Leaf										
Macrophyte										
Unidentified										

Table 5.16: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}) and organic matter variables (whole-reach total, g). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, is 18, # indicates n=17, and ## indicates n=16.

	Vegetated					Non-vegetated				
	AP	LAP	β-glu	α-glu	xylo	AP	LAP	β-glu	α-glu	xylo
CBOM										
FBOM										
Needle										
Wood			m = -0.27# *r* = 0.26*		m = 0.29 *r* = 0.23*					
C – repro					m = 0.41 *r* = 0.38**					
Leaf										
Macrophyte										
Unidentified										

Table 5.17: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between mean individual EEA (μM 4-MUB L^{-1} h^{-1}) and retained organic matter fractions (g day^{-1}). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size, n, is 18, # indicates n=17, and ## indicates n=16.

	Vegetated					Non-vegetated				
	AP	LAP	β-glu	α-glu	xylo	AP	LAP	β-glu	α-glu	xylo
CBOM							m = 0.02 *r* = 0.33*			
FBOM										
Needle										
Wood										
C – repro										
Leaf			m = -0.13 *r* = 0.33*				m = 0.08 *r* = 0.44**			
Macrophyte				m = -0.11 *r* = 0.32*						
Unidentified							m = 0.04 *r* = 0.41**			

The DISTLM tests could not identify any statistically significant multiple variable models to explain the variation in the activity of β-glu, α-glu and xylo enzymes within the vegetated reaches. The DOC:TN ratio was found to be the most significantly correlated variable with β-glu (p = 0.004) and α-glu (p = 0.001) activity, explaining 38 and 52.5% of the variation within the vegetated reaches, respectively (Tables 5.18 and 5.19). The activity of xylo was most significantly correlated with TP concentration (p = 0.003), explaining 52.9% of the variation within the vegetated reaches (Table 5.20).

Table 5.18: Results of conditional test from DISTLM for mean β-glu activity (μM 4-MUB L^{-1} h^{-1}) from the vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
DOC : TN	4.631	9.824	**0.011**	0.380	0.380	16
Wetted area	0.645	1.402	0.245	$5.294e^{-2}$	0.433	15
TN : TP	0.244	0.513	0.504	$2.002e^{-2}$	0.453	14
Leaf (retained)	$6.425e^{-2}$	0.127	0.706	$5.277e^{-3}$	0.459	13
Wood (WR)	$2.188e^{-4}$	$3.983e^{-4}$	0.989	$1.797e^{-5}$	0.459	12

Table 5.19: Results of conditional test from DISTLM for mean α-glu activity (μM 4-MUB L^{-1} h^{-1}) from the vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
DOC : TN	7.244	17.697	**0.001**	0.525	0.525	16
DOC : SRP	0.126	0.294	0.606	$9.137e^{-3}$	0.534	15
Wetted area	0.152	0.339	0.555	$1.099e^{-2}$	0.545	14
Grass (retained)	$6.307e^{-2}$	0.132	0.751	$4.572e^{-3}$	0.550	13
Velocity	$5.743e^{-2}$	0.112	0.755	$4.163e^{-3}$	0.554	12
Flow percentile	0.465	0.899	0.360	$3.368e^{-2}$	0.588	11

Table 5.20: Results of conditional test from DISTLM for mean xylo activity (μM 4-MUB L^{-1} h^{-1}) from the vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
TP	5.459	17.996	**0.002**	0.529	0.529	16
C-repro	0.934	3.575	0.068	$9.059e^{-2}$	0.620	15
DOC : TN	0.167	0.624	0.470	$1.622e^{-2}$	0.636	14
Flow percentile	$6.083e^{-2}$	0.214	0.650	$5.899e^{-3}$	0.642	13
TN	$9.962e^{-2}$	0.333	0.543	$9.660e^{-3}$	0.652	12
C-repro (WR)	$3.544e^{-2}$	0.110	0.730	$3.436e^{-3}$	0.655	11
DOC : SRP	$2.500e^{-3}$	$7.036e^{-3}$	0.927	$2.425e^{-4}$	0.655	10
Wood (WR)	$1.073e^{-3}$	$2.718e^{-3}$	0.966	$1.040e^{-4}$	0.656	9
SRP	$3.294e^{-5}$	$7.418e^{-5}$	0.995	$3.194e^{-6}$	0.656	8

There were also no statistically significant combinations of multiple variables to explain the variation in the activity of LAP, β-glu and α-glu enzymes within the non-vegetated reaches. The activity of LAP was most significantly correlated with the retained load of leaves (p = 0.005, Table 5.21). The DOC:TN ratio was the most significantly correlated variable with β-glu (p = 0.007) and explained 34.7% of the variation in activity within the non-vegetated reaches (Table 5.22). The activity of the α-glu enzyme was most significantly correlated with TN concentration (p = 0.036) within the non-vegetated reaches, explaining 24.3% of the variation (Table 5.23). A combination of flow percentile and TP concentration was significantly correlated with xylo activity (p = 0.034). Flow percentile explained 56.4% of the variation while TP explained 10.7% (Table 5.24).

Table 5.21: Results of conditional test from DISTLM for mean LAP activity (μM 4-MUB L^{-1} h^{-1}) from the non-vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Leaf (retained)	1.511	10.639	**0.005**	0.385	0.385	17
COM (retained)	0.157	1.111	0.307	$3.995e^{-2}$	0.425	16
UI (retained)	$1.005e^{-3}$	$6.680e^{-3}$	0.937	$2.560e^{-4}$	0.425	15

Table 5.22: Results of conditional test from DISTLM for mean β-glu activity (μM 4-MUB L^{-1} h^{-1}) from the non-vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
DOC : TN	5.978	9.025	**0.007**	0.347	0.347	17
CBOM	1.273	2.040	0.160	$7.387e^{-2}$	0.421	16
Flow percentile	0.698	1.128	0.296	$4.051e^{-2}$	0.461	15
TN	0.493	0.784	0.387	$2.858e^{-2}$	0.490	14
FBOM	$8.086e^{-2}$	0.121	0.757	$4.691e^{-3}$	0.494	13

Table 5.23: Results of conditional test from DISTLM for mean α-glu activity (μM 4-MUB L^{-1} h^{-1}) from the non-vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
TN	3.624	5.469	**0.032**	0.243	0.243	17
TP (retained)	0.906	1.399	0.252	$6.082e^{-2}$	0.304	16
Flow percentile	0.217	0.321	0.577	$1.455e^{-2}$	0.319	15

Table 5.24: Results of conditional test from DISTLM for mean xylo activity (µM 4-MUB L^{-1} h^{-1}) from the non-vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Flow percentile	3.720	22.032	**0.001**	0.564	0.565	17
TP	0.704	5.195	**0.034**	0.107	0.671	16
Velocity	0.118	0.864	0.332	$1.791e^{-2}$	0.689	15
TN	$5.057e^{-2}$	0.354	0.533	$7.672e^{-3}$	0.697	14
DOC : SRP	$4.142e^{-2}$	0.275	0.616	$6.284e^{-3}$	0.703	13
SRP	0.122	0.797	0.379	$1.849e^{-2}$	0.722	12
DOC : TN	$4.200e^{-2}$	0.258	0.633	$6.372e^{-3}$	0.728	11
NOx : SRP	$1.747e^{-2}$	$9.842e^{-2}$	0.735	$2.651e^{-3}$	0.731	10

5.4 Discussion

The activities of the C-hydrolysing extracellular enzymes: α-glu, β-glu and xylo, were analysed in this study to explore differences in the sources of DOC between vegetated and non-vegetated reaches, and the variation in their activity during different discharges. In contrast, the activities of the nutrient acquiring enzymes, AP and LAP, were analysed to explore nutrient limitation within vegetated and non-vegetated reaches during different discharges.

Patterns in C-hydrolysing enzymes

Within aquatic systems, the presence of α-glu indicates the processing of starch compounds from vascular macrophytes/plants and unicellular green algae (Jones & Lock 1989). In this study, the activity of α-glu was significantly higher within the non-vegetated reaches during the no-flow and both high-flow periods, which suggests there was a larger contribution of autochthonous DOC sources and low molecular-weight plant polysaccharides within non-vegetated reaches compared to vegetated reaches. These results vary from those of a similar study examining the effects of urbanisation on DOC sources and bioavailability that found relatively consistent dissolved α-glu activity between urbanised and less-urbanised streams (Harbott & Grace 2005). This would suggest that there were no differences in the contribution of algal derived starch to the DOC pool with urbanisation, however, the study found high LAP (a peptidase hydrolysing enzyme, Jones & Lock 1989) and esterase (a non-specific extracellular hydrolysing enzyme, Jones & Lock 1989) activity within urbanised streams relative to less urbanised streams. The authors suggested that peptides from filamentous algae may be a dominant C source in urbanised streams (Harbott & Grace 2005). As the removal of riparian vegetation can increase PAR penetrating the water column with a concomitant increase in autochthonous production (McTammany et al. 2007, Julian et al. 2010), it is likely that the difference in α-glu activity in this study is due to an increase in algal and macrophyte production within the non-vegetated reaches. However, there was only a relatively small difference in α-glu activity between vegetated and non-vegetated reaches during the low-flow period, which coincided with the austral summer. High temperatures and extreme light conditions have been shown to cause algal photoinhibition (see Chapter 3, Üveges & Padisák

2011). This suggests that algal production may have been similar within vegetated and non-vegetated reaches during the low-flow period, and other DOC sources may have dominated within the non-vegetated reaches during this period.

The activity of β-glu extracellular enzyme indicates the processing of low molecular-weight cellulose substrates from both allochthonous and autochthonous sources (Jones & Lock 1989, Romaní & Sabater 2000). The activity of β-glu was also generally higher within the non-vegetated reaches compared to the vegetated reaches during all sampling periods, although this was not statistically significant. These results indicate that there was a similar contribution of plant polysaccharides to the DOC pool within vegetated and non-vegetated streams. The activity of β-glu in biofilms was also not significantly correlated with canopy cover within agricultural streams located in Pennsylvania, USA (Rier et al. 2011). In a study of differences in biofilm enzyme activity in streams draining native, pasture and pine catchments in New Zealand, the activity of β-glu was found to be higher in pasture catchments although this was also not statistically different (Findlay et al. 1997). Similarly, within urbanised streams the activity of β-glu within stream water was also found to be slightly but not significantly higher (approximately 0.7 μM 4-MUB h^{-1}) compared to less-urbanised streams (Harbott & Grace 2005). The urban streams did show an increase in DOC concentration and esterase activity with urbanisation, which the authors suggested to indicate higher rates of C processing (Harbott & Grace 2005). Esterase activity was not measured in this study, so it is difficult to determine whether the increase in β-glu activity in some reaches was due to higher rates of C processing of low-molecular weight cellulose substrates. There were significant positive correlations between β-glu activity with CBOM and FBOM within the non-vegetated reaches which could also suggest that the variation in activity was due to differences in C processing. Alternatively, high-molecular weight organic matter can undergo photodegradation during high-light conditions (Weigner et al. 2001, Howitt et al. 2008). This may mean that during the low-flow periods when large amounts of PAR would have penetrated the water column at non-vegetated reaches, high-molecular weight organic matter may have been subject to photodegradation leading to an increase in β-glu activity.

The presence of xylo activity indicates the processing of xylooligosaccharide substrates usually derived from allochthonous plant material (Romaní & Sabater 2000). There was minimal xylo activity during the low and no-flow periods, however, the activity then increased within both vegetated and non-vegetated reaches during the high-flow periods. There was slightly higher xylo activity within the vegetated reaches during the second high-flow sampling period. This indicates that there were generally similar processing rates of plant-derived xylooligosaccharide substrates occurring within the vegetated and non-vegetated reaches. Similarly, there was no significant difference in the xylo activity of biofilms in streams draining native, pasture and pine catchments in New Zealand (Findlay et al. 1997). In contrast, xylo activity was found to be higher within less-urbanised streams and although no results were presented, this was suggested to be attributed to larger amounts of litter input from riparian vegetation (Harbott & Grace 2005). The streams in this study showed that there was no significant difference in total coarse benthic organic matter (CBOM) between vegetated and non-vegetated reaches during the four different sampling times (see Chapter 4), which adds support to why there was no significant difference in xylo activity between vegetated and non-vegetated reaches. However, there was some variation in the composition of CBOM (although found to be non-significant) between vegetated and non-vegetated reaches with larger amounts of wood, and *Casuarina cunninghamiana* needles and reproductive structures at some vegetated reaches (see Chapter 4). The results of the linear regressions showed that there was a positive significant correlation between xylo activity and *C. cunninghamiana* reproductive structures within the vegetated reaches, but there were no other significant correlations between xylo activity and the organic matter variables within the vegetated and non-vegetated reaches. This suggests that the xylo activity may not necessarily be related to the quantity of CBOM present within these streams and that the increase during the high-flow periods is attributed to leaching from organic matter and soils in upland and riparian zones as the wetted area increases. In addition, xylo activity has also shown to increase in intermittent streams during periods of fragmentation when there is a low supply of low-molecular weight DOC available to heterotrophs (Ylla et al. 2010). Therefore, the low activity of xylo during the low and no-flow periods shows that low-molecular weight

carbon is not limiting heterotrophic activity within the vegetated or non-vegetated reaches of the current study.

Comparison of C-hydrolysing enzyme activities can provide insight into the dominant source of DOC within reaches. Within this study, there was a significant effect of hydrology or time on the rates of all C-hydrolysing enzymes. During the low-flow period, the β-glu and α-glu enzymes had relatively equal rates of activity at both vegetated and non-vegetated reaches, which indicates that there were similar quantities of autochthonous and allochthonous contributions of DOC present. The rate of xylo activity did not increase to similar or higher activity rates until the high-flow periods suggesting allochthonous contributions of DOC increased once flow resumed. These results are similar to a study using amino acid composition to determine the sources of DOC within an intermittent Mediterranean stream (Ylla et al. 2011). The study found that DOC was predominantly from autochthonous sources as surface water contracted, while during the expansion phase following the dry period, DOC was predominantly from allochthonous sources (Ylla et al. 2011). Similarly, in a study of a dry-land river waterhole, the sources of DOC supporting heterotrophic metabolism were from autochthonous production during the fragmented period (Fellows et al. 2007). This indicates that there was a larger allochthonous contribution of DOC during the high-flow periods and suggests that the quality of DOC is lower during the low and no-flow periods compared to the high-flow periods, as it would primarily consist of low-molecular weight compounds.

Patterns in AP and LAP nutrient acquiring enzymes

In this study, there was no significant difference in the activity of AP and LAP enzymes between vegetated and non-vegetated reaches. Other studies that have been more focused on the effects of riparian vegetation on sources of DOC have examined the activity of LAP and/or AP to determine the contribution of peptidases and phosphatases to the dissolved organic matter or DOC pool (Harbott & Grace 2005, Ylla et al. 2010). As previously discussed, a significant difference in LAP activity existed between urban and less-urbanised streams and was attributed to the release of peptidases from algae to the DOC pool (Harbott & Grace 2005).

In other studies, activities of the AP and LAP extracellular enzymes have previously been used to indicate nutrient limitation (Sinsabaugh et al. 2010, Hill et al. 2010a), however, these studies have been developed along a pre-determined nutrient gradient. Additionally, to estimate the stoichiometric balance of heterotrophic EEA, the activity rates were normalised to the microbial/bacterial biomass present in the sample in these studies (Sinsabaugh et al. 2010, Hill et al. 2010a). In this study, biomass was not measured so the stoichiometric balance of EEA cannot be determined or compared to other studies. The results of the nutrient surveys showed that there were no significant differences in surface water nutrient concentrations between vegetated and non-vegetated reaches used in this study (see Chapter 3). Therefore, the lack of any significant difference in AP and LAP enzyme activity is not surprising.

Despite the lack of a nutrient gradient between reaches in this study, there were very low concentrations of NOx present within both the vegetated and non-vegetated reaches on most sampling occasions and N was also identified as the most limiting nutrient for autotrophic production (see Chapter 3). Analysis of the overall rates of EEA showed that the LAP enzymes, which are associated with microbial uptake of N, exhibited the highest rates of activity within both the vegetated and non-vegetated reaches. Increased LAP activity relative to other enzymes have also been observed in systems that are N-limited (Hill et al. 2006, Hill et al. 2010a). This suggests that N is also the most demanded resource by the microbial communities within both vegetated and non-vegetated reaches.

There were also no significant correlations between the nutrient related enzymes and the nutrient concentrations in this study. A study of 31 streams across an agricultural nutrient (TP and TN) gradient in Pennsylvania, USA, found a significant correlation between AP activity (denoted as APA in their study) and surface water TP concentration (Rier et al. 2011). There were no significant correlations between TN and LAP activity (denoted as LAMP in their study), which may have been due to the streams not being N limited. In contrast, a study of EEA associated with microbial communities within river sediments across 447 sites characterised by different land uses found no correlation between the LAP and other peptidase enzymes with N-limitation (Hill et al. 2010a). The authors suggested that may have been due to N only being

limited relative to elevated C and P concentrations. Alternatively, other studies of nutrient limitation have also used a wider range of EEA's that are related to N and P uptake by heterotrophic communities including NAG (Hill et al. 2006), valine aminopeptidase and cystine aminopeptidase (Tiqua 2011) for N-limitation and acid phosphatase and phosphohydrolase for P-limitation (Tiqua 2011). In this study, the activity of NAG could not be detected and no other N or P associated substrates were used. This may explain why there were no significant correlations with the activity of LAP or AP with any of the particulate or dissolved nutrient concentrations and suggests that a range of N and P associated substrates may need to be employed.

EEA as a method of determining DOC sources

Only a limited number of studies have used rates of EEA to assess sources of DOC within the water column, which makes it difficult to make comparisons between the rates of this study and other EEA studies. Many studies focussing on rates of EEA within biofilm communities growing on various substrates (Jones & Lock 1989, Romaní & Sabater 2000, Rier et al. 2007, Rier et al. 2011) or within sediments (Burns & Ryder 2001, Hill et al. 2006, Hill et al. 2010a), which are normalised to weights or area rather than volume. Compared to other published studies of water column rates of dissolved EEA, the rates of EEA in this study were up to an order of magnitude higher in some cases (Chappell & Goulder 1995, Harbott & Grace 2005, Ylla et al. 2010).

These differences in EEA rates highlight a potential limitation of using EEA, in that direct comparison of rates of EEA between studies can be difficult even when comparing samples from similar fractions of microbial activity. This is mostly due to there being inconsistencies in laboratory methods (Boon 1990). For example, the concentrations of 4-MUB substrates added to samples or the substrate to sample ratio can vary between studies (Boon 1990).

Differences in the temperature of substrate and sample incubation can also affect the rates of enzyme activity (Boon 1991), which can present issues when relating the data back to the natural systems. This is because the effects of differences in in-stream water temperature may not be detected within the laboratory analyses. However, in this study the large differences in

C-hydrolysing enzyme activities between sampling times is most likely a good representation of the change in dominant C sources.

Determining the contributions of allochthonous and autochthonous sources to the DOC pool can potentially illustrate the rates of ecosystem productivity (GPP) and respiration (ER). In this context, the α-glu EEA results would suggest that there are higher levels of GPP within the non-vegetated reaches compared to the vegetated reaches. If this suggestion was true the very high levels of α-glu activity within the downstream vegetated control reach, Booralong Bridge, and the non-vegetated Moredun Creek reach would have the highest rates of GPP across all sampling periods. Additionally, heterotrophic bacteria will aim to acquire carbon and nutrients in the most energetically efficient way (Sinsabaugh & Moorehead 1994), with preference for low-molecular weight organic matter sources over high-molecular weight sources (Tolunen et al. 1992). Therefore, the higher rates of xylo EEA during the high-flow period relative to β-glu and α-glu EEA could also suggest that in-stream GPP may have been reduced during this period. This would lead to an increase in the demand for high-molecular weight allochthonous organic matter sources and lower productivity/respiration (P/R) ratios. Further investigation into the rates of in-stream GPP and ER, and the metabolic balance of the system would provide more evidence of the relationships between metabolic processes and EEA.

Alternative methods apart from EEA have used to determine sources of DOC include phospholipid-fatty acid analyses (Boon 1991, Boon & Sorrel 1991), amino acid analyses (Ylla et al. 2011), solid phase microextraction-gas chromatography (Zander et al. 2007), and excitation-emission analyses (McKnight et al. 2001, McDonald et al. 2004). Although these methods can provide a more detailed analysis of the type of DOC present within systems (i.e. the aromacity), they cannot give insight into the heterotrophic utilisation of DOC. Furthermore, the same limitations in using laboratory equipment with different sensitivities can apply when comparing results between studies (McKnight et al. 2001). Stable isotope analyses have also been widely employed to determine the sources of DOC driving food webs (e.g. Bunn & Boon 1993); however, this technique again may not also give insight into the heterotrophic utilisation of DOC present within the system. This limitation is further supported by the lack of significant

correlations between DOC concentration and activities of any of the three C-hydrolysing enzymes found in this study. This result indicates that the lability of DOC can vary within equal concentrations present within the water column.

Correlation of biophysical variables to variation in enzyme activity

Following the temporary-river ecology theory, a larger variation in C-hydrolysing EEA's was expected within and between vegetated and non-vegetated reaches during the low and no-flow period. The results of this study showed that the largest variation in the three C-hydrolysing EEA's between reaches was during the no-flow period followed by the first high-flow period (Figure 6.1).

During the no-flow period, the variation between reaches in the three C-hydrolysing EEA's was driven by differences in the rates of α-glu and β-glu activity. The results of the DISTLM tests showed that the variation in β-glu activity was most correlated with the DOC:TN ratio within both vegetated and non-vegetated reaches, while the activity of α-glu was most correlated with the DOC:TN within the vegetated reaches and TN within the non-vegetated reaches. The DOC:TN ratio decreased during the high-flow periods which was driven by a decline in the TN concentration (see Chapter 3). As the N concentration measured in the TN samples includes the concentration of phytoplankton and microbial seston (Dodds 2003), the variation during the low-flow periods and the decline in the α-glu and β-glu EEA's during the high-flow periods may be attributed to a change in the phytoplankton and seston biomass within the reaches. Exploration of the rates of productivity and respiration would provide further support for this prediction (see Chapter 6).

During the first high-flow period sampled in this study, which was one-month after continuous surface water flow, the variation between reaches was driven by differences in the rates of xylo activity. This indicates that there was an increase in allochthonous sources of DOC present within reaches, and therefore it was expected that this would be positively correlated with CBOM fractions. Within the vegetated reaches, xylo activity was positively correlated with wood and *Casuarina* reproductive features at the whole-reach scale, which increased during the high-flow periods at this spatial scale (see Chapter 4). However, at the non-vegetated

reaches there were no correlations between xylo activity and CBOM fractions. In addition, there were strong positive correlations between flow percentile and TP concentrations within both vegetated and non-vegetated reaches. A number of studies have shown increases in allochthonous contributions to DOC due to increased run-off during high-precipitation events (e.g. Wilson & Xenopolous 2008, Saraceno et al. 2009, Bass et al. 2011). In these study reaches, although there was a decrease in TP concentration during the high-flow events there was actually an increase in TP imported loads, which was suggested to be also due to an increase in soil surface run-off. The significant correlations with flow percentile suggest that the increases in both xylo activity and TP are largely attributed to desorption of DOC and TP from terrestrial soils or the incorporation of these nutrients with eroded and transported soil.

5.5 Summary

There was a significant difference in rates of α-glu activity between vegetated and non-vegetated reaches. There was also a significant effect of hydrology (time) on all three C-acquiring enzymes. The activities of the α-glu and β-glu enzymes were highest within the vegetated and non-vegetated reaches during the low and no-flow periods, and generally decreased during the high-flow sampling periods. The α-glu and β-glu activity during the low and no-flow periods indicates that high-quality DOC sources were not limiting during these periods of surface water fragmentation. During the high-flow periods, the rate of xylo activity increased within both vegetated and non-vegetated reaches, which suggests that organic matter is being imported from upstream terrestrial or adjacent riparian zones during these periods.

There was no significant difference in rates of AP and LAP activity between vegetated and non-vegetated reaches. There was a significant effect of hydrology (time) on the rates of AP and LAP activity. The activity of AP was very low during the low-flow period. The increase in AP activity varied across all reaches during the no-flow and first high-flow sampling periods. However, the AP activity was consistently higher across all reaches during the second high-flow sampling period compared to the low-flow period. There was a lot of variation in the rates of LEU activity within and between vegetated and non-vegetated reaches. The activity of LEU generally

increased within most vegetated and non-vegetated reaches during the no-flow and second high-flow sampling period.

The variation in the three C-hydrolysing EEA's between reaches was during the no-flow period followed by the first high-flow period. As the sources of DOC can indicate the dominant metabolic processes present within reaches, the high activity of the α-glu and β-glu enzymes during this period suggests that there will be increased chlorophyll *a* levels and rates of primary productivity. The high activity of xylo enzymes during the high-flow periods suggests that there will also be an increased rates of respiration during this period that may be attributed to CPOM that has become retained within reaches. These predictions will be further explored in Chapter 6.

Chapter 6: Ecosystem metabolism dynamics within vegetated and non-vegetated reaches.

6.1 Introduction

Ecosystem metabolism is the term used to describe the ecological processes of carbon production and respiration by autotrophic and heterotrophic biota. In-steam autotrophs including phytoplankton, benthic and epiphytic algae, and macrophytes are responsible for the photosynthetic production of autochthonous sources of organic carbon within the water column, referred to as gross primary production (GPP). Autotrophic respiration and heterotrophic decomposition of organic matter are the main processes of carbon utilisation within aquatic systems and together are referred to as ecosystem respiration (ER). The process of ER consumes dissolved oxygen (DO) from the sediments or water column, while DO is produced during GPP processes. As both rates of GPP and ER are governed by nutrient availability, predominantly N and P, ecosystem metabolism is therefore an important facilitator of biotic carbon and nutrient cycling at the basal food web level in aquatic systems (Sterner & Elser 2002, Cross et al. 2005). The rates at which ecosystem metabolism occur in aquatic systems can be influenced by many biophysical variables including: C:nutrient stoichiometry (C:N:P), organic matter quantity and quality, and hydro-geomorphic/environmental parameters (see Chapters 3, 4, and 5). Therefore, exploring the response of ecosystem metabolism to altered environmental parameters can provide insight into the dominant ecosystem processes in streams.

The balance between the rates of GPP and ER, which is calculated as the P/R ratio, can indicate the dominant processes within aquatic systems (Rosenfeld & Mackay 1987, Meyer 1989). A study across eight different biomes in North America found that most streams had a P/R ratio of < 1, indicating that ER was the dominant metabolic process (Mulholland et al. 2001). The P/R ratios can also provide some insight into the sources of organic carbon that are driving heterotrophic respiration (Rosenfeld & Mackay 1987, Meyer 1989).

Net ecosystem production (NEP) is the difference between the rates of GPP and ER. When the rate of GPP (i.e. the autotrophic production of carbon) exceeds the rate of ER (i.e. the respiration of carbon) the NEP will be positive and the system will be considered autotrophic.

Within autotrophic systems, the quantity of organic carbon produced through GPP (autochthonous sources) can sustain the heterotrophic demand for organic carbon. Conversely, when the rate of ER exceeds GPP, NEP will be negative and the system will be considered heterotrophic. This may mean that the heterotrophic community is reliant on other sources of organic matter being transported to the system such as, allochthonous sources or other autochthonous sources from upstream reaches (Ylla et al. 2010).

In aquatic systems, different autotrophic functional groups (i.e. groups that differ according to vegetative form, role or habitat) can contribute to the whole-system metabolic rates (Acuña et al. 2011). For example, in upland streams, various autotrophic functional groups such as benthic algae, floating macroalgae and macrophytes can all have differing rates of GPP and therefore also regulate the quantities of available organic carbon within the system (Acuña et al. 2011). Similarly, different heterotrophic communities can be associated with different habitats such as biofilms (Tank & Webster 1998, Cardinale et al. 2002) or accumulations of benthic organic matter (Gessner et al. 1999, Bärlocher et al. 2011, Bärlocher et al. 2012 – see Appendix 1). As the physical template is important in determining the types of habitats available for autotrophic and heterotrophic activity, then differences in channel morphology and features between reaches may lead to varying rates of GPP and ER, as well as, the dominant processes that control nutrient retention and cycling.

Anthropogenic disturbances

A number of studies have shown that the removal of riparian vegetation and other land-use practices have altered metabolic rates of streams. In some cases, this has led to changes to the trophic state of the system, predominantly from heterotrophic to autotrophic (Fellows et al. 2006a, McTammany et al. 2007, Bernot et al. 2010). As removal of riparian vegetation can increase the amount of light reaching the water column and lower the surface area:volume ratio of reaches through channel widening, conditions for autotrophic growth are optimised, particularly for benthic algae and macrophytes (Bunn et al. 1999, Julian et al. 2010). The increase in GPP is therefore responsible for the increase in P/R ratio and the shift to autotrophy.

In contrast, streambank erosion can increase with the removal of riparian vegetation, resulting in the transport and deposition of large amounts of sediment or inorganic material through the system (Prosser et al. 2001, Pusey & Arthington 2003). Increases in surface water turbidity can reduce the amount of light penetrating the water column and reaching the benthos, which may decrease rates of GPP (Rier & King 1996). In addition, unstable sediments may smother biofilm communities and produce anoxic habitats, which may lead to reduced rates of GPP and ER, and shift NEP to heterotrophy (Rier & King 1996, Uehlinger 2000).

The removal of riparian vegetation can also lead to reduced quantities of allochthonous organic matter imported to streams (Campbell et al. 1992, Sabater et al. 2000, Reid et al. 2008). Streambank erosion has also caused reductions in reach heterogeneity, which subsequently leads to decreases in retention and standing stocks of organic matter (Reid et al. 2008, Watson & Barmuta 2010). In some aquatic systems where the heterotrophic community depends on allochthonous organic matter, this decrease has been shown to lead to lower rates of heterotrophic respiration (Aldridge et al. 2009).

The concentration and stoichiometric balance of nutrients can influence both production and respiration by autotrophic and heterotrophic communities (see Chapter 3, Redfield 1958, Elser et al. 2007, Menéndez et al. 2011). In some agricultural streams, nutrient concentrations have increased due to the removal of riparian vegetation and the wide-spread application of fertilisers (Townsend & Riley 1999, Harris 2001). The increase in stream concentrations of N and P can lead to decreases in C:nutrient stoichiometry (also referred to as C:nutrient ratios), which has been shown to increase GPP through enhanced autotrophic growth and production of high-quality low-molecular weight C (Rier & Stevenson 2002, Sabater et al. 2011). Similarly, a general increase in the availability of N and P within the water column can lead to an increase in heterotrophic decomposition of organic matter and therefore rates of ER (Stelzer et al. 2003, Benstead et al. 2009).

Hydrology

The metabolic state of some temporary streams have shown to alternate from autotrophy during periods of surface water fragmentation to heterotrophy during high-flow or expansion periods (Fisher et al. 1982, Uehlinger 2000). Increases in surface water velocity can scour biofilms (Uehlinger & Naegeli 1998, Uehlinger 2000) and flush or dilute phytoplankton (Mitrovic et al. 2011) leading to decreased rates of GPP, while unstable bed substrates can inhibit the formation of biofilms and smother organic matter, which can decrease rates of GPP and ER (see Chapter 4, Rier & King 1996, Atkinson et al. 2008).

In contrast, some studies have found that the increase in wetted area can initiate microbial activity (Wilson et al. 2011), although these patterns have been difficult to identify in some intermittent streams (Larned et al. 2007). Large quantities of allochthonous DOC can be transported to streams during precipitation and storm events (Kaplan & Newbold 1993, Boyer et al. 1997). As this allochthonous DOC is characterised by high C:nutrient ratios (Kaplan & Newbold 1993, Boyer et al. 1997), which requires a higher rate of heterotrophic decomposition (Tulonen et al. 1992), there may be an increase in rates of ER (Gibson & O'Reilly 2012). This provides further suggestion that the metabolic state of some reaches may shift to heterotrophy during high-flow or expansion periods.

The reaches used in the present study showed that there was evidence of large contributions of algal-derived DOC and high rates of C processing during the low and no-flow periods (see Chapter 5). This suggests that there will be higher rates of GPP and ER during these periods, and the reaches would be autotrophic. In contrast, the contribution of algal derived DOC decreased during the high-flow periods with a higher contribution from allochthonous sources. It would be expected that the rates of GPP may be reduced during the high-flow periods, however, the rates of ER may increase leading to reaches dominated by heterotrophic processes.

Aims

The aim of this chapter is to explore differences in reach-scale ecosystem metabolism between vegetated and non-vegetated reaches, across four different discharges.

1) I aim determine how the presence of riparian vegetation affects rates of ecosystem metabolism and trophic state. I predict higher rates of GPP within non-vegetated reaches compared to vegetated reaches. I expect that there will be higher rates of ER within vegetated reaches compared to non-vegetated reaches, however, the larger quantities of labile DOC present within the non-vegetated reaches could increase ER rates in these reaches. Additionally, I predict that NEP will be positive within non-vegetated reaches, and negative within vegetated reaches.
2) Through sampling macrophyte biomass and water column chlorophyll *a* concentrations, I aim to explore whether there is a correlation between macrophyte biomass and/or chlorophyll *a* concentrations with GPP rates. I expect there to be larger biomass of macrophytes and chlorophyll *a* at non-vegetated reaches. I also aim to explore if there is an interaction between these autotrophic functional groups and hydro-geomorphic variables.
3) Using a step-wise regression technique, a further objective is to determine the relationships and interactions between the metabolic parameters (net change in DO, GPP, ER, P/R ratio) with hydro-geomorphic variables, nutrient concentration and stoichiometry, organic matter quantity and quality, nutrient and organic matter retention, and chlorophyll *a* concentration and macrophyte biomass. I also aim to explore the relationships between the metabolic parameters and DOC sources using the extracellular enzyme activity (EEA) data from Chapter 5. I predict that GPP will be more strongly positively correlated with chlorophyll *a* concentration, macrophyte biomass and α-glu activity within the non-vegetated reaches compared to vegetated reaches. I also predict that ER will be more strongly correlated with the organic matter variables and xylo activity within the vegetated reaches.

6.2 Methods

Ecosystem metabolism

Ecosystem metabolism was determined by monitoring the change in dissolved oxygen (DO) for 36 hours at each site, during four discharge events of different magnitudes. An open-system, two-station technique was used when surface water was flowing to monitor the DO change in the same parcel of water travelling through the reach (Grace & Imberger 2006). During no-flow periods, a single station method was used (Grace & Imberger 2006). Both the upstream and downstream sites on a single stream were monitored concurrently and the stations were set up at approximately 12:00 hrs at a stream and deployment was for one full diel cycle (i.e. two nights). The equipment was collected at approximately 07:00 hrs and moved to the next stream, so that the metabolism of all five streams could be monitored within a 10 day period.

The DO concentrations were measured with membrane probes attached to meters (Orion Star Series, Thermo Fisher Scientific, USA), which were set at 15 minute logging intervals. Each probe was calibrated in water-saturated air at each site, immediately before deployment. To correct for probe drift and differences in calibration, the probes were placed together for 30 to 60 minutes at the start of each deployment. The meters were attached to steel pickets in well-mixed areas of the stream channel at the start and end of each reach, with the probes reading at approximately half the surface water depth. During no-flow periods the probes were placed in the deepest area of the largest pool within each study reach.

Determination of the reaeration coefficient

I used the surface renewal method (SRM, Owens et al. 1964) to estimate K_2, which is based on mean reach depth and velocity, and is suitable for streams with depth and velocities of between 0.12 – 3.55 m s^{-1} and 0.3 – 1.50 m s^{-1}, respectively (Grace & Imberger 2006). The reaeration coefficient at 20°C, $K_{2(20°C)}$ in cm h^{-1}, is given by the equation: $K_{2(20°C)} = 50.8 \times (v^{0.67}) \times (D^{-0.85})$, where v = velocity (cm s^{-1}) and D = depth (cm). The mean reach depth, D, was calculated using the equation D = Q / (v x W), where Q = discharge, v = velocity, and W = average stream wetted width. The average stream wetted width, W, was estimated from the

measuring the water height along a known transect across the stream. These values were used to extract an outline of the area inundated at that particular water height from the reach DEMs (as outlined in Chapter 2). During each deployment, average reach velocity across a defined downstream transect was recorded at approximately 1 m intervals to calculate discharge in m^3 sec^{-1} using a flowmeter (Marsh McBirney Flowmate, model 2000, see Chapter 2). I used the equation of Elmore and West (1961), $K_{2(t°C)} = K_{2(20°C)} \times 1.024^{(t-20)}$ to adjust $K_{2(20°C)}$ to the average surface water temperature at hourly intervals, where *t*, is the average hourly stream temperature calculated from each of the DO probes.

Analysis of dissolved oxygen change

The temperature corrected K_2 values and the DO saturation deficits were used to correct for the change in DO attributable to reaeration at hourly intervals (Marzolf et al. 1994, Young & Huryn 1998). The DO saturation deficit was determined as the difference between the measured DO values and the 100% saturation value (defined as the equilibrium concentration at the recorded surface water temperature and barometric pressure), which were recorded by the DO meters.

The reaeration-corrected change in DO was used to calculate reach metabolic parameters (net change, GPP, ER and P/R ratio). From the 36 hour deployment period, the 24 hr period between midnight and midnight was selected to represent the diel light-dark cycle. The reaeration-corrected DO measurements were then plotted against time (from midnight to midnight) and fitted with linear curves ($y = mx + c$). The total net DO change (mg L^{-1}) over 24 hrs for each meter deployed (upstream and downstream) was determined by summing the slope value (m) of each linear curve fitted. Linear curves were fitted to time intervals that gave the best r^2 values (< 0.90), which were usually 3-5 hr intervals (for e.g. 0900-1200 hrs). This method is similar to that of Ryder et al. (2004) and allows for the correction of probe drift by calculating the trend in DO change rather than the absolute values. The values of daily GPP and ER were the sum of the positive and negative slope values (m), respectively. The daily net change in DO was estimated by subtracted the reaeration-corrected rates of ER from GPP. Daily rates of C-fixation were estimated by multiplying the net change in DO values by 0.375 (Ryder 2004). Whole-reach estimates of C-fixation (NEP) were determine by multiplying the daily rates of C-

fixation by the corresponding reach volumes as determined in Chapter 2. No net change in DO, GPP or ER could be calculated at the Gwydir non-vegetated reach during September, as the changes in DO detected could not separate probe drift from very low DO metabolism.

Light measurements

During the February 2010 field trip, a quantum sensor attached to a data logger (Starlogger model 2004C, Unidata, Australia) recorded photosynthetic active radiation (PAR) at 5 minute intervals, however, this dataset was unfortunately lost when there was a fault with the logger. Throughout the May, September and November 2010 field trips, light measurements (lumens m^{-2}) were recorded at the stream surface using HOBO pendant light data loggers (onsetcomp.com), set at 15 minute intervals.

Chlorophyll a surveys and analysis

Triplicate water column samples for chlorophyll *a* analysis were collected at fixed upstream and downstream transects of each reach at each deployment. The water samples were processed within 24 hours of collection, where approximately 500 mL of water was filtered through a 47 µm glass fibre filter (Whatman GF/F) and stored at -4°C until further analysis. Chlorophyll *a* was extracted using 90% acetone, and the concentrations determined by spectrophotometric absorption, following the method of Parsons et al. (1984). Reach-scale chlorophyll *a* mass-balance and retention was estimated for the low and high-flow periods by multiplying the mean concentrations by daily discharge. Discharge was calculated for each sampling occasion following the methods outlined in Chapter 2. Retention or export was then determined by subtracting the exported (downstream) load from the imported (upstream) load (Von Schiller et al. 2011b).

Macrophyte biomass surveys and processing

The biomass of dominant macrophyte species at each site was determined from six permanent transects. On two of the four sampling periods (February and May 2010), percent cover was estimated at six random locations along each of the six transects, using a 0.25 m^2 quadrant (n=36 per site). At each random location, the percent cover and height of the major taxa were

recorded. The six permanent transects at each reach were chosen randomly and the six quadrat measurements represented approximately 10 – 30% of the reach width. Unfortunately the high water levels during the September and November 2010 sampling meant that the original sampling method could not be employed, as the macrophytes along transects were predominantly submersed. Alternatively, I measured the length and width of visible macrophyte patches and estimated the percent cover and height of the major taxa from three randomly placed quadrats (0.25 m^2). As these surveys were only conducted on macrophyte patches visible at each reach, the total number of samples (n) varied between reaches.

On all four sampling occasions, percent cover was converted to biomass using samples of each plant species collected from each study reach. New samples were collected on each survey to account for the difference in growth. At each site, above-ground plant material of each species present in the particular survey was collected. For each species, triplicate samples were collected for three different values of percent cover (25%, 50%, and 100%). Samples were placed in sealed bags and frozen upon return from the field until analysis. For processing, the plant material was rinsed to remove inorganic material, epiphytes and macroinvertebrates, and then dried at 65°C for 48 hours to constant weight. To obtain ash-free dry weights (AFDW), the dried material was weighed, ashed at 540°C for three hours and then re-weighed. The AFDW biomass (g m^{-2}) was then related to percent cover using linear regression (Appendix 6). Macrophyte biomasses were calculated for each transect or patch within each reach during each sampling occasion by averaging values from quadrants within each transect or patch. Total biomass for each entire reach during the February and May sampling times was calculated by averaging the six transect measurements and then multiplying by the channel wetted area. The total biomass of each reach during the September and November sampling times was calculated by multiplying the patch average by the dimensions of the patch, and then adding the biomass of all patches within the reach together.

Statistical analyses

Metabolic parameters

A mixed-model ANOVA was used to identify significant differences in the change of metabolic parameters (net DO change, GPP, ER and P/R ratio) between vegetated and non-vegetated reaches and across time. The model consisted of three factors: i) Treatment – vegetated and non-vegetated (a=2; fixed), ii) Stream – Moredun Creek, Gwydir River, Roumalla Creek, Booralong Creek and Laura Creek (b=5; random), iii) Time – four sampling occasions (c=4; random). Individual analyses were run for each metabolic parameter using PERMANOVA. The statistical power of the main test was increased through replication at the treatment level, whereby the data from both the control and treatment streams were combined so that the analyses included five vegetated and five non-vegetated reaches. This meant that the statistical design became unbalanced (an unequal number of replicate samples within each factor level) and as such the formulae used for a balanced design (Table 6.1) were invalid. The correct formulae used for the unbalanced design as determined by the PERMANOVA program are listed in Appendix 5 and will be referred to throughout the results section. The net DO change, GPP, and ER data were square-root transformed with the sign reinstated prior to analysis to reduce skewness. PERMDISP analyses were used to test for significant differences in dispersions.

To discriminate between the effects of the main treatment (vegetation) and the natural longitudinal change in the different metabolic parameters, multivariate one-way ANOVAs were used to test for significant differences between locations (Lo) on each of the control streams. All four metabolic parameters were included in the multivariate dataset. Three ANOVAs were performed in which two tested the difference between locations on each of the two control streams separately, and a third test combined the data from both control streams. Data were pooled across time (hydrology) within each test and Monte-Carlo *p*-values were used when less than 100 unique permutations could be attained (see Appendix 3).

Linear regression analyses were conducted to explore the relationships between the four individual metabolic parameters and various biogeochemical variables. The rates of net DO change, GPP and ER were square-root transformed with the sign reinstated prior to analysis.

The predictor variables included: hydro-geomorphic variables, surface water nutrient concentration and nutrient stoichiometry, benthic organic matter variables, nutrient and organic matter mass-balance and retention, extracellular enzyme analyses (EEA), chlorophyll *a* concentration, chlorophyll *a* retention, mean daily surface water temperature, total suspended solids (TSS) concentration and macrophyte biomass, which are listed with their respective transformations in Table 6.2. Before conducting the analyses, residual plots were examined for outliers and datasets were checked for samples with large leverage. If identified, these values were removed and the new sample size reported within the results table. All analyses were conducted using SYSTAT (Version 12.0). As the risk of making Type I errors increase with the number of single linear regressions (Quinn & Keough 2002), the regressions were only accepted as significant if $p \leq 0.025$, following Bonferroni's principle (Quinn & Keough 2002). All analyses were conducted using SYSTAT (Version 12.0).

Following the single linear regressions, all predictor variables that were significantly correlated to the metabolic parameters were combined into a dataset, which was specific to each of the four metabolic parameters in vegetated and non-vegetated reaches. The datasets were then used to perform conditional tests within the DISTLM routine in PERMANOVA (outlined in Chapter 4). This was in order to determine which of the significant predictor variable(s) were most correlated with the variation in each metabolic parameter.

Light, chlorophyll a and macrophyte biomass

The three-factor ANOVA outlined above (Table 6.1) was also used to test for significant differences in light, chlorophyll *a* concentration and macrophyte biomass between vegetated and non-vegetated reaches, and the four sampling periods. Analyses were performed using the PERMANOVA program. Prior to analysis chlorophyll *a* and macrophyte biomass data were $\log_{(x+1)}$ and Box-Cox transformed, respectively, to reduce skewness. The light data were also $\log_{(x+1)}$ transformed prior to analysis. PERMDISP analyses were used to test for significant differences in dispersions.

To discriminate between the effects of the main treatment (vegetation) and the natural longitudinal change in light, chlorophyll *a* concentration and macrophyte biomass, multivariate

one-way ANOVAs were used to test for significant differences between locations (Lo) on each of the control streams. Three ANOVAs were performed in which two tested the difference between locations on each of the two control streams separately, and a third test combined the data from both control streams. Data were pooled across time (hydrology) within each test and Monte-Carlo *p*-values were used when less than 100 unique permutations could be attained (Appendix 3).

Table 6.1: Table of formulae for a balanced statistical design. Treatment is a fixed factor and has two levels (a=2), stream is a random factor and has five levels (b=5), and time is a random factor with four levels (c=4). Tables of correct formulae for the unbalanced design are listed in Appendix 5 and will be referred to in the results section.

	Source of variation	Multipliers				Degrees of freedom	Expected mean square	Variance component
		i	j	k	r			
1	**Treatment = Tr**	0	b	C	n	a-1	$\sigma^2_e + n\sigma^2_{TrStTi} + bn\sigma^2_{TrTi} + cn\sigma^2_{TrSt} + bcn\sigma^2_{Tr}$	$(MS_{Tr} - MS_e)/bcn$
2	**Stream = St**	a	1	C	n	b-1	$\sigma^2_e + an\sigma^2_{StTi} + acn\sigma^2_{St}$	$(MS_{St} - MS_e)/acn$
3	**Time = Ti**	a	b	1	n	c-1	$\sigma^2_e + an\sigma^2_{StTi} + abn\sigma^2_{Ti}$	$(MS_{Ti} - MS_e)/abn$
4	**Tr x St**	0	1	C	n	(a-1)(b-1)	$\sigma^2_e + n\sigma^2_{TrStTi} + cn\sigma^2_{TrSt}$	$(MS_{TrSt} - MS_e)/cn$
5	**Tr x Ti**	0	b	1	n	(a-1)(c-1)	$\sigma^2_e + n\sigma^2_{TrStTi} + bn\sigma^2_{TrTi}$	$(MS_{TrTi} - MS_e)/bn$
6	**St x Ti**	a	1	1	n	(b-1)(c-1)	$\sigma^2_e + an\sigma^2_{StTi}$	$(MS_{StTi} - MS_e)/an$
7	**Tr x St x Ti**	0	1	1	n	(a-1)(b-1)(c-1)	$\sigma^2_e + n\sigma^2_{TrStTi}$	$(MS_{TrStTi} - MS_e)/n$
8	**Residual = e**	1	1	1	1	abc(n-1)	σ^2_e	MS_e

Table 6.2: List of predictor variables and their respective transformations used in the regression analyses. SA:V = surface area:volume ratio, C-repro = *C. cunninghamiana* reproductive structures, UI = unidentified fraction, temperature = mean daily surface water temperature, TSS = total suspended solids.

Variable	Transformation	Variable	Transformation	Variable	Transformation
No. of features	-	Wood (g)	$Log_{(x+1)}$	DOC (g day^{-1})	√x with sign reinstated
Flow percentile	$Log_{(x+1)}$	C-repro (g)	$Log_{(x+1)}$	NOx (g day^{-1})	√x with sign reinstated
Velocity	$Log_{(x+0.1)}$	Leaf (g)	$Log_{(x+1)}$	SRP (g day^{-1})	√x with sign reinstated
SA:V	-	Macrophyte (g)	$Log_{(x+1)}$	TN (g day^{-1})	$x^{.33}$ with sign reinstated
Wetted area	-	UI (g)	$Log_{(x+1)}$	TP (g day^{-1})	√x with sign reinstated
CBOM (g m^{-2})	$Log_{(x+1)}$	DOC	-	CBOM (g day^{-1})	√x with sign reinstated
FBOM (g m^{-2})	$Log_{(x+1)}$	NOx	Box-Cox	FBOM (g day^{-1})	√x with sign reinstated
Needle (g m^{-2})	$Log_{(x+1)}$	SRP	1/(x+1)	Needle (g day^{-1})	√x with sign reinstated
Wood (g m^{-2})	$Log_{(x+1)}$	TN	$Log_{(x)}$	Wood (g day^{-1})	√x with sign reinstated
C-repro (g m^{-2})	$Log_{(x+1)}$	TP	1/(x+1)	C-repro (g day^{-1})	√x with sign reinstated
Leaf (g m^{-2})	$Log_{(x+1)}$	DOC:NOx	-	Leaf (g day^{-1})	√x with sign reinstated
Macrophyte (g m^{-2})	$Log_{(x+1)}$	NOx:SRP	-	Macrophyte (g day^{-1})	√x with sign reinstated
UI (g m^{-2})	$Log_{(x+1)}$	DOC:SRP	-	UI (g day^{-1})	√x with sign reinstated
CBOM (g)	$Log_{(x+1)}$	DOC:TN	-	AP	$Log_{(x+1)}$
FBOM (g)	$Log_{(x+1)}$	TN:TP	-	Leu	$Log_{(x+1)}$
Needle (g)	$Log_{(x+1)}$	DOC:TP	-	β-glu	$Log_{(x+1)}$
Temperature	$Log_{(x+1)}$	Chlorophyll *a*	$Log_{(x+1)}$	α-glu	$Log_{(x+1)}$
TSS	$Log_{(x+1)}$	Macrophyte biomass	Box-Cox	xylo	$Log_{(x+1)}$

6.3 Results

Ecosystem metabolism

Gross primary productivity

There was no clear difference in GPP rates between vegetated and non-vegetated reaches, and was supported by the non-significant result from the PERMANVOA test (Table 6.3). Despite this result, there was a trend in some treatment streams during the February and May deployment periods, where GPP rates at the Gwydir and Roumalla vegetated reaches decreased and their paired non-vegetated reaches increased. The PERMANOVA test showed that there was a significant effect of hydrology on daily GPP rates (p = 0.001, Table 6.3). Daily rates of GPP were highest at all reaches during February and May, the low and no-flow periods, and ranged between 1.64 and 10.36 mg O_2 L^{-1} day^{-1} (Figure 6.1). The positive values during February indicate that transport through each reach increased the rate of GPP, relative to the upstream surface water entering the reach. During September and November, the high-periods, the daily rates of GPP decreased to between -1.56 and 1.06 mg O_2 L^{-1} day^{-1}, with the exception of Booralong CS in November (Figure 6.1). The change in GPP rates relative to upstream surface water varied between reaches during these high-flow periods.

The tests for significant differences between locations on the control streams showed that there were no significant effects of location on the four metabolic parameters. The result was consistent for each test when data were combined between the two control streams and on each individual control stream (Appendix 4, Table A4.21).

Table 6.3: Results of PERMANOVA main test for significant differences in reach GPP (mg O_2 L^{-1} day^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	0.503	0.503	0.486	0.742
St	4	4.824	1.206	2.926	0.069
Ti	3	34.531	11.510	28.210	**0.001**
TrxSt**	2	0.556	0.278	0.856	0.511
TrxTi	3	4.321	1.440	4.433	0.087
StxTi	12	4.713	0.393	0.605	0.748
TrxStxTi**	4	1.300	0.325	0.501	0.701
Res	7	4.543	0.649		
Total	36	55.241			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.12 for correct formulae.

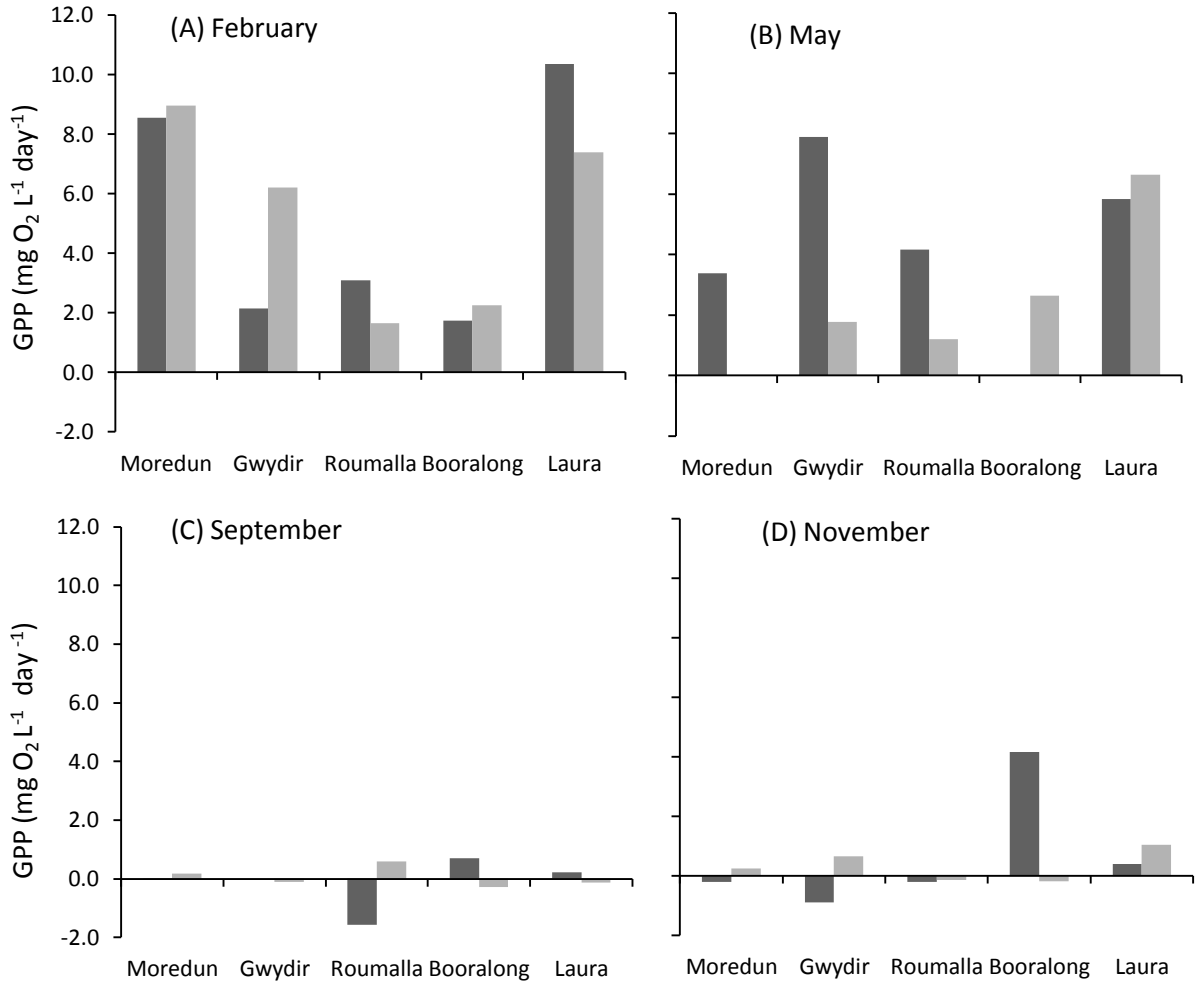

Figure 6.1: Reach GPP (mg O_2 L^{-1} day^{-1}) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Ecosystem respiration

There was also no significant difference in daily rates of ER between vegetated and non-vegetated reaches (Table 6.4). Between sampling times, the pattern of ER rates was very similar to the pattern of GPP rates and there was also a significant effect of hydrology (time) on ER rates (p = 0.001, Table 6.4). A negative ER value indicates that there was an increase in ER rates as surface water moved through reaches, while a positive value indicates that the rates of ER decreased as surface water moved through reaches (Figure 6.2). With the exception of the upstream non-vegetated control reach, Laura Homestead, the highest rates of ER occurred during February, the low-flow period, and ranged between -8.24 and -2.37 mg O_2 L^{-1} day^{-1} (Figure 6.2). The negative values during February show that all reaches increased in ER rates, with the exception of Laura Homestead, relative to the upstream surface water. During May, the rates of ER decreased across all reaches and ranged between -4.05 and -1.38 mg O_2 L^{-1} day^{-1} (Figure 6.2). During September and November, the high-flow periods, rates of ER ranged between -2.02 and 1.17 mg O_2 L^{-1} day^{-1} (Figure 6.2). Therefore, the change in rates of ER relative to upstream surface water varied across reaches during September and November. In addition, the low rates of GPP and ER during September and November across the reaches meant that the positive or negative net changes could not be consistently attributed to fluctuations in GPP or ER.

Table 6.4: Results of PERMANOVA main test for significant differences in reach ER (mg O_2 L^{-1} day^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	2.532	2.532	2.479	0.171
St	4	6.568	1.642	2.648	0.086
Ti	3	24.310	8.103	15.777	**0.001**
TrxSt**	2	$7.811e^{-2}$	$3.906e^{-2}$	0.171	0.843
TrxTi	3	3.229	1.077	4.715	0.091
StxTi	12	5.982	0.499	0.237	0.959
TrxStxTi**	4	0.913	0.228	0.109	0.859
Res	7	14.722	2.103		
Total	36	61.325			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.12 for correct formulae.

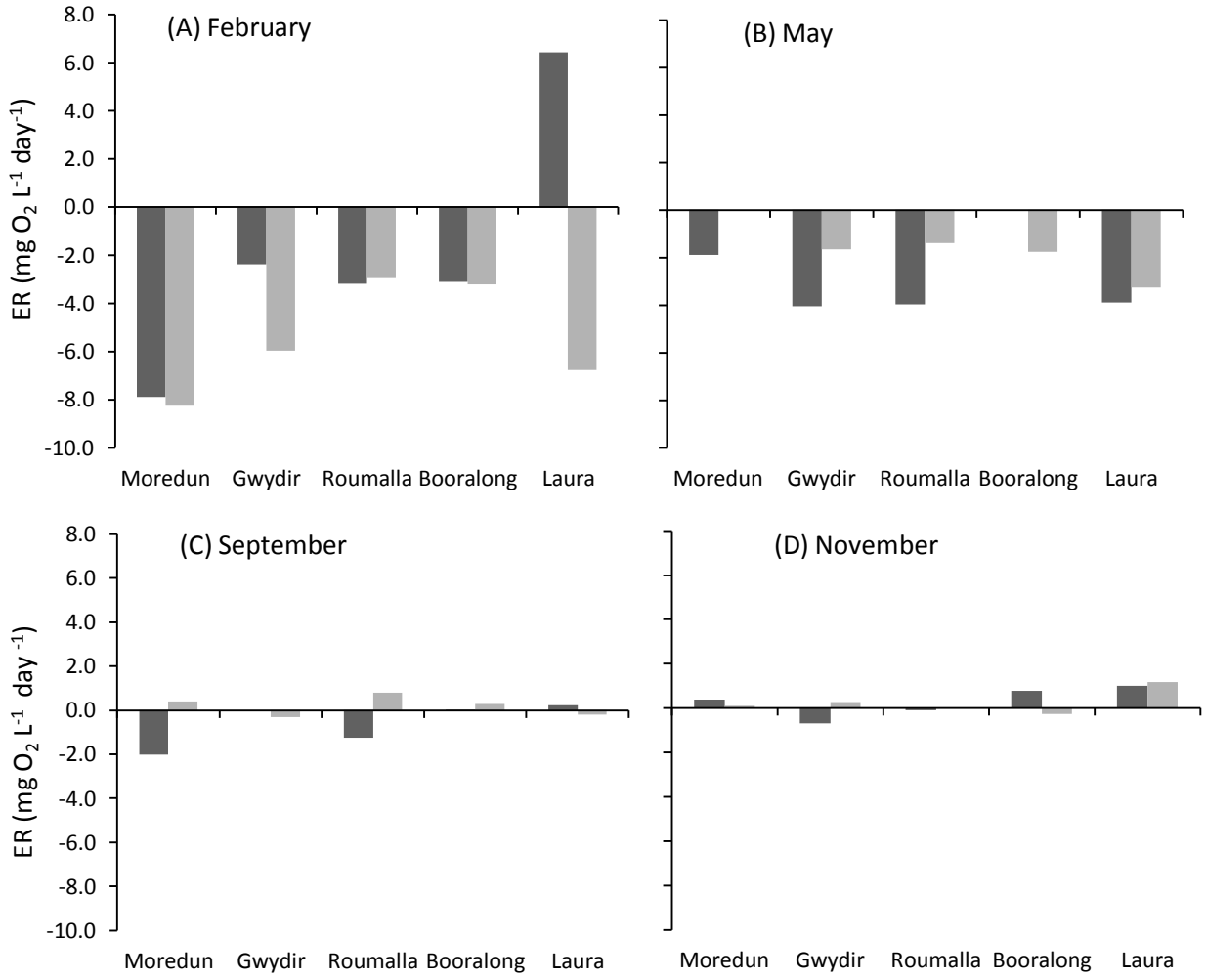

Figure 6.2: Reach ER (mg O_2 L^{-1} day^{-1}) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Net DO change

The net change in DO was highly variable across time and there appeared to be no clear pattern within or between vegetated and non-vegetated reaches (Figure 6.3). This was supported by the PERMANOVA test that showed that there was no significant effect of riparian vegetation (Table 6.5). There did appear to be a trend within some reaches across time, for example, the net change in DO was consistently negative at the Roumalla vegetated reach during all sampling times. At the Moredun and Gwydir vegetated reaches, the net change in DO was consistently positive during all sampling periods (Figure 6.5). There was a significant effect of hydrology on net change in DO (p = 0.029, Table 6.5). During May, the no-flow period, the net change in DO was positive at all non-vegetated reaches. With the exception of the upstream non-vegetated control reach, Laura Homestead, the positive net DO values also increased between February and May (Figure 6.3). The majority of net DO changes were between -2.00 and 2.00 mg O_2 L^{-1} day^{-1} across all sampling periods, however, these low values generally decreased to between -1.00 and 1.00 mg O_2 L^{-1} day^{-1} during September and November, the high-flow periods (Figure 6.3).

Table 6.5: Results of PERMANOVA main test for significant differences in reach net DO change (mg O_2 L^{-1} day^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	$9.137e^{-2}$	$9.137e^{-2}$	0.524	0.731
St	4	4.902	1.226	2.390	0.140
Ti	3	6.514	2.171	4.099	**0.029**
TrxSt**	2	0.276	0.137	0.384	0.724
TrxTi	3	2.220	0.740	2.069	0.215
StxTi	12	6.162	0.514	1.022	0.521
TrxStxTi**	4	1.431	0.358	0.712	0.614
Res	7	3.516	0.502		
Total	36	32.017			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.12 for correct formulae.

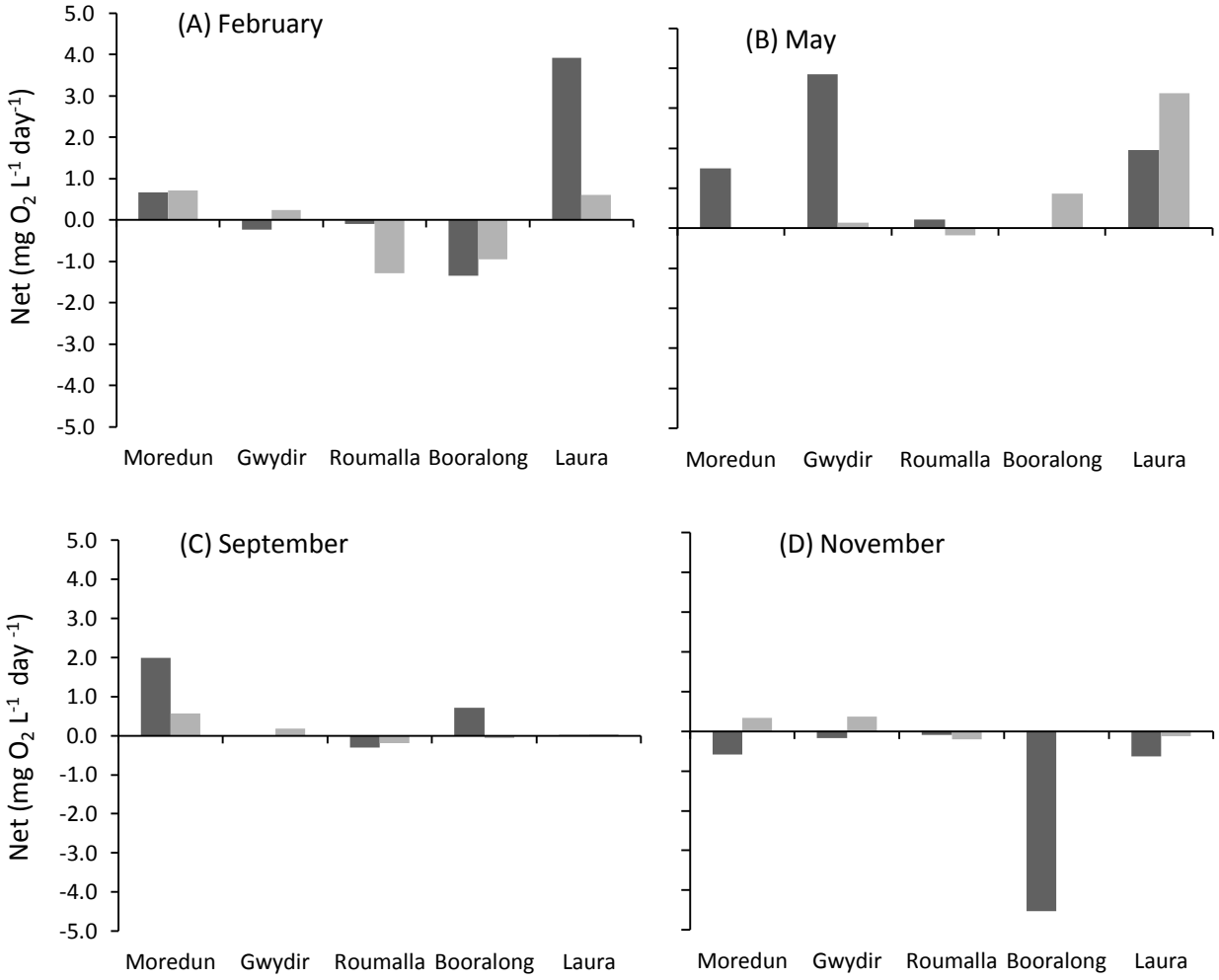

Figure 6.3: Reach net change in DO concentration (mg O_2 L^{-1} day^{-1}) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Net C-fixation and consumption

The rates of net C-fixation and consumption (also known as net ecosystem production, NEP) were identical to the pattern of net change in DO (discussed above). The highest rate of C-fixation was within the upstream non-vegetated control reach, Laura HD (1.47 g C L^{-1} day^{-1}), during February, the low-flow period (Figure 6.4). The highest rate of C-consumption was within the upstream vegetated control reach, Booralong CS (-4.53 g C L^{-1} day^{-1}), during November, the second high-flow sampling period (Figure 6.4).

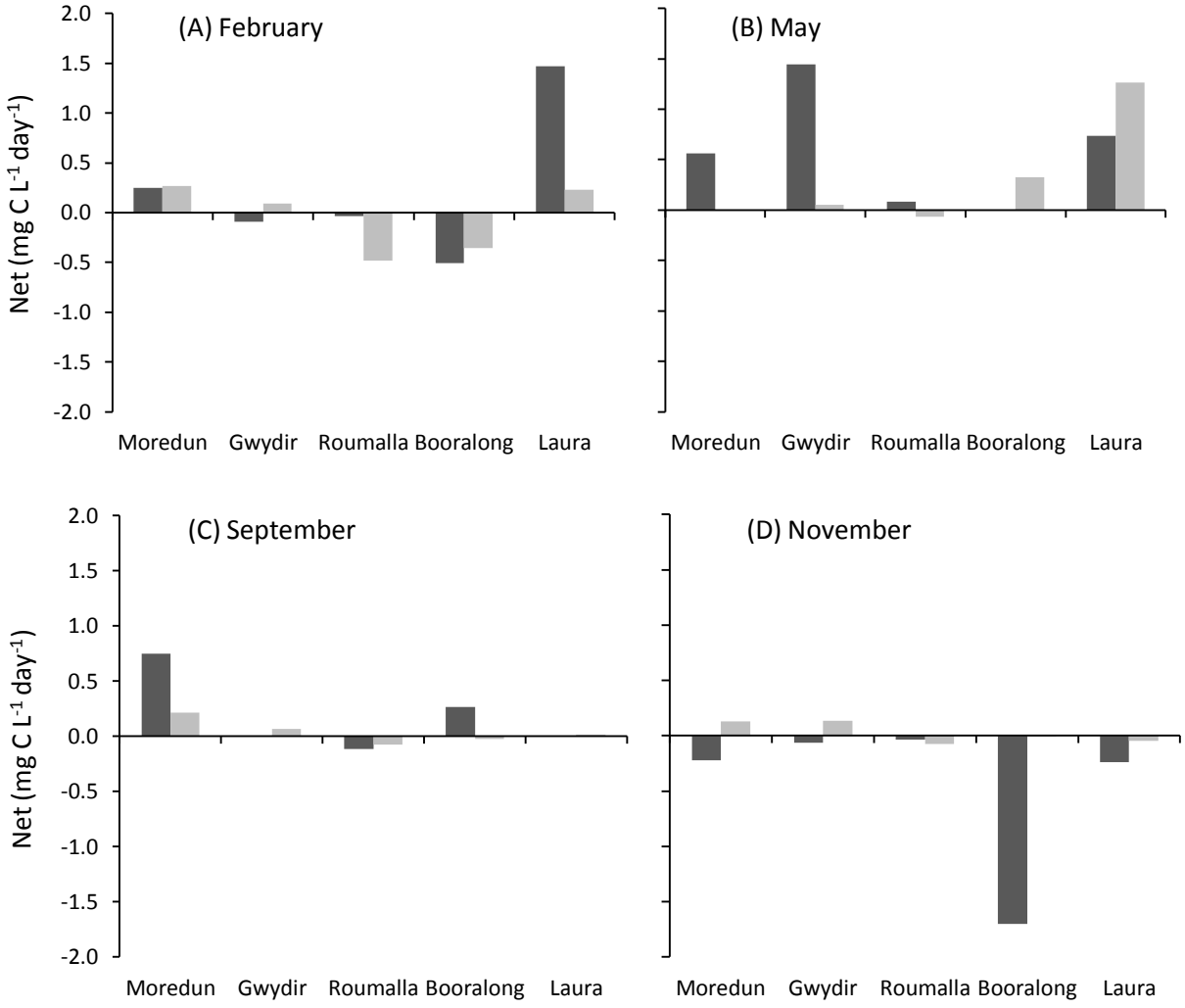

Figure 6.4: Reach net change in C (mg C L^{-1} day^{-1}) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

During February, the low-flow period, the largest net C-fixation at the whole-reach scale was 55,795 mg day^{-1} at the upstream non-vegetated control reach, Laura HD (Table 6.6). This was more than five times the next largest rate of net C-fixation, which was 10,216 mg day^{-1} at the downstream non-vegetated control reach, Laura Bridge. The largest net consumption of C was estimated to be -8,275 mg day^{-1} at the Gwydir non-vegetated reach (Table 6.6). Three of the vegetated reaches, Roumalla vegetated and the vegetated control reaches, Booralong CS and Booralong Bridge were all net consumers of C with rates ranging between -3,365 and -5,793 mg day^{-1} in February. Whole-reach estimates of C-fixation rates were reduced during the no-flow period and all reaches except the Roumalla vegetated reach were autotrophic with net C-fixation rates ranging between 3 and 5,252 mg day^{-1} (Table 6.6). There was large variation between all reaches as to whether there was net C-fixation or consumption during September, the first high-flow sampling period. With the exception of both reaches on the non-vegetated control stream, Laura HD and Laura Bridge, the whole-reach rates of net C-fixation and consumption increased during September (Table 6.6). During November, the second high-flow sampling period, both Moredun vegetated and Gwydir vegetated reaches had rates of net C-fixation of 105,680 and 172,676 mg day^{-1}. The other eight reaches were heterotrophic, with rates of C-consumption ranging from -439 to -168,594 mg day^{-1} (Table 6.6).

Table 6.6: Whole-reach estimates of net C-fixation and consumption (mg day^{-1}).

Reach	February	May	September	November
Moredun V	1,485		282,004	105,680
Gwydir V	7,627	3	92,394	172,676
Roumalla V	-4,201	-25	-29,948	-8,700
Booralong CS	-5,793		56,743	-83,265
Booralong Bridge	-3,365	74	-6,428	-439
Moredun NV	3,206	342	965,487	-168,594
Gwydir NV	-8,275	5,252		-82,056
Roumalla NV	-870	55	-47,631	-4,411
Laura HD	55,795	2,188	3,691	-69,554
Laura Bridge	10,216	2,462	7,287	-13,042

Productivity:respiration ratios

There was no clear difference in P/R ratios between vegetated and non-vegetated reaches. The Roumalla vegetated P/R ratio was consistently lower than the Roumalla non-vegetated reach, however, this trend was not evident within the two other treatment streams. These disparate results were further supported by the non-significant result of the PERMANOVA analyses (Table 6.7). During February, the P/R ratios were lowest (< 0.75) within the Roumalla vegetated and both vegetated control reaches, Booralong CS and Booralong Bridge (Figure 6.5). All P/R ratios increased when flow ceased during May and ranged between 0.86 and 1.95. There was large variation in reach P/R ratios between all ten reaches during September, the first high-flow period. The variation in P/R ratios then decreased in November, the second high-flow period, with the majority of P/R ratios were less than 1 during November (Figure 6.5). Despite the evidence of some patterns in P/R ratios between sampling periods, the PERMANOVA test showed that the effect of hydrology was marginally non-significant on P/R ratio (p = 0.051, Table 6.7).

Table 6.7: Results of PERMANOVA main test for significant differences in reach P/R ratio between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	0.270	0.270	4.499	0.077
St	4	1.027	0.257	1.772	0.156
Ti	3	1.520	0.507	3.328	0.051
TrxSt**	2	$2.071e^{-2}$	$1.036e^{-2}$	0.233	0.797
TrxTi	3	0.179	$5.952e^{-2}$	1.337	0.368
StxTi	12	1.770	0.148	1.310	0.412
TrxStxTi**	4	0.178	$4.451e^{-2}$	0.395	0.781
Res	7	0.788	0.113		
Total	36	6.048			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.12 for correct formulae.

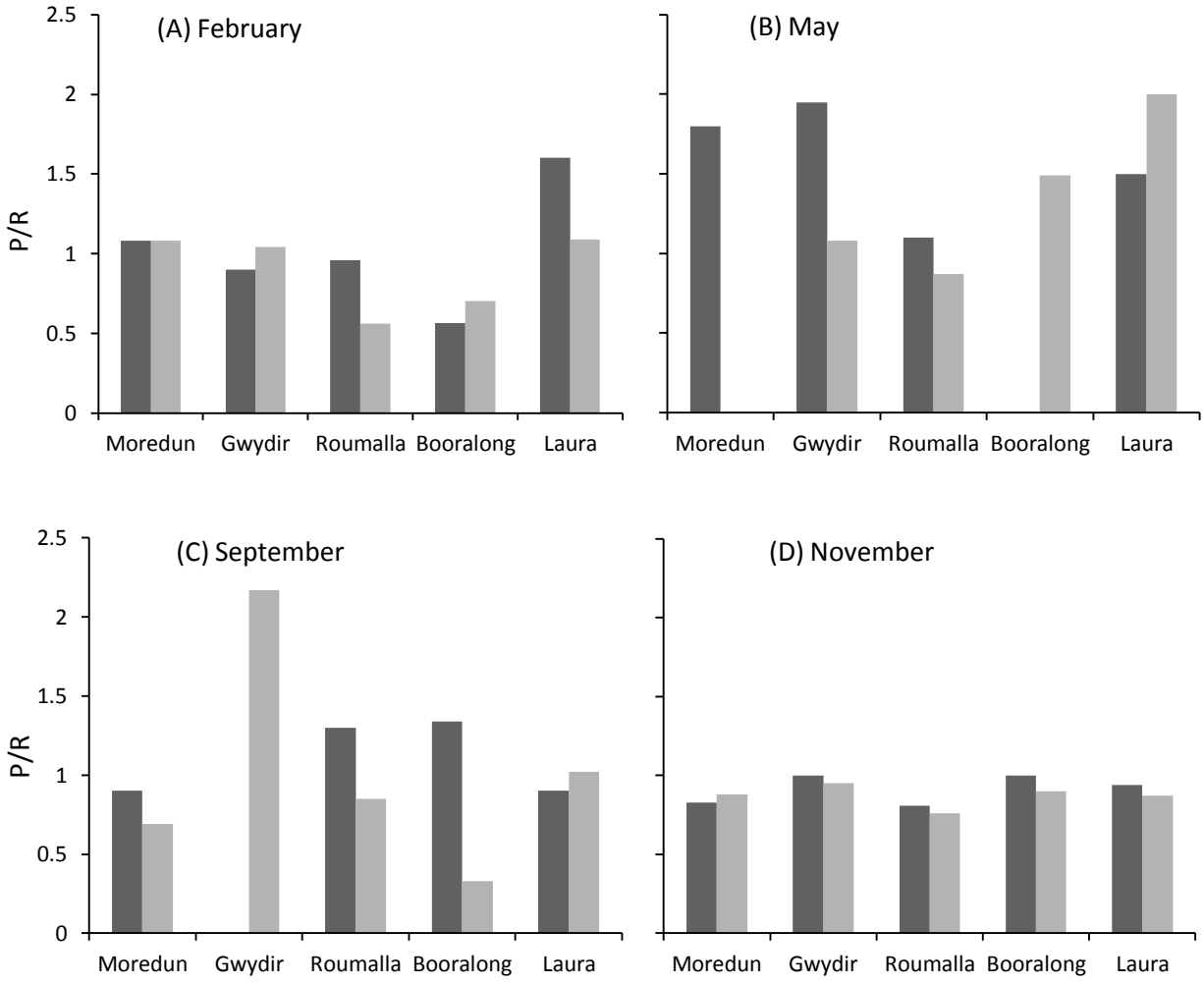

Figure 6.5: Reach P/R ratio for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively.

Light

There was a significant negative effect of riparian vegetation on the average daily light reaching the reach surface water (p = 0.015, Table 6.8). It was expected that the highest light would occur during February, which was during the austral summer and low-flow sampling period, however, equipment issues meant that these values were irretrievably lost. There was no significant effect of location on PAR within the control streams (Appendix 4, Table A4.22). The highest average light recorded at both vegetated and non-vegetated reaches occurred during November, the second high-flow sampling period. At the non-vegetated reaches, the highest average daily light ranged between 175,555 and 573,076 lumens m^{-2}, while average daily light ranged between 72,438 and 323,251 lumens m^{-2} at the vegetated reaches during November (Table 6.9). The largest difference between vegetated and non-vegetated reaches on the same treatment stream was recorded at Moredun Creek during September, the first high-flow sampling period. During September, the average daily light at the Moredun vegetated and non-vegetated reaches was 3,843 and 15,285 lumens m^{-2}, respectively (Table 6.9). The range of average daily light values were lowest during May, the no-flow period, at both vegetated (983 to 6,060 lumens m^{-2}) and non-vegetated reaches (2,978 to 23,034 lumens m^{-2}), with the exception of the downstream non-vegetated control reach, Laura Bridge (Table 6.9).

Table 6.8: Results of PERMANOVA main test for significant differences in average daily light values (lumens m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	28.083	28.083	15.178	**0.015**
St	4	2.860	0.715	0.463	0.832
Ti	2	10.831	5.416	3.193	0.101
TrxSt**	2	1.851	0.926	2.395	0.304
TrxTi	2	1.657	0.829	2.144	0.305
StxTi	8	13.386	1.673	31.182	**0.001**
TrxStxTi**	2	0.773	0.387	7.203	**0.024**
Res	5	0.268	$5.366e^{-2}$		
Total	26	111.030			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.13 for correct formulae.

Table 6.9: Average daily light values (lumens m^{-2}) for each vegetated and non-vegetated reaches during each deployment time (n = 96).

Reach	May	September	November
Moredun V		3,843	169,418
Gwydir V	6,060	8,891	323,251
Roumalla V	4,099	8,272	83,684
Booralong CS		192	72,438
Booralong Bridge	983	278	99,343
Moredun NV	2,978	15,285	371,743
Gwydir NV	23,899		354,494
Roumalla NV	9,852	32,513	175,555
Laura HD	23,034	16,651	573,076
Laura Bridge	22,042	25,458	443,750

Chlorophyll a surveys

There was no clear difference in mean chlorophyll *a* concentration between vegetated and non-vegetated reaches during May, November and September, and was supported by the non-significant result of the PERMANOVA analyses (Table 6.10). There was no significant effect of location on chlorophyll *a* concentration within the control streams (Appendix 4, Table A4.23). During February, the low-flow period, there was no clear pattern between the vegetated and non-vegetated reaches on the treatment streams. Moredun and Gwydir vegetated reaches had lower mean chlorophyll *a* concentrations of 0.01 and 0.02 mg L^{-1}, compared to the Moredun and Gwydir non-vegetated reaches which had mean chlorophyll *a* concentrations of 0.04 and 0.038 mg L^{-1}, respectively (Figure 6.6). Conversely, the Roumalla vegetated reach had a higher mean chlorophyll *a* concentration of 0.04 mg L^{-1} compared to the Roumalla non-vegetated reach which had a concentration of 0.01 mg L^{-1} during February (Figure 6.6).

The effect of hydrology on chlorophyll *a* concentration was determined to be significant (p = 0.001, Table 6.10). Mean water column chlorophyll *a* concentration ranged between 0.009 and 0.079 mg L^{-1} during February. Chlorophyll *a* concentration increased at Moredun non-vegetated (0.172 mg L^{-1}), Booralong Bridge (0.253 mg L^{-1}) and Laura Bridge (0.195 mg L^{-1}) reaches during May, the no-flow period. The concentration decreased to below 0.010 mg L^{-1} during September and November across all reaches (Figure 6.6).

There was also a significant interaction between treatment (Tr), stream (St) and time (Ti) determined by the PERMANOVA test (p = 0.013, Table 6.10) and also between streams (St) and time (Ti) (p = 0.001, Table 6.10). It was evident from the large standard error bars (Figure 6.6) that there was large variation within samples from each reach at each sampling time. Although the variation appeared to be consistent between sampling times the results of the PERMDISP analysis showed that there were significant differences in dispersions between sampling times (p = 0.001). This significant PERMDISP result means that the significant effects and interation tests with hydrology (Table 6.10) may be partly driven by differences in dispersions, however, the results suggest that hydrology is driving the differences in chlorophyll *a* concentration between treatments and streams.

Table 6.10: Results of PERMANOVA main test for significant differences in chlorophyll a concentration (mg L^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	$5.663e^{-3}$	$5.663e^{-3}$	0.889	0.524
St	4	0.164	$4.100e^{-2}$	1.262	0.366
Ti	3	0.938	0.313	8.631	**0.004**
TrxSt**	2	$1.740e^{-2}$	$8.700e^{-3}$	2.359	0.194
TrxTi	3	$5.495e^{-2}$	$1.832e^{-3}$	0.497	0.679
StxTi	12	0.406	$3.385e^{-2}$	27.861	**0.001**
TrxStxTi**	5	$1.844e^{-2}$	$3.688e^{-3}$	3.036	**0.013**
Res	83	0.101	$1.215e^{-3}$		
Total	113	1.563			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.13 for correct formulae.

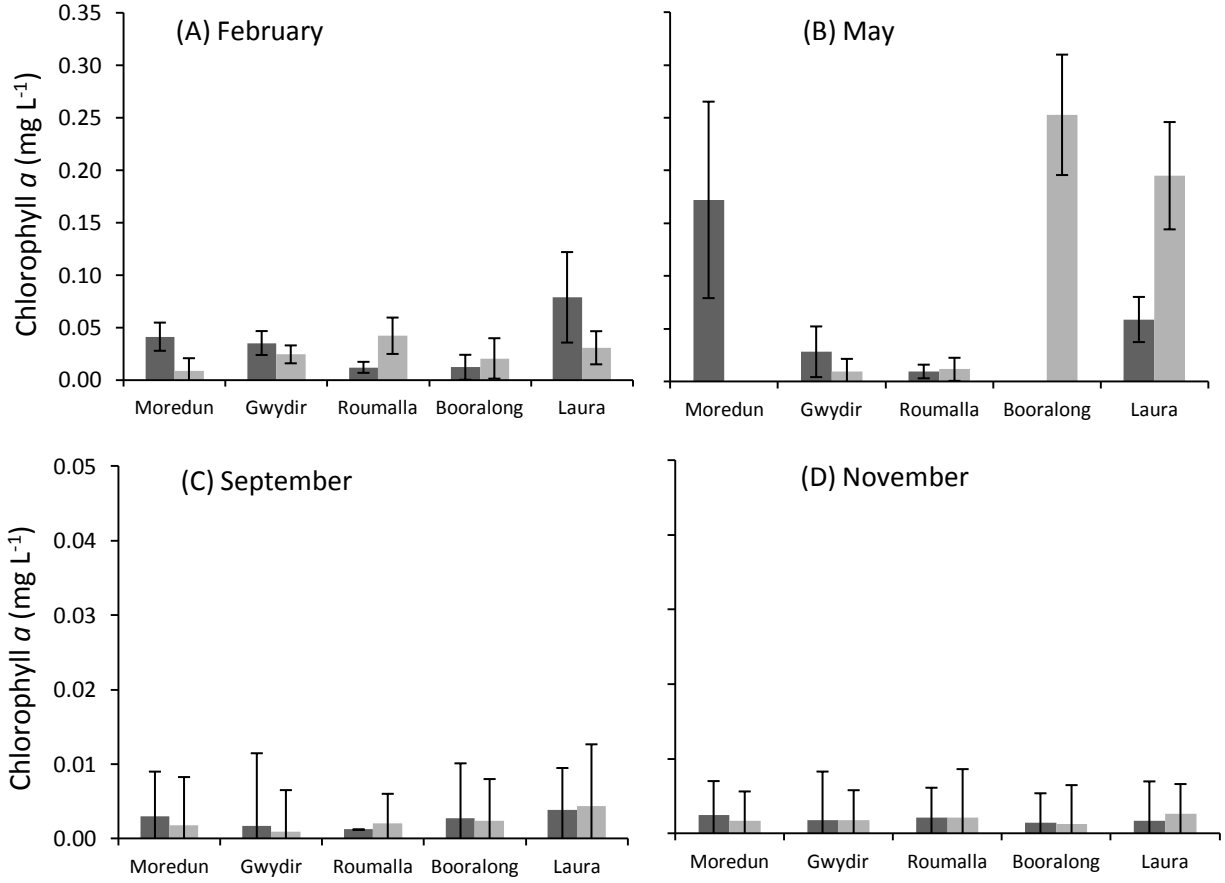

Figure 6.6: Mean surface water chlorophyll *a* concentration (mg L^{-1}) for each stream during (A) February, (B) May, (C) September, and (D) November sampling periods. Error bars are standard error of the sample mean (n=3). The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively. Note that the axis scales differ between the top (A and B) and bottom figures (C and D).

Chlorophyll a mass-balance

The load of chlorophyll *a* entering the study reaches increased at all sites between the February (low-flow) and September (first high-flow) periods, except at the Gwydir vegetated and non-vegetated reaches, and the upstream non-vegetated control reach, Laura Homestead (Table 6.11). The imported loads then decreased across all reaches during, November, the second high-flow period. There was a net export or increase of chlorophyll *a* from both vegetated and non-vegetated reaches across all sampling periods. The largest exported load was from the non-vegetated control stream reaches, Laura Homestead (284 g day^{-1}) and Laura Bridge (219 g day^{-1}) during February, the low-flow period. The largest retained load or decrease in mass-balance of chlorophyll *a* was within the Gwydir non-vegetated reach (-163 g day^{-1}) during September, the first high-flow period (Table 6.11).

Table 6.11: The mass-balance and retained loads of chlorophyll *a* (g day^{-1}) at each reach during the February, September and November sampling periods. Standard error of the mean are in parentheses (n=3). V = vegetated, NV = non-vegetated and Br = Bridge.

	February			September			November		
	Import	**Export**	**Retained**	**Import**	**Export**	**Retained**	**Import**	**Export**	**Retained**
Moredun NV	201 (0.6)	200 (0.9)	-1	1226 (3.2)	1439 (4.2)	213	697 (2.5)	697 (2.5)	0
Moredun V	12 (0.3)	19 (0.5)	7	776 (2.8)	898 (4.6)	122	462 (0.0)	514 (2.2)	52
Gwydir NV	1020 (2.3)	1089 (2.0)	69	1021 (2.8)	858 (6.9)	-163	861 (2.7)	861 (4.5)	0
Gwydir V	733 (2.8)	755 (1.5)	22	449 (4.0)	449 (4.0)	0	782 (2.7)	861 (2.7)	79
Roumalla NV	101 (1.2)	107 (0.5)	6	177 (1.6)	189 (0.0)	12	98 (0.9)	102 (0.9)	4
Roumalla V	92 (2.1)	137 (1.0)	45	239 (3.0)	314 (1.6)	75	112 (0.8)	94 (1.4)	-18
Laura HD	841 (1.8)	1124 (5.2)	284	666 (2.5)	764 (2.5)	98	171 (1.9)	189 (1.7)	18
Laura Br	296 (2.2)	515 (2.0)	219	753 (5.6)	815 (3.6)	62	273 (2.4)	265 (1.3)	-8
Booralong CS	63 (1.5)	52 (0.8)	-11	178 (0.0)	218 (2.1)	40	26 (0.8)	26 (0.5)	0
Booralong Br	48 (0.8)	74 (1.2)	26	250 (1.3)	234 (1.8)	-16	32 (0.9)	32 (0.9)	0

Macrophyte biomass

The results of the PERMANOVA test showed that macrophyte biomass was significantly larger at non-vegetated reaches compared to vegetated reaches, indicating that there was a negative effect of riparian vegetation (Table 6.12). There was no significant effect of location on macrophyte biomass within the control streams (Appendix 4, Table A4.24). Overall, the Moredun non-vegetated reach had the largest total macrophyte biomass during February (228 kg), May (3036 kg) and November (27 kg) sampling periods (Figure 6.7). The largest total biomass present within the vegetated reaches was < 1 kg during February at the Gwydir vegetated reach. The results of the PERMDISP analyses show that there was a significant difference in dispersions within treatment (p = 0.001) and streams (p = 0.001), however, it is clear in Figure 6.7 that only very small amounts of macrophyte biomass were recorded at vegetated reaches on a few occasions, in comparison to the non-vegetated reaches.

Table 6.12: Results of PERMANOVA main test for significant differences in total macrophyte biomass (kg) between treatment (Tr), streams (St), time (Ti), and their significant interactions. All ten reaches were used in the analysis. Significant results are in bold type.

Source	df	SS	MS	Pseudo-F	P(perm)
Tr	1	11.779	11.779	6.305	**0.030**
St	4	2.764	0.691	1.313	0.346
Ti	3	2.619	0.873	1.489	0.277
TrxSt**	2	3.789	1.894	8.326	**0.030**
TrxTi	3	0.146	$4.862e^{-2}$	0.214	0.874
StxTi	12	6.579	0.548	26.582	**0.001**
TrxStxTi**	5	1.138	0.228	11.031	**0.005**
Res	7	0.144	$2.063e^{-2}$		
Total	37	43.296			

**Some cells were empty due to an unbalanced design – see Appendix 5, Table A5.14 for correct formulae.

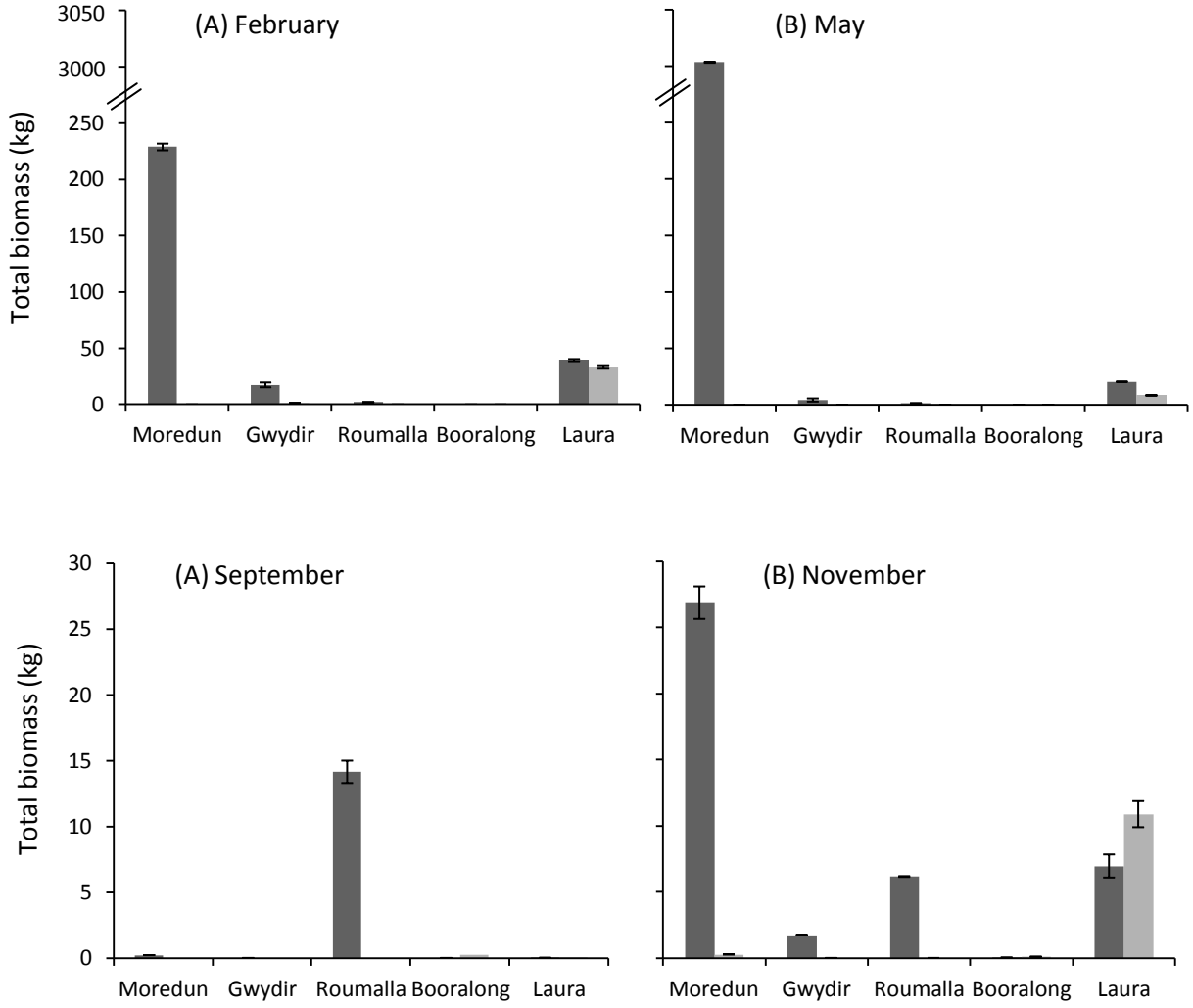

Figure 6.7: Total macrophyte biomass (kg) for each stream during (A) September and (B) November sampling periods. Error bars are standard error of the sample mean. The two columns for each stream represent the upstream (dark grey) and downstream (light grey) reaches, which are the non-vegetated and vegetated reaches in the treatment streams, respectively. Note that the axis scales are different between the top (A and B) and bottom (C and D) figures.

The BEST analyses showed that flow percentile and surface area:volume ratio were significantly correlated with chlorophyll *a* concentration within the vegetated reaches (p = 0.03), and explained 60% of the variation within the model (Table 6.13). Similarly, within the non-vegetated reaches, a combination of flow percentile, velocity and surface:area ratio were significantly correlated with chlorophyll *a* concentration (p = 0.02) and explained 47.9% of the variation (Table 6.14). The variation retained/exported chlorophyll *a* loads were not significantly correlated with any combination of the hydro-geomorphic variables within either the vegetated or non-vegetated reaches, although a combination of velocity and wetted area were close to the 0.05 significance level (p = 0.13) within the vegetated reaches. There was no significant correlation between the hydro-geomorphic variables and total macrophyte biomass within the vegetated reaches (Table 6.13). Within the non-vegetated reaches, wetted area explained 22.1% of the variation in total macrophyte biomass, however, this was only close to the 0.05 significance level (p = 0.16, Table 6.14).

Table 6.13: Results of the BEST analyses between chlorophyll *a* and macrophyte data from the vegetated reaches and their corresponding hydro-geomorphic variables. Significant results are in bold type.

Nutrient	Correlation level	Correlation variables	Significance level
Chl. *a* concentration	0.606	Flow percentile, surface area:volume	**0.03**
Chl. *a* retention	0.320	Velocity, wetted area	0.13
Macrophyte biomass	0.104	No. of features, wetted area	0.60

Table 6.14: Results of the BEST analyses between chlorophyll *a* and macrophyte data from the non-vegetated reaches and their corresponding hydro-geomorphic variables. Significant results are in bold type.

Nutrient	Correlation level	Correlation variables	Significance level
Chl. *a* concentration	0.479	Flow percentile, velocity, surface area:volume	**0.02**
Chl. *a* retention	-0.083	Velocity	0.99
Macrophyte biomass	0.221	Wetted area	0.16

Correlation of hydro-geomorphic and biochemical variables with metabolic parameters

Within the vegetated reaches, the significant positive correlations with the variation in GPP rates included: flow percentile (r^2 = 0.42, Table 6.15), surface area:volume ratio (r^2 = 0.35, Table 6.15), DOC:SRP (r^2 = 0.37, Table 6.17) and TN:TP ratios (r^2 = 0.25, Table 6.17), α-glu EEA (r^2 = 0.44, Table 6.18) and mean daily surface water temperature (r^2 = 0.26, Table 6.19). The significant negative correlations with the variation in GPP rates included: SRP (r^2 = 0.37, Table 6.16) and TP concentration (r^2 = 0.52, Table 6.16), and DOC:TN ratio (r^2 = 0.38, Table 6.17).

The significant positive correlations with the variation in ER rates at the vegetated reaches included: velocity (r^2 = 0.55, Table 6.15), SRP (r^2 = 0.70, Table 6.16) and TP concentrations (r^2 = 0.82, Table 6.16), DOC:SRP (r^2 = 0.71, Table 6.17) and DOC:TN ratios (r^2 = 0.75, Table 6.17), xylo EEA (r^2 = 0.87, Table 6.18), and whole-reach mass estimates of CBOM (r^2 = 0.25, Table 6.21), wood (r^2 = 0.35, Table 6.21) and *C. cunninhamiana* reproductive features (r^2 = 0.39, Table 6.21). The significant negative correlations included: flow percentile (r^2 = 0.79, Table 6.15), surface area:volume ratio (r^2 = 0.39, Table 6.15), TN concentration (r^2 = 0.64, Table 6.16), TN:TP ratio (r^2 = 0.32, Table 6.17), α-glu EEA (r^2 = 0.51, Table 6.18), chlorophyll *a* concentration (r^2 = 0.70, Table 6.19), and mean daily surface water temperature (r^2 = 0.32, Table 6.19).

The variation in GPP rates within the non-vegetated reaches were significantly positively correlated with: flow percentile (r^2 = 0.80, Table 6.15), surface area:volume ratio (r^2 = 0.32, Table 6.15), wetted area (r^2 = 0.39, Table 6.15), TN concentration (r^2 = 0.23, Table 6.16), chlorophyll *a* concentration (r^2 = 0.42, Table 6.19), macrophyte biomass (r^2 = 0.44, Table 6.19), and TSS concentration (r^2 = 0.33, Table 6.19). The significant negative correlations with GPP rates included: velocity (r^2 = 0.51, Table 6.15), SRP (r^2 = 0.44, Table 6.16) and TP concentration (r^2 = 0.80, Table 6.16), NOx:SRP (r^2 = 0.58, Table 6.17), DOC:SRP (r^2 = 0.53, Table 6.17) and DOC:TN ratios (r^2 = 0.68, Table 6.17), and xylo EEA (r^2 = 0.80, Table 6.18).

Within the non-vegetated reaches, the variation in ER rates were significantly positively correlated with velocity (r^2 = 0.34, Table 6.15), SRP (r^2 = 0.59, Table 6.16) and TP concentration (r^2 = 0.76, Table 6.16), and significantly negatively correlated with flow percentile (r^2 = 0.28, Table 6.15) and TN concentration (r^2 = 0.60, Table 6.16).

The variation in P/R ratios at the vegetated reaches was significantly positively correlated with TP concentration (r^2 = 0.27, Table 6.16), xylo EEA (r^2 = 0.44, Table 6.18), aerial unit estimates of benthic *C. cunninghamiana* reproductive (r^2 = 0.40, Table 6.19) and unidentifiable organic matter fractions (r^2 = 0.57, Table 6.19), and whole-reach estimates of benthic *C. cunninghamiana* reproductive features (r^2 = 0.27, Table 6.21).

There were no significant correlations between reach P/R ratios and the predictor variables within the non-vegetated reaches. However, there were a number of significant correlations between the net change in DO concentration with the predictor variables within the non-vegetated reaches. These included significant positive relationships with flow percentile (r^2 = 0.57, Table 6.15), wetted area (r^2 = 0.23, Table 6.15), TN concentration (r^2 = 0.51, Table 6.16), DOC:SRP ratio (r^2 = 0.29, Table 6.17), β-glu (r^2 = 0.61, Table 6.18) and α-glu EEA (r^2 = 0.57, Table 6.18), benthic macrophyte biomass (r^2 = 0.33, Table 6.19), chlorophyll *a* concentration (r^2 = 0.47, Table 6.19) and TSS concentration (r^2 = 0.72, Table 6.19). The significant negative correlations between net change in DO and predictor variables included: velocity (r^2 = 0.32, Table 6.15), SRP (r^2 = 0.26, Table 6.16) and TP concentrations (r^2 = 0.49, Table 6.16), DOC:TN (r^2 = 0.45, Table 6.17) and DOC:TP ratios (r^2 = 0.23, Table 6.17), xylo EEA (r^2 = 0.23, Table 6.18), and daily retained/exported loads of FBOM (r^2 = 0.23, Table 6.20) and chlorophyll *a* (r^2 = 0.23, Table 6.20).

There were no significant relationships between net change in DO concentration and the predictor variables within the vegetated reaches. There were also no significant correlations between any of the metabolic parameters within vegetated and non-vegetated reaches with retained nutrient loads.

Table 6.15: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between reach metabolic parameters and hydro-geomorphic variables. Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size is n=18 for vegetated and n=19 for non-vegetated datasets; # indicates n=17 (vegetated) or n=18 (non-vegetated), ## indicates n=16 (vegetated) or n=17 (non-vegetated).

	Vegetated				Non-vegetated			
	Net	GPP	ER	P/R	Net	GPP	ER	P/R
No. feature								
Flow percentile		m = 0.48 *r* = 0.42**	m = -0.76# *r* = 0.79***		m = 0.58## *r* = 0.57***	m = 0.99# *r* = 0.80***	m = -0.50# *r* = 0.28*	
Velocity		m = -0.36 *r* = 0.26*	m = 0.55# *r* = 0.55***		m = -0.38 *r* = 0.32*	m = -0.73 *r* = 0.51***	m = 0.48# *r* = 0.34**	
SA:V		m = 0.48# *r* = 0.35**	m = -0.60# *r* = 0.39**			m = 1.58## *r* = 0.32*		
Wetted area			m < 0.01# *r* = 0.38**		m < 0.01 *r* = 0.23*	m < -0.01 *r* = 0.39**		

Table 6.16: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between reach metabolic parameters and mean nutrient concentrations (mg L^{-1}). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size is n=18 for vegetated and n=19 for non-vegetated datasets; # indicates n=17 (vegetated) or n=18 (non-vegetated), ## indicates n=16 (vegetated) or n=17 (non-vegetated).

	Vegetated				Non-vegetated			
	Net	GPP	ER	P/R	Net	GPP	ER	P/R
DOC								
NOx								
SRP		m = -0.01# *r* = 0.37**	m = 0.02# *r* = 0.70***		m = -0.02 *r* = 0.26*	m = -0.03 *r* = 0.44**	m = 0.03# *r* = 0.59***	
TN			m = -3.86## *r* = 0.64***		m = 2.45# *r* = 0.51***	m = 2.63 *r* = 0.23*	m = -3.11### *r* = 0.60***	
TP		m = -0.12# *r* = 0.52***	m = 0.16## *r* = 0.82***	m = 0.04 *r* = 0.27*	m = -0.12 *r* = 0.49***	m = -0.20## *r* = 0.80***	m = 0.17## *r* = 0.76***	

Table 6.17: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between reach metabolic parameters and nutrient ratios. Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size is n=18 for vegetated and n=19 for non-vegetated datasets; # indicates n=17 (vegetated) or n=18 (non-vegetated), ## indicates n=16 (vegetated) or n=17 (non-vegetated).

	Vegetated				Non-vegetated			
	Net	GPP	ER	P/R	Net	GPP	ER	P/R
DOC : NOx								
NOx : SRP						m = -3.50# *r* = 0.58***		
DOC : SRP		m < 0.01 *r* = 0.37**	m < 0.01# *r* = 0.71***		m < 0.01 *r* = 0.29*	m < -0.01 *r* = 0.53***		
DOC : TN		m = -0.09# *r* = 0.38**	m = 0.14## *r* = 0.75***		m = -0.10 *r* = 0.45**	m = -0.18# *r* = 0.68***		
TN : TP		m = 0.05# *r* = 0.25*	m = -0.06# *r* = 0.32*					
DOC : TP					m < -0.01 *r* = 0.23*			

Table 6.18: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between reach metabolic parameters and EEA (μM 4-MUB L^{-1} h^{-1}). Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size is n=18 for vegetated and n=19 for non-vegetated datasets; # indicates n=17 (vegetated) or n=18 (non-vegetated), ## indicates n=16 (vegetated) or n=17 (non-vegetated).

	Vegetated				Non-vegetated			
	Net	GPP	ER	P/R	Net	GPP	ER	P/R
AP								
LAP								
β-glu					m = 0.71# *r* = 0.61***			
α-glu		m = 0.98 *r* = 0.44**	m = -1.48## *r* = 0.51**		m = 1.11## *r* = 0.57***			
xylo			m = 1.52### *r* = 0.87***	m = 0.34# *r* = 0.44**	m = -0.77 *r* = 0.23*	m = -1.98# *r* = 0.80***		

Table 6.19: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between organic matter variables, chlorophyll *a* concentration, macrophyte biomass, mean daily surface water temperature and TSS concentration with reach metabolism variables. Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size is n=18 for vegetated and n=19 for non-vegetated datasets; # indicates n=17 (vegetated) or n=18 (non-vegetated), ## indicates n=16 (vegetated) or n=17 (non-vegetated).

	Vegetated				Non-vegetated			
	Net	GPP	ER	P/R	Net	GPP	ER	P/R
CBOM								
FBOM								
Needle								
Wood								
C - repro				m = 1.01# *r* = 0.40**				
Leaf								
Macrophyte					m = 2.47## *r* = 0.33*			
Unidentified				m = 0.65## *r* = 0.57***				
Chlorophyll *a*			m = -24.59## *r* = 0.70***		m = 5.30 *r* = 0.47***	m = 7.54 *r* = 0.42**		
Macrophyte biomass						m = 1.74 *r* = 0.44**		
Temperature		m = 0.11 *r* = 0.26*	m = -0.14 *r* = 0.32*					
TSS					m = 0.29## *r* = 0.72***	m = 0.34 *r* = 0.33**		

Table 6.20: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between retained organic matter fractions and chlorophyll *a* (g day^{-1}) with reach metabolism variables. Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size is n=18 for vegetated and n=19 for non-vegetated datasets; # indicates n=17 (vegetated) or n=18 (non-vegetated), ## indicates n=16 (vegetated) or n=17 (non-vegetated).

	Vegetated				Non-vegetated			
	Net	GPP	ER	P/R	Net	GPP	ER	P/R
CBOM								
FBOM					m = -0.12 *r* = 0.23*			
Needle								
Wood								
C - repro								
Leaf								
Macrophyte								
Unidentified								
Chlorophyll *a*					m = -0.12 *r* = 0.23*			

Table 6.21: Slope (m) and squared multiple *r* (*r*) values of significant regression analyses between organic matter variables (whole-reach total, g) with reach metabolism variables. Asterisk indicates level of significance (* $p < 0.025$, ** $p < 0.01$, *** $p < 0.0001$). Sample size is n=18 for vegetated and n=19 for non-vegetated datasets.

	Vegetated				Non-vegetated			
	Net	GPP	ER	P/R	Net	GPP	ER	P/R
CBOM			m = 0.58 *r* = 0.25*					
FBOM								
Needle								
Wood			m = 0.54 *r* = 0.35**					
C - repro			m = 0.63 *r* = 0.39**	m = 0.19 *r* = 0.27*				
Leaf								
Macrophyte								
Unidentified								

The DISTLM tests could not identify any statistically significant models based on multiple variables to explain the variation in net change of DO, GPP and P/R ratios within the vegetated reaches. Flow percentile was found to be the most significantly correlated variable with GPP (p = 0.002), explaining 41.5% of the variation within the vegetated reaches (Table 6.22). A combination of flow percentile, xylo activity and mean daily temperature was significantly correlated with ER (p = 0.005), explaining 90% of the variation within the vegetated reaches (Table 6.23). However, xylo activity was significantly correlated with P/R ratio (p = 0.015) and explained 30.2% or the variation (Table 6.24).

Table 6.22: Results of conditional test from DISTLM for GPP (mg L^{-1} day^{-1}) from the vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Flow percentile	7.011	11.390	**0.002**	0.416	0.416	16
Temperature	1.618	2.949	0.100	$9.597e^{-2}$	0.512	15
SA:V	0.759	1.422	0.277	$4.502e^{-2}$	0.557	14
α-glu	0.569	1.071	0.348	$3.372e^{-2}$	0.591	13
Velocity	0.481	0.899	0.366	$2.853e^{-2}$	0.619	12
DOC : SRP	0.286	0.513	0.501	$1.696e^{-2}$	0.636	11
TN : TP	0.479	0.847	0.400	$2.841e^{-2}$	0.665	10
DOC : TN	0.500	0.873	0.378	$2.967e^{-2}$	0.694	9
TP	0.178	0.286	0.614	$1.054e^{-2}$	0.705	8
SRP	$4.546e^{-4}$	$6.391e^{-4}$	0.984	$2.696e^{-5}$	0.705	7

Table 6.23: Results of conditional test from DISTLM for ER (mg L^{-1} day^{-1}) from the vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Flow percentile	17.202	40.075	**0.001**	0.715	0.715	16
Xylo	2.529	8.743	**0.011**	0.105	0.820	15
Temperature	1.941	11.334	**0.005**	$8.065e^{-2}$	0.900	14
TP	0.181	1.061	0.328	$7.513e^{-3}$	0.908	13
SRP	0.375	2.440	0.138	$1.556e^{-2}$	0.924	12
TN	0.423	3.282	0.115	$1.759e^{-2}$	0.941	11
Wetted area	0.129	0.996	0.331	$5.341e^{-3}$	0.946	10
Wood (WR)	0.171	1.370	0.259	$7.085e^{-3}$	0.954	9
TN : TP	0.194	1.671	0.241	$8.040e^{-3}$	0.962	8
Chlorophyll *a*	$8.145e^{-2}$	0.675	0.457	$3.384e^{-3}$	0.965	7
DOC : TN	0.165	1.455	0.264	$6.852e^{-3}$	0.972	6
DOC : SRP	0.108	0.946	0.386	$4.493e^{-3}$	0.976	5
C-repro (WR)	0.235	2.794	0.153	$9.771e^{-3}$	0.986	4
SA:V	0.292	19.819	**0.016**	$1.215e^{-2}$	0.998	3
Velocity	$4.267e^{-3}$	0.213	0.703	$1.773e^{-4}$	0.998	2
α-glu	$1.187e^{-2}$	0.422	0.627	$4.931e^{-4}$	0.999	1

Table 6.24: Results of conditional test from DISTLM for P/R ratio from the vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Xylo	0.996	6.926	**0.015**	0.302	0.302	16
UI	0.528	4.471	0.059	0.160	0.462	15
C-repro (WR)	$3.826e^{-2}$	0.309	0.563	$1.160e^{-2}$	0.474	14
TP	$8.069e^{-3}$	$6.075e^{-2}$	0.816	$2.447e^{-3}$	0.476	13
C-repro	$1.923e^{-3}$	$1.338e^{-2}$	0.916	$5.831e^{-4}$	0.477	12

A combination of TP concentration and β-glu activity was significantly correlated with the net change in DO (p = 0.006), explaining 68.6% of the variation within the non-vegetated reaches (Table 6.25). The combination of flow percentile and the DOC:SRP ratios were significantly correlated with GPP (p = 0.002), explaining 86.3% of the variation within the non-vegetated reaches (Table 6.26). The concentration of TN was found to be the most significantly correlated variable with ER (p = 0.013) within the non-vegetated reaches (Table 6.27) and explained 29.6% of the variation. No statistically significant multiple variable models were correlated with the P/R ratio within the vegetated reaches.

Table 6.25: Results of conditional test from DISTLM for net change in DO (mg L^{-1} day^{-1}) from the non-vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
TP	8.308	16.530	**0.003**	0.493	0.493	17
β-glu	3.262	9.880	**0.006**	0.194	0.687	16
FBOM (retained)	0.535	1.691	0.212	$3.175e^{-2}$	0.718	15
Velocity	0.733	2.554	0.118	$4.347e^{-2}$	0.762	14
Grass	0.798	3.226	0.098	$4.737e^{-2}$	0.809	13
Flow percentile	0.847	4.288	0.051	$5.025e^{-2}$	0.859	12
DOC : TP	0.322	1.732	0.208	$1.913e^{-2}$	0.879	11
TSS	0.451	2.824	0.116	$2.675e^{-2}$	0.905	10
TN	0.181	1.148	0.323	$1.071e^{-2}$	0.916	9
Chlorophyll *a*	0.172	1.103	0.330	$1.018e^{-2}$	0.926	8
DOC : SRP	0.106	0.649	0.463	$6.262e^{-3}$	0.932	7
xylo	$7.380e^{-2}$	0.416	0.534	$4.379e^{-3}$	0.937	6
DOC : TN	$4.671e^{-2}$	0.229	0.645	$2.772e^{-3}$	0.940	5
SRP	$1.819e^{-2}$	$7.273e^{-2}$	0.792	$1.079e^{-3}$	0.941	4
α-glu	$2.160e^{-2}$	$6.623e^{-2}$	0.824	$1.282e^{-3}$	0.942	3
Wetted area	$4.958e^{-3}$	$1.018e^{-2}$	0.934	$2.942e^{-4}$	0.942	2

Table 6.26: Results of conditional test from DISTLM for GPP (mg L^{-1} day^{-1}) from the non- vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Flow percentile	27.138	42.526	**0.001**	0.714	0.714	17
DOC : SRP	5.635	17.291	**0.002**	0.148	0.863	16
NOx : SRP	0.680	2.249	0.139	$1.790e^{-2}$	0.881	15
SA:V	0.642	2.309	0.140	$1.690e^{-2}$	0.898	14
Wetted area	0.447	1.687	0.199	$1.177e^{-2}$	0.909	13
Chlorophyll *a*	0.798	3.618	0.093	$2.101e^{-2}$	0.930	12
TN	0.832	5.044	0.052	$2.191e^{-2}$	0.952	11
xylo	0.543	4.266	0.068	$1.429e^{-2}$	0.967	10
Macrophytes	0.146	1.165	0.328	$3.837e^{-3}$	0.970	9
TSS	0.333	3.357	0.120	$8.764e^{-3}$	0.979	8
Velocity	0.170	1.904	0.220	$4.467e^{-3}$	0.984	7
DOC : TP	$8.845e^{-2}$	0.991	0.332	$2.329e^{-3}$	0.986	6
SRP	$5.783e^{-2}$	0.606	0.498	$1.523e^{-3}$	0.987	5
TP	$4.848e^{-2}$	0.452	0.528	$1.276e^{-3}$	0.989	4

Table 6.27: Results of conditional test from DISTLM for ER (mg L^{-1} day^{-1}) from the non-vegetated reaches with significant biophysical variables.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
TN	10.917	7.164	**0.013**	0.297	0.297	17
SRP	3.304	2.339	0.137	$8.972e^{-2}$	0.386	16
Velocity	0.217	0.145	0.728	$5.892e^{-3}$	0.392	15
TP	0.141	$8.900e^{-2}$	0.751	$3.840e^{-3}$	0.396	14

6.4 Discussion

Rates of ecosystem metabolism

The rates of whole-system GPP ranged between -1.56 and 10.36 mg L^{-1} day^{-1} across all reaches in this study, which fell within the lower range suggested as typical of upland streams of 0.2 to 50 mg L^{-1} day^{-1} (see Grace & Imberger 2006). In contrast, the rates of ER ranged between 1.17 and -8.24 mg L^{-1} day^{-1}, which were lower than the suggested typical range of -8 to -100 mg L^{-1} day^{-1} (see Grace & Imberger 2006). It is difficult to compare the rates of GPP and ER found in this study, which are reported in volumetric units (mg L^{-1} day^{-1}), to other published studies as the majority of results are reported in aerial units (g m^{-2} day^{-1}). However, Grace and Imberger (2006) put together a table of whole-system rates of GPP and ER that were converted into volumetric units (mg L^{-1} day^{-1}). The metabolic rates reported for Sycamore Creek, a North American semi-arid intermittent stream, were much higher than those reported in the present study, with mean GPP and ER rates estimated as 280 and -200 mg L^{-1} day^{-1}, respectively (Mulholland et al. 2001). Similarly, the rates of GPP and ER reported for South Kings Creek, a North American semi-arid agricultural upland stream, were 70 and -53 mg L^{-1} day^{-1}, respectively (Mulholland et al. 2001). The metabolic rates reported for other agricultural streams and rivers were closer to the rates estimated in the present study. In a study on the effects of land-use on stream metabolism in New Zealand, the mean rates of GPP and ER were reported as 3 and -5 mg L^{-1} day^{-1}, respectively, for the developed pasture stream (Young & Huryn 1999). The range of GPP and ER rates for the Latrobe River in southeastern Australia were estimated between 0.3 and 1.2 mg L^{-1} day^{-1}, and -1.5 and -7.0 mg L^{-1} day^{-1}, respectively (Chessman 1985). Rates of metabolism reported for the Little Tennessee River, USA, were also similar to those found in the present study, with rates of GPP and ER ranging between 1.5 and 3.0 mg L^{-1} day^{-1}, and -2.4 and -5.4 mg L^{-1} day^{-1}, respectively (McTammany et al. 2003). These low rates of GPP and ER reported in the present study suggest that these ecosystem processes are being limited or disturbed in some way.

Riparian vegetation

In this study, there was no overall effect of riparian vegetation on whole-ecosystem GPP or ER. These results contrast to those observed in similar studies of the effects of riparian vegetation on metabolism. In paired forested and grassland reaches along 13 streams in Pennsylvania and Maryland, USA, rates of whole-ecosystem GPP and ER (g m^2 day^{-1}) were higher during both the warmer and colder seasons (Bott et al. 2006). In upland areas of southern Tasmania, Australia, whole-ecosystem GPP and ER estimates were up to 10 times and two times greater, respectively, in cleared than forested streams (Clapcott & Barmuta 2010). Benthic GPP and respiration were found to increase up to two orders of magnitude across a land-use gradient (including riparian vegetation removal) in southeast Queensland, Australia (Fellows et al. 2006a). Similarly, a study which spanned across three different biomes within Australia, the rates of benthic GPP and respiration were found to increase with decreasing riparian canopy cover (Bunn et al. 1999). An increase in GPP after the removal of riparian vegetation was also observed within a Mediterranean stream (Sabater et al. 2000). Within these studies, the higher rates of GPP have been attributed to an increase in PAR reaching the streambed creating optimal conditions for autotrophic photosynthesis/production (Bunn et al. 1999, Sabater et al. 2000, Bott et al. 2006, Clapcott & Barmuta 2010).

This study showed that the amount of light (measured as lumens m^{-2}) reaching the water column was significantly higher at the non-vegetated reaches compared to vegetated reaches, however, unlike previously published studies, this did not result in significant differences in the rates of GPP and ER, or chlorophyll *a* concentrations. These disparate findings may be due to differences in the structural composition of riparian vegetation or width of riparian zone between the vegetated reaches in this study and those used in other studies, as these differences may have produced more shade or cover over the streams. For example, a range of riparian canopy covers from 0 to 100% were used in studies by Fellows et al. (2006a) and Bunn et al. (1999), while only a single to a few lines of trees were present in the riparian zones in the vegetated reaches of this study. In addition, there have been a number of studies that have modelled relationships between the extent of riparian canopy cover and autotrophic biomass

or production (Steinman et al. 1989, Graham et al. 1995, Bunn et al. 1999). This suggests that there may be a threshold of width for the influence of riparian vegetation on metabolic rates that was not reached in the current study streams.

The suggestion that there may be a threshold of width for the influence of riparian vegetation on GPP is reflected in other variables measured in this study. The results of the chlorophyll *a* surveys showed that there was no significant difference in chlorophyll *a* concentrations between vegetated and non-vegetated reaches. Riparian cover of between 60 to 90% has been suggested as an optimal threshold for reducing filamentous algal growth (Quinn et al. 1997), which implies that this threshold was not reached in some of the vegetated streams. Furthermore, the rates of GPP and ER can also be affected by surface water temperatures (Mulholland et al. 2001, Demars et al. 2011). The amount of light reaching the water column can alter temperatures, however in the present study there was no significant difference in water temperatures during any of the sampling periods (see Chapter 4). This also suggests that the cover (shade) of riparian vegetation provided in the vegetated reaches was not effective enough at reducing water temperatures or plankton/algal growth, which has been reflected in the rates of whole-system GPP.

In contrast to these results, there was a significant negative effect of riparian vegetation on macrophyte biomass. Increased macrophyte biomass with decreasing riparian vegetation has been shown in many studies, and has also been attributed to the increase in light or PAR available to the reaches (Canfield & Hoyer 1988, Fletcher et al. 2000, Hoyer et al. 2004, Julian et al. 2010). A threshold of only 50% cover by riparian vegetation has been found to negatively affect submerged macrophyte production and biomass (Canfield & Hoyer 1988). Lower surface area:volume ratios were also found within the non-vegetated reaches compared to the vegetated reaches and this has also been shown to promote macrophyte biomass (Carr et al. 1997). However, the results of this study show that the significant difference in macrophyte biomass did not lead to differences in whole-system GPP, and may also suggest that there is some limitation of macrophyte growth occurring within the non-vegetated reaches. Alternatively, this may also suggest that water column chlorophyll *a* contributed more to

whole-ecosystem GPP, although other functional groups such as, biofilms and epiphytic algae were not measured in this study.

Nutrient limitation leading to increases in the C:nutrient ratios can reduce rates of metabolism (Guasch et al. 1995, Tank & Dodds 2003, Westhorpe et al. 2010). Nutrient surveys found that all study reaches had low concentrations of oxidised nitrogen, and nutrient enrichment experiments showed that autotrophic growth at all reaches was N-limited during all sampling periods (see Chapter 3). Therefore, nutrient limitation may have prevented maximum growth within reaches and may explain why there was no significant difference in rates of GPP between vegetated and non-vegetated reaches across all sampling periods. The nutrient enrichment experiments also showed evidence of photoinhibition of benthic autotrophic communities during the low-flow period (see Chapter 3), which suggests that photoinhibition may have interacted with nutrient limitation to reduce rates of GPP during the low-flow period.

Net ecosystem production

The low rates of GPP and ER in this study was also reflected in the estimates of net C-fixation (NEP) rates, which ranged between -1.70 to 1.47 mg C L^{-1} day^{-1} across all reaches. The net C-fixation rates presented in volumetric units (mg C L^{-1} day^{-1}) for other metabolism studies could be estimated from the table listed in Grace and Imberger (2006). The rate of net C-fixation for South Kings Creek, a North American semi-arid agricultural upland stream was estimated to 17 mg C L^{-1} day^{-1}, while Sycamore Creek, a North American semi-arid intermittent stream, had a net C-fixation rate of 80 mg C L^{-1} day^{-1} (Mulholland et al. 2001, adapted from Grace & Imberger 2006). These rates show that the streams are strongly net autotrophic (Mulholland et al. 2001). In contrast, rates of net C-fixation were reported as -2 mg C L^{-1} day^{-1} in a developed pasture stream in New Zealand (Young & Huryn 1999, adapted from Grace & Imberger 2006), and between -0.3 to -6.7 mg C L^{-1} day^{-1} in the Latrobe River, southeastern Australia (Chessman 1985), indicating that these streams were net heterotrophic. The low net C-fixation rates reported in the present study adds further support to the suggestion that ecosystem metabolism is being limited or disturbed in some way.

Similar to the rates of GPP and ER, there were also inconsistencies in NEP patterns within the vegetated and non-vegetated reaches. For instance, it was predicted that during the low and no-flow periods the vegetated reaches would be net heterotrophic and the non-vegetated reaches would be net autotrophic. The results showed that two of the vegetated reaches and three of the non-vegetated reaches were net autotrophic during the low-flow period, and all reaches with the exception of the Roumalla vegetated were net autotrophic during the no-flow period. This implies that the C available to support the heterotrophic communities at these reaches was from autochthonous sources during these periods (Jones & Lock 1989, Ylla et al. 2010, see Chapter 5), predominantly from algae in both the vegetated and non-vegetated reaches, as well as, macrophytes in the non-vegetated reaches. The concentrations of chlorophyll *a* were highest within both vegetated and non-vegetated reaches during this no-flow period, and there was no significant difference in concentrations between vegetated and non-vegetated reaches. These results are also supported by the extracellular enzyme analyses (EEA), which showed high β-glu activity relative to the activity of xylo enzymes within the reaches, and a significant difference in α-glu activity between vegetated and non-vegetated reaches (see Chapter 5). Essentially, this means that there is highly labile C present within reaches during no-flow periods regardless of the presence of riparian vegetation. However, these results show that despite NEP being the same between vegetated and non-vegetated reaches, the overall sources of C and metabolic rates are through different pathways (i.e. algae in vegetated reaches, algae and macrophytes in non-vegetated reaches). This highlights the advantage of exploring the sources of C through EEA to provide further insight into the dynamics of metabolism, as this conclusion could not be reached with estimates of metabolism alone.

Further inconsistencies in patterns of NEP rates were evident in the three vegetated reaches and two non-vegetated reaches that were heterotrophic during the low-flow period. This implies that the rates of ER were higher than GPP within these reaches and there was not enough autochthonous produced C available to support the heterotrophic community. However, the chlorophyll *a* concentrations were still high at these reaches, particularly the Roumalla vegetated and Gwydir non-vegetated reaches. The inconsistencies may be a

reflection of the types and number of habitats present within both vegetated and non-vegetated reaches, in addition to an issue relating to measuring whole-ecosystem rates of ER and the sampling design in this study. The addition of woody debris from riparian vegetation can facilitate the accumulation of CBOM, which are optimal habitats for microbial processes and can lead to increased rates of ER (Aldridge et al. 2009, Newham et al. 2011). In these habitats it would be expected that water column sources of highly labile organic matter would not be a suitable source of C to benthic heterotrophic communities as coarse organic matter would be preferred, and therefore would not be related to patterns of chlorophyll *a* concentrations. In the present study, a large number of logs were present in the Roumalla vegetated and Gwydir non-vegetated reaches (see Chapter 2), which may explain the high rates of ER despite the large concentrations of chlorophyll *a* present. Alternatively, the hyporheic zone can have high rates of heterotrophic activity (Jones et al. 1995, Naegeli & Uehlinger 1997, Fellows et al. 2001) and the potential for hyporheic exchange of DO to occur can be enhanced in reaches with sandy bed substrates compared to those constrained by bedrock (Jones & Holmes 1996). The Roumalla vegetated and Gwydir non-vegetated reaches also had large sections of sandy substrates, which may also explain the inconsistencies in ER. Therefore, this suggests that benthic and/or hyporheic respiration may have contributed to whole-system rates of ER in these heterotrophic reaches, however, benthic rates of respiration would need to be measured separately to add further support to this suggestion.

The results of this study also showed that five and nine of the ten reaches were net autotrophic with P/R ratios more than 1 during the low and no-flow periods, respectively. During the high-flow periods, the P/R ratios decreased to less than 1 across all reaches, however, not all reaches were net heterotrophic. Similarly, a study of the recovery of ecosystem metabolism after flash flooding in the semi-arid intermittent stream, Sycamore Creek, also showed that P/R ratios were less than 1 immediately after flooding and then increased to more than 1 once discharge decreased to less than 0.25 $m^3 s^{-1}$ (Fisher et al. 1982). A study on the metabolism of an in-channel waterhole of a dryland river in Queensland, Australia, also showed that the P/R ratio was more than 1 during periods of surface water fragmentation, however, metabolism during periods of connectivity were not reported (Fellows et al. 2007). There have been few other

studies reporting the shift in trophic state with changes in discharge in intermittent streams (but see Uehlinger 2000), therefore, the results of this study provide further evidence that ecosystem metabolism will shift from autotrophy to heterotrophy between periods of surface water fragmentation and connectivity.

Hydrology

The regression analyses showed that rates of GPP and ER were significantly negatively correlated with flow percentile within vegetated and non-vegetated reaches. Large increases in discharge have been shown to decrease rates of GPP through removing filamentous algae and macrophytes (Biggs 1995, Acuńa et al. 2011). Surface water velocities can also dilute phytoplankton and microbial biomass in addition to reducing growth rates (Mitrovic et al. 2003, Mitrovic et al. 2011). Reduced rates of GPP and ER have also been attributed to bed substrates becoming destabilised (Uehlinger 2000, Atkinson et al. 2008). In the present study, both macrophyte biomass and chlorophyll *a* decreased during the high-flow periods, and this reflects the decrease in rates of GPP among reaches. It was evident that in some reaches of the present study, channel beds that consisted of sand became destabilised during high-flow periods (see Chapter 4), while other reaches showed that channel bed areas that previously consisted of cobble became covered in sand and fine particles during these periods (see Chapter 4). As these physical changes can lead to organic matter and heterotrophic communities becoming smothered (Bunn 1988, Benfield et al. 2001, see Chapter 4), this may reflect the decrease in rates of ER during the high-flow periods in this study. These results offer further explanation for the lack of significant difference in GPP and ER rates between vegetated and non-vegetated reaches was because the rates had significantly decreased from the effects of increased discharge.

During the high-flow periods, there was no consistent pattern in the rates of GPP or ER between and within vegetated and non-vegetated reaches. However, a larger decrease in rates of GPP compared to ER meant that eight of the 10 study reaches did shift towards heterotrophy during the high-flow periods. Other studies have also shown that there will be a shift to heterotrophy during and after high-flow events, which has been attributed to ER being more

resilient to the negative or disturbance effect of increased discharge (Uehlinger & Naegeli 1998, Uehlinger 2000). The differences in the resistance of GPP and ER processes during the high-flow events will additionally determine how resilient or the recovery of these processes are after the high-flow periods (Uehlinger 2000). However, because of the many different ways in which discharge can disturb rates of GPP and ER this adds further complexity as to how individual reaches will recover. For example, rates of ER may have been reduced in some reaches such as the Gwydir vegetated and Roumalla vegetated reaches, because of unstable bed substrates (Uehlinger 2000, Atkinson et al. 2008). In contrast, the decreased rates of ER within reaches that have cobble bed substrates may have been attributed to scouring from cobbles (Reid et al. 2006). The inconsistent results of this study therefore reflect the complexity of the different ways in which discharge can affect reach-scale metabolism and the need to improve understanding of the relative contribution of habitats to overall stream metabolism.

Methodology considerations

The majority of published metabolism studies present the rates of GPP and ER in aerial units (g m^{-2} day^{-1}), rather than volumetric units (mg L^{-1} day^{-1}) as reported in this study. The use of aerial units can be for several reasons depending on the study. For instance, some studies may compare metabolic rates measured by benthic chambers to those determined for the water column (Fellows et al. 2006a, Oliver & Merrick 2006). Also, as this appears to be the more common way of presenting results, it may be easier for whole-ecosystem studies to convert to aerial units for comparison to the published literature. In other studies, whole-system rates of GPP and ER are converted to aerial units because water depths are less than 30 cm, therefore, the metabolic rates are largely attributed to the benthic community rather than the water column (Grace & Imberger 2006). As such, some studies will aim to standardise the rates of GPP and ER to benthic chlorophyll *a* or biofilm biomass, or alternatively, these related measurements will also be expressed in aerial units (Mulholland et al. 2001, Oliver & Merrick 2006, Bernot et al. 2010). In this study, the average reach water depths at each reach ranged from 0.14 m during the low-flow period to 1.16 m during the high-flow periods, so it was

assumed that the change in DO can be assumed to be attributed to planktonic algae or macrophytes (which would affect water column changes) rather than benthic communities.

Calculating the reaeration coefficient (K_2) is an important part of separating the true metabolic flux of DO from that caused by natural turbulence and absorption. Comparative studies have shown that the most accurate method of calculating reaeration within a reach is through the use of volatile gas and conservative tracer techniques (Young & Huryn 1996). Reviews of empirical based methods based on oxygen-light records or hydrological variables such as the surface renewal method (SRM) used in this study, have shown that there can be large variation in the calculated reaeration coefficients (Cox 2003, Iwata et al. 2007, Aristegi et al. 2009). However, empirical based calculations based on hydrological variables have been used as surrogate methods to calculate reaeration coefficients in many studies that have used the tracer-gas as their primary method, including a study on the role of geomorphology on stream metabolism (Bott et al. 2006), or to assist building reaeration coefficient regression models over different discharges (Mulholland et al. 2001, Ortiz-Zayas et al. 2005). Similarly, empirical calculations using hydrological variables were used by McTammany et al. (2003) in their study of longitudinal patterns of ecosystem metabolism along a 37 km stretch of the Little Tennessee River, North Carolina, salt injections were used to give greater accuracy to stream velocity or travel time, rather than the less accurate method of recording velocity at stream transects with a current meter. The energy dissipation method (EDM) is another empirical based technique based on hydrological variables (Grace & Imberger 2006). The suitability of the EDM over the 'dome' method (Copeland & Duffer 1964) for water bodies with higher turbulence is best shown in a study of the response of ecosystem metabolism to restoration (Colangelo 2007). Prior to restoration, the river system was considered lentic, so the best suited method was the 'dome' method, while the EDM was used post restoration due to the switch to a lotic system and therefore, a more turbulent system (Colangelo 2007). Although it is acknowledged that using volatile gas and conservative tracer injections to estimate K_2 provides the most accurate measurements, this method was not feasible due to the remote location of the streams and cost of equipment. Using oxygen-light based equations requires detailed records of the diel change in DO (Kosinski 1984, Chapra & Di Toro 1991) which were not present for these study

reaches. As the sampling periods occurred over different discharges, it was more likely that the change in hydrological variables would be stronger drivers of reaeration rather than light attenuation, within these study reaches. The significant effect of hydrology on nutrient concentrations (see Chapter 3) and benthic organic matter (see Chapter 4) mass offers further support for this suggestion. The SRM method used in this study was selected as the preferred technique over the EDM method because it was more suited to the hydrological conditions (surface water depth and velocity) found within the present study reaches (Grace & Imberger 2006).

In contrast to benthic chamber techniques, the open-system two-station technique for determining rates of ecosystem metabolism cannot provide an estimate of within reach variance. This means that there is a limited capacity to interpret ambiguous data as thoroughly as the chamber methods (Grace & Imberger 2006). This issue with ambiguity is evident from data collected from the Gwydir non-vegetated reach during September where no metabolic parameters could be calculated due to measurements having large variation between the 15 minute logging percentiles, which meant no linear curve could be confidently fitted (r^2 less than 0.90). The aim of the present study was to determine if whole-reach ecosystem metabolism was different between vegetated and non-vegetated reaches, therefore it was necessary to employ the open-system two station technique. Furthermore, as opposed to a single-station method, the two-station technique allowed for the change in metabolic rates measured to be ascribed to within each reach (Grace & Imberger 2006).

Effects of vegetation and hydrology on functional groups

Macrophytes

There was a significant negative effect of riparian vegetation on macrophyte biomass, with the largest difference between vegetated and non-vegetated reaches occurring during the low and no-flow periods. Other studies have shown similar results of increased biomass of submerged, semi-submerged and emergent macrophytes with decreasing riparian vegetation (Canfield & Hoyer 1988, Fletcher et al. 2000, Julian et al. 2010). The enhanced biomass and growth was attributed to an increase in light or PAR reaching the streambed (Canfield & Hoyer 1988,

Fletcher et al. 2000, Julian et al. 2010). A study in a second-order stream in central Wisconsin, USA, showed that a three-fold increase in PAR in non-forested reaches resulted in a four-fold increase in submerged macrophyte biomass compared to forested reaches (Julian et al. 2010). In addition to the change in riparian vegetation, the increase in PAR reaching non-forested reaches was also attributed to a wider stream channels (Julian et al. 2010). In the present study, the amount of light (measured as lumens m^{-2}) was significantly higher and channels were also wider within non-vegetated reaches compared to vegetated reaches. This suggests that the increase in light reaching the streams due to decreased riparian vegetation and channel width has led to increased macrophyte biomass in these agricultural streams.

It is important to note that different sampling methods were used to estimate biomass between the low and no-flow periods with the high-flow periods. The high-flow events meant that part of the channel could not be sampled due to safety concerns and high concentrations of TSS meant that visual observations could also not occur. Unfortunately this means that macrophyte biomass may have been underestimated during the high-flow periods, however, the large difference in biomass between vegetated and non-vegetated reaches during the low and no-flow periods still provides evidence of the significant effect of vegetation on biomass.

The largest total macrophyte biomass was present within the non-vegetated reaches during the low and no-flow periods and decreased during the high-flow periods. Many other studies have shown that increases in surface water velocity and discharge can decrease macrophyte biomass and in some cases shoot density (see review by Carr 1997). Although there was a general decrease detected in this study, there was no significant linear effect of hydrology on macrophyte biomass or correlations using a non-linear analysis with other hydro-geomorphic variables. However, most submerged macrophyte species exhibit seasonal growth, which means that their biomass would be reduced during their period of senescence, and therefore the effects of hydrology may not be relevant during these periods (Dawson 1976, Clarke 2002). During the low and no-flow period in this study, the dominant macrophytes were submerged species (see Appendix 6), which suggests that changes in hydrology during the high-flow periods could not have a strong affect on biomass due to their growth characteristics.

Chlorophyll a

The chlorophyll *a* concentrations present in the study reaches ranged between 1.2 to 195 µg L^{-1}, which are up to 39 times the suggested concentration of 5 µg L^{-1} for slightly disturbed lowland rivers and streams in southeastern Australia (ANZECC 2000). No chlorophyll *a* concentrations have currently been set for temperate upland streams in Australia. Another study on the Gwydir River downstream of the major water storage, Copeton Dam, pelagic chlorophyll *a* concentrations have ranged between 4 and 16 µg L^{-1} (Hadwen et al. 2010). An experimental study of plankton limitation in the nearby lowland Namoi River, Australia, showed that pelagic chlorophyll *a* concentrations ranged from 14.23 µg L^{-1} in summer to 4.37 µg L^{-1} in winter (Westhorpe et al. 2010). The study found that an increase in inorganic nutrients and glucose increased pelagic chlorophyll *a* concentrations to 120 µg L^{-1} in summer to 70 µg L^{-1} in winter (Westhorpe et al. 2010). Although concentrations of NOx in the reaches used in this study were below detection limits on most occasions, the concentrations of SRP were up to 18 times higher than the suggested concentration of 15 µg L^{-1} for slightly disturbed upland streams in southeastern Australia (ANZECC 2000). This indicates that high nutrient availability has led to increased algal growth and suggests that streams in the upper Gwydir River catchment have been impacted by agricultural practices.

In this study, there was no significant effect of vegetation on pelagic chlorophyll *a* concentration and the results do not support the study predictions. Most of the published literature for upland or small streams has focused on the significant negative effects of riparian vegetation on benthic autotrophic production (Sabater et al. 2000, Mulholland et al. 2001, Mosisch et al. 2001, Fellows et al. 2006a, Hagen et al. 2010), as the benthic community is assumed to be the main contributor to whole-ecosystem GPP. Although it has been suggested that the effects of riparian vegetation will have similar effects on phytoplankton productivity (Dodds & Welch 2000), there appears to be only a single study that has investigated the effects of riparian vegetation on phytoplankton in streams (Vähälato et al. 2005). The study focussed on the absorption of PAR by phytoplankton throughout the Neuse Basin, an agricultural area of North Carolina, USA, and showed that the interaction riparian shading and short flushing times

could reduce phytoplankton production in streams (Vähälato et al. 2005). Their study only had a single sampling period during autumn and the system was not considered to be nutrient limited (Vähälato et al. 2005). In this study, with the exception of the Roumalla vegetated reach, the chlorophyll *a* concentration was slightly higher within the non-vegetated reaches during the low-flow period. This suggests that during low-flow conditions riparian vegetation can reduce chlorophyll *a* concentrations within some reaches, and also highlights the need for repeated sampling in studies. Additionally, as the reaches in this study are N-limited, this may have also contributed to the overall non-significant difference between vegetated and non-vegetated reaches.

Discharge had a negative effect on mean chlorophyll *a* concentrations and as such, there was little difference in concentrations between vegetated and non-vegetated reaches during this period. The significant effect of discharge on chlorophyll *a* concentrations is similar to other studies in temporary streams, but these studies have looked at the effects on biofilm communities rather than pelagic communities (Uehlinger & Naegeli 1998, Biggs et al. 1999, Acuńa et al. 2004). However, increased flows in larger regulated rivers have also been shown to negatively affect phytoplankton growth (Webster et al. 2000, Mitrovic et al. 2011). The significant effect of hydrology in this study could suggest that discharge is more important in controlling autotrophic growth than shading by riparian vegetation.

Correlation of hydro-geomorphic and biochemical variables to metabolic parameters

In the context of temporary-river ecology, the largest variation in the rates of GPP and ER between all reaches was expected to occur during the no-flow period (Larned et al. 2010). From the results it is hard to determine when the most variation occurred between reaches on the same stream as this was not consistent in either the low and no-flow periods. However, during the low and no-flow periods the magnitude of variation was greater than during the high-flow periods, but this resulted from the low metabolic rates during the high-flow periods.

As previously discussed, hydrology was found to have a significant negative effect on the rates of GPP and ER. In addition, step-wise multiple regression analyses showed that flow percentile was most correlated with GPP at both the vegetated and non-vegetated reaches, and also with

ER at the vegetated reaches. The multiple regression models showed that DOC:SRP ratio also explained 14.8% of the variation in GPP within the non-vegetated reaches. During the high-flow periods, the concentration of SRP decreased (see Chapter 3). The decrease in SRP concentration caused an increase in the stoichiometric balance of DOC:SRP and led to ratios higher than the Redfield ratio of 100:1 (Redfield 1958), which could be limiting to autotrophic growth (Guasch et al. 1995, Tank & Dodds 2003, Westhorpe et al. 2010). Therefore, the results suggest that the increased DOC:SRP ratios during the high-flow periods could negatively affect GPP through nutrient limitation within the non-vegetated reaches.

Within the vegetated reaches, rates of ER were most correlated with flow percentile, xylo activity and temperature, while the concentration of TN was most correlated with ER at the non-vegetated reaches. Although the presence of high-molecular weight xyloside substrates can lead to higher ER activity (Tolunen et al. 1992), there was actually a decrease in ER when xylo activity increased during the high-flow periods in this study. A number of studies that have shown the negative effect of increased discharge on ER attributed to reduced habitat or substrate availability (Uehlinger 2000, Acuña et al. 2004, Atkinson et al. 2008). This implies that in this study, the effects of increased discharge had a disturbance effect on overall heterotrophic activity, and therefore may have masked any effects arising from the change in DOC quality. Therefore, given that the direction of the correlation was not as expected, this suggests that xylo activity is not independent from ER within the vegetated reaches.

Similarly, rates of ER and TN concentration within the non-vegetated reaches were negatively correlated. Increases in nitrogen have shown to lead to increased rates of ER in similar streams (Benstead et al. 2009). However, although the study reaches were shown to be N-limited and both rates of ER and TN concentration were shown to decrease with increased discharge, the total load of TN imported to reaches increased during these high-flow periods (see Chapter 3). This suggests that there was also a lack of independence between rates of ER and TN concentration rather than a meaningful ecological relationship. However, this does also suggest the importance of hydrology in driving the rates of ER in both the vegetated and non-vegetated reaches.

6.5 Summary

There were no significant effects of the presence of riparian vegetation on whole-system GPP or ER. Both GPP and ER rates were significantly affected by hydrology and decreased during the high-flow periods. There was a trend in some treatment streams between February and May, where GPP rates at the Gwydir and Roumalla vegetated reaches decreased and their paired non-vegetated reaches increased. The NEP estimates showed that three vegetated and two non-vegetated reaches were net heterotrophic during February, the low-flow period. During May, the no-flow period, nine of the ten reaches were autotrophic, while there was no clear pattern in trophic state within vegetated and non-vegetated reaches during September and November, the high-flow periods.

There was a significant negative effect of vegetation on in-stream macrophyte biomass, which was attributed to the increased amount of light reaching the non-vegetated reach channels. The largest macrophyte biomasses were present at the non-vegetated reaches during May, the no-flow period, however, there were no significant correlations between macrophyte biomass and hydro-geomorphic variables.

There were no significant differences in chlorophyll *a* concentrations between vegetated and non-vegetated reaches. Chlorophyll *a* concentrations were significantly affected by hydrology, and the largest variation in concentration between vegetated and non-vegetated reaches occurred during February and May. Despite the large chlorophyll *a* concentrations present, the rates of C-fixation were considered very low compared to those reported in the literature, which suggests that autotrophic production was being limited or disturbed in some way.

Chapter 7: Synthesis

7.1 Introduction

Biogeochemical processes represent the mass-balance and cycling of nutrients within aquatic ecosystems. Reach-scale riparian revegetation is a commonly used technique in stream restoration often aimed at returning ecosystem processes such as nutrient and organic matter cycling to a more natural condition. Measuring the response of these processes to restoration is important as they provide an understanding of fundamental ecosystem functions that support stream foodwebs rather than inferring process-level responses from the return of structural habitats (Ryder & Miller 2005). The role of the riparian zone in regulating organic matter and nutrient dynamics in streams has been the focus of much research; however the influence of the riparian zone on longitudinal transport and retention processes is far less understood. Riparian revegetation techniques often aim to increase nutrient and organic matter retention through increasing organic matter input and enhancing retention through increased geomorphic complexity (Bernhardt & Palmer 2011). Within stream reaches, nutrient and organic matter retention can occur through abiotic processes such as physical entrainment by protruding features (Quinn et al. 2007), or through biotic processes including heterotrophic uptake (Aldridge et al. 2009). As the rates of different biogeochemical processes can vary with channel geomorphology and hydrology, it is necessary to explore their effects and interactions with riparian vegetation, particularly in intermittent streams where discharge can vary from floods to cease-to-flow events over relatively short timeframes.

The main aim of this study was to explore the biogeochemistry of vegetated and non-vegetated reaches within agricultural intermittent streams in the upper Gwydir River catchment, Australia. Understanding channel complexity is an integral part of quantifying biogeochemical processes in streams. I used on-ground surveying techniques to develop high-resolution DEMs and spatially explicit habitat maps to identify channel morphology and key geomorphic features such as flood debris accumulation points and wetted area that can influence organic matter and nutrient retention (Chapter 2). The channel parameters derived from the DEMs allowed reach-scale hydrologic models to be constructed to produce nutrient and organic matter mass-

balances (Chapters 2, 3 and 4). The quantity and quality of organic matter can play a pivotal role in the nutrient retention and stoichiometry (Aldridge et al. 2009, Gibson & O'Reilly 2012). I quantified reach-scale nutrient and organic matter mass-balances to identify differences in organic matter retention between vegetated and non-vegetated reaches, and to explore the relationship with nutrient stoichiometry and retention under different hydrological conditions (Chapter 3 and 4). As rates of organic matter breakdown can affect organic matter retention (Webster et al. 1999), I conducted a litterbag experiment with *Casuarina cunninghamiana* needles, the dominant riparian vegetation species in the study streams, to explore the difference in rates and biophysical drivers of organic matter breakdown between vegetated and non-vegetated reaches (Chapter 4). The quality of organic matter is largely determined from its' source and can alter rates of reach-scale heterotrophic respiration and nutrient retention (Findlay & Sinsabaugh 1999); therefore, extracellular enzyme techniques were used to determine dominant sources of DOC regulating heterotrophic metabolism (Chapter 5). Rates of dominant extracellular enzyme activities were linked to metabolic processes measured using an open-system technique to estimate daily rates of whole-reach GPP, ER and net C-fixation (Chapter 6).

This final chapter synthesises the main research findings to advance the conceptual understanding of dominant biogeochemical processes in vegetated and non-vegetated reaches of intermittent streams. I also address the advantages and limitations to the study design used in this thesis and discuss the implications of this study for future restoration projects.

7.2 Riparian vegetation

In this study, the presence of riparian vegetation did not consistently increase the geomorphic complexity of reaches. There was no consistent difference in the amount of logs present between vegetated and non-vegetated reaches but there were some differences in the types of other geomorphic features present. Flood debris accumulation points were only present within vegetated reaches, while there were a larger number of macrophyte beds found within non-vegetated reaches. It was also evident that non-vegetated reaches had larger wetted areas, particularly during periods of surface flow connectivity. The larger wetted areas meant that the

surface area:volume ratio and surface water velocities were lower within the non-vegetated reaches. As geomorphic complexity and physical channel parameters can regulate organic matter and nutrient retention (Acuńa et al. 2004, Brookes et al. 2005, Aldridge et al. 2009), there is potential for differences in biogeochemical processes between vegetated and non-vegetated reaches.

Potential differences in reach scale nutrient retention or nutrient stoichiometry were not evident in this study. Increased wetted area and lower surface area:volume ratios have been shown to increase rates of abiotic and biotic nutrient uptake, and therefore decrease nutrient spiraling lengths (Aldridge et al. 2009). An increase in the number of geomorphic features and therefore channel complexity, have also been shown to enhance nutrient retention (see Brookes et al. 2005). In this study, hydro-geomorphic parameters were not correlated with the retention and/or export of DOC, TN, TP and NOx in any reaches. The exception was the correlation of SRP retention with surface area:volume ratio and wetted area within the non-vegetated reaches, with larger SRP loads being retained when surface area:volume ratio decreased and wetted area increased. Although increases in wetted area have been associated with increased heterotrophic activity such as organic matter decomposition (Boulton 1991, Langhans & Tockner 2006), the decreased rates of GPP and ER during the high-flow periods meant that there would have been an actual decrease in biotic demand. Unchanged rates of AP enzyme activity provide further evidence that an increase in SRP retention at non-vegetated reaches was not due to an increase in heterotrophic activity. However, the significant correlation still provides support for the suggestion the increases in wetted area can lead to an increase in SRP retention within some reaches.

The C:nutrient ratios and nutrient enrichment experiments identified that autotrophic production was consistently N-limited. Nutrient spiraling concepts suggest that there will be an increase in biotic nutrient uptake if biota are limited by a particular nutrient (Newbold et al. 1981, Fisher et al. 1998). The nutrient mass-balances showed that most reaches retained NOx during the majority of flow conditions, which supports this concept. During the first high-flow sampling period, there was an increase in the quantity of NOx retained within several of the

vegetated reaches. This may be in part due to an increase in imported load of NOx within all study reaches at this time. The increase in NOx loads imported from upstream reaches during the first high-flow period are linked to a concomitant decrease in reach-scale biotic demand, as shown by the reduced rates of GPP and ER during these periods (Fellows et al. 2006b). Therefore, the results of this study support the suggestion that nutrients are more likely to be retained within reaches if biota are limited by the particular nutrient.

Reaches with riparian vegetation did not have a larger total mass of FBOM or CBOM measured as aerial units or whole-reach scales. This is in contrast to the findings of many other studies that have compared organic matter mass between vegetated and non-vegetated reaches (Reid et al. 2008, Gilling et al. 2009, Watson & Barmuta 2010). Vegetated reaches are often thought to have larger CBOM masses because there is a larger input of allochthonous organic matter (Campbell et al. 1992, Sabater et al. 2000, Reid et al. 2008) in addition to a greater capacity to retain organic matter through increased geomorphic complexity (James & Henderson 2005, Watson & Barmuta 2010). The results of this study have demonstrated no significant difference in FBOM or CBOM imported or retained between vegetated and non-vegetated reaches. However, as there were different geomorphic features present between vegetated and non-vegetated reaches, the biophysical processes leading to organic matter retention varied between reaches. The experimental organic matter releases were able to show that the flood debris accumulation points and bank edges were important in retaining organic matter in vegetated reaches, while the macrophyte beds and pools were important for retaining organic matter in non-vegetated reaches. While the results showed that these different structures are performing the same function it is important to note that the impact of disturbance may not act equally on the different structures. For example, macrophyte beds may be more easily disturbed (removed) compared to flood debris accumulation points by floods (Carr 1997), which suggests that organic matter retention may not be the same over time. Increased benthic organic matter has been shown to increase the rate of nutrient retention through enhanced heterotrophic activity (Aldridge et al. 2009). However, there was no effect of riparian vegetation on rates of organic matter breakdown demonstrating that the organic matter retained within the vegetated and non-vegetated reaches will undergo similar rates of

breakdown. The lack of significant difference in organic matter mass and rates of breakdown between vegetated and non-vegetated reaches was also reflected in the no differences in other reach-scale ecosystem functions such as nutrient stoichiometry, nutrient retention and metabolic rates. These findings do not support the current predictions that riparian vegetation will increase benthic organic mass within reaches, as non-vegetated reaches are still able to retain imported organic matter through different biophysical processes.

Trees that grow in the riparian zone can provide shade over stream channels (Gregory et al. 1991), which may lead to reduced autotrophic growth (Bunn et al. 1999, Fellows et al. 2006a, Julian et al. 2010). In addition to the increase in the surface area of macrophyte beds as geomorphic features, there was also an increase in macrophyte biomass found within the wetted channel at non-vegetated reaches, which is similar to the findings of other studies (Bunn et al. 1998, Julian et al. 2010). The difference in macrophyte growth is most likely attributed to an increase in the amount of light reaching the channels, larger wetted channel areas and low surface water velocities (Canfield & Hoyer 1988, Bunn et al. 1998, Julian et al. 2010). Structural changes to macrophyte biomass and distribution were reflected in the increased β-glu activity within the non-vegetated reaches, indicating that macrophytes are providing an important (and different) source of DOC to heterotrophic communities within the non-vegetated reaches. In some intermittent streams, the high-quality low-molecular weight DOC can become limiting to heterotrophs during periods of surface water fragmentation, which causes the heterotrophic communities to switch to high-molecular weight DOC that is lower in quality (Ylla et al. 2010). In this study, the activity of both β-glu and α-glu during these periods meant that high-quality DOC was not limiting to heterotrophic metabolism in disconnected pools. These results provide evidence that riparian vegetation can alter the metabolic pathways contributing to GPP and therefore the sources of DOC available to heterotrophic communities between vegetated and non-vegetated reaches.

Despite the significant difference in macrophyte biomass, there were no significant effects of riparian vegetation on rates of whole-system GPP and ER, which is in contrast to many other studies that show increased rates of GPP with decreasing riparian vegetation (Sabater et al.

2000, Fellows et al. 2006a, Clapcott & Barmuta 2010). Rates measured in this study were within the lower range suggested for upland streams of 0.2 to 50 mg L^{-1} day^{-1} and the rates of ER were lower than the suggested range of -8 to -100 mg L^{-1} day^{-1} (see Grace & Imberger 2006). Similarly, the low rates of GPP and ER also led low rates of net-C fixation within both the vegetated and non-vegetated reaches. These rates are surprising given that the chlorophyll *a* concentrations were relatively high, ranging between 0.009 and 0.195 mg L^{-1} during the low and no-flow periods, and between 0.001 and 0.004 mg L^{-1} during the high-flow periods. This may suggest that metabolic rates are being limited or disturbed in some way. Furthermore, the mass-balance results for chlorophyll *a* showed that these high concentrations meant large loads of phytoplankton were being longitudinally transported through both vegetated and non-vegetated reaches. It would be expected that these large concentrations would lead to all reaches being autotrophic, however, the net-C fixation rates indicated that three vegetated and two non-vegetated reaches were heterotrophic. These results indicate that the presence of riparian vegetation at the reach-scale may not always significantly lower rates of GPP or increase rates of ER to produce heterotrophic systems, particularly if the rates of GPP and ER are already low within the reaches. The low rates of GPP and ER within both the vegetated and non-vegetated reaches may also suggest that other parameters that are not controlled through riparian vegetation may have more influence over reach metabolism.

Previous studies have suggested that riparian vegetation will lead to increased biogeochemical cycling of key elements within reaches. It is currently understood that this increase in cycling will be reflected in an increase in organic matter and nutrient retention attributed to enhanced channel geomorphic complexity and lead to higher C:nutrient ratios within reaches. In addition, the presence of riparian vegetation is thought to produce heterotrophic systems through lowering rates of GPP and increasing rates of ER. The results of this study show that riparian vegetation can alter the channel morphology and the types of geomorphic features present within reaches. However, these differences do not lead to increased organic matter or nutrient retention and therefore, higher C:nutrient ratios at reach scales in these intermittent streams. This study further shows that although the presence of riparian vegetation can reduce the

abundance of emergent and submerged macrophytes, this will not always alter the rates of metabolism or trophic state within reaches.

7.3 Hydrology

Changes in hydrology can create new and different habitats that are linked to biogeochemical processes including nutrient and organic matter retention (Brookes et al. 2005, Quinn et al. 2007, Larned et al. 2010). In this study, the wetted area inundated at each reach increased with discharge leading to lower surface area:volume ratios. The increase in wetted area also meant that a larger number of geomorphic features were inside the wetted perimeter of the main channel at some reaches. In the vegetated reaches, these features were predominantly flood debris accumulation points while at non-vegetated reaches the increased discharge meant that macrophyte beds became inundated. The whole-reach estimates of CBOM and FBOM showed that there was no clear pattern as to whether the increased wetted area with discharge led to an increase or decrease in CBOM and FBOM masses within either the vegetated or non-vegetated reaches. Other studies focused on the effects of discharge on CBOM and FBOM at the aerial unit scale in intermittent streams have found similar results (Gurtz et al. 1988, Boulton & Lake 1992), while others have shown a general decrease with increased discharge (Acuńa et al. 2004). Increases in discharge led to an increase in the magnitude of COM and FOM imported to reaches. However, these increases did not always lead to increased retention within the reaches. This complexity could stem from increases in water height changing the capacity of submersed geomorphic features to intercept and retain transported organic matter. Some geomorphic features may also reach a threshold in the quantity of organic matter retained (Jones 1997, Quinn et al. 2007), therefore causing more organic matter to be exported from some reaches. Furthermore, organic matter that has been previously retained within reaches may be resuspended with increased surface water velocities and exported from reaches. Therefore, the results suggest that although changes in hydrology will lead to new and different habitats being inundated and increases in discharge can transport larger organic matter loads to reaches, this will not necessarily lead to an increase in the amount of organic matter retained or stored within reaches.

Organic matter that is retained within reaches will undergo biotic and abiotic breakdown processes (Webster et al. 1999). At reach-scales, increased heterotrophic respiration has been related to increased benthic organic matter mass (Acuńa et al. 2004, Aldridge et al. 2009). In this study, some of the largest quantities of benthic organic matter were present during the high-flow periods, however, the rates of ER actually decreased during these sampling periods. The litterbag experiments in this study found that the abiotic process of fragmentation by increased surface water velocities was found have a significant negative effect on organic matter breakdown, particularly within vegetated reaches. Other studies have found similar negative relationships between surface water velocities and the breakdown of organic matter (Heard et al. 1999, Lepori et al. 2005, Hoover et al. 2006). The litterbag experiment also indicated that large surface water velocities and unstable bed substrates led to organic matter being smothered by inorganic material. These results indicate that hydrology and the interaction with channel geomorphology will be important in determining the fate of organic material that is retained within vegetated and non-vegetated reaches and that increases in organic matter present within reaches will not always lead to increased rates of ER at reach-scales.

Increases in discharge and wetted area have been shown to transport large quantities of allochthonous DOC to streams, which can alter the dominant sources of DOC available to heterotrophic communities (Ylla et al. 2011). The extracellular enzyme analyses showed that during the low and no-flow periods the main sources of DOC available to heterotrophs were high-quality low-molecular weight DOC from allochthonous and autochthonous sources, while during the high-flow periods the DOC pool was dominated by low-quality high-molecular weight substrates from allochthonous sources. The rate of ER can change with the quality of DOC available, with high-molecular weight DOC increasing the rates of ER (Tulonen et al. 1992, Gibson & O'Reilly 2012). However, the results of this study showed that the rates of ER did not increase during the high-flow periods when high-molecular weight DOC was present in this study. This suggests that although the increase in discharge did lead to a change in the dominant sources of DOC (which agrees with the findings of other studies), this shift in DOC

quality did not have an effect on the heterotrophic activity within vegetated or non-vegetated reaches.

The quantity of nutrients available to biota can also increase the rates of GPP and ER within reaches (Rier & Stevenson 2002, Benstead et al. 2009, Sabater et al. 2011). In this study, there was an increase in the total quantity (i.e. load) of nutrients transported to reaches during the high-flow periods. However, the rates of GPP and ER decreased within both the vegetated and non-vegetated reaches during these high-flow periods. This negative effect of discharge has also been shown for similar metabolism studies (Fisher et al. 1982, Uehlinger et al. 2000, Acuña et al. 2011). The decrease in rates of GPP and ER were likely to be attributed to a decrease in the algal and macrophyte production, as discharge also had a negative impact on chlorophyll *a* concentration and macrophyte biomass. These results indicate that discharge has a disturbance effect on the autotrophic community and that hydrology plays an important role in controlling the rates of GPP and ER through decreasing autotrophic production, regardless of the increase in nutrient availability.

Despite the mass-balance estimates showing that there were larger loads of nutrients being transported through reaches during the high-flow periods, the nutrient surveys actually showed that there was a decrease in the concentration across all reaches. This indicates that there was a dilution effect of increased discharge on surface water nutrient concentrations and is similarly to other studies on the effects of discharge on nutrient dynamics (Triska et al. 1990, Dent & Grimm 1999). In many regulated systems, flow releases are used to decrease autotrophic growth through diluting nutrient concentrations (Mitrovic et al. 2003, Ryder & Vink 2007, Mitrovic et al. 2011). In this study, although there was a negative effect of increased discharge on the rates of GPP, ER and net C-fixation when estimated at the volumetric unit scale, the whole-reach estimates showed that the rates of C-fixation and consumption were up to 5000 times higher during the high-flow periods compared to low-flow periods. Similarly, the concentration of chlorophyll *a* decreased but the mass-balance estimates showed that there was an increase in the transported chlorophyll *a* loads across all reaches during the first high-flow period. Although this initial increase in the chlorophyll *a* loads may indicate an initial

'flushing' effect of discharge within the reaches (Triska et al. 1990), the whole-reach estimates of C-fixation and consumption show that the dilution of nutrients was not having an effect on the rates of metabolism within the reaches. The results also adds further support for the above suggestion that the physical disturbance of hydrology may have a greater impact on metabolic rates than biochemical parameters such as nutrient concentration in these unregulated systems.

In contrast to the dilution effect of increased discharge on nutrient concentrations and metabolic rates, the rates of EEA did not differ in magnitude between the low and high-flow periods, which highlights a potential limitation of using EEA to infer rates of heterotrophic and autotrophic activity. As rates of ER decreased during the high-flow periods at the volumetric unit scale it would be assumed that there would also be a concurrent decrease in rates of EEA, which was not the case in this study. Additionally, the rates of EEA in the present study were very high in comparison to those reported in other studies, which would suggest that the rates of ER would also be much higher; however, the ER rates were found to be very low (see Grace & Imberger 2006). In this study, the enzyme activities analysed were from a dissolved surface water fraction, which shows the activity of 'free' extracellular enzymes (i.e. not attached to microbial seston). This suggests that the activity of these enzymes may be not be released by the microbes when required for hydrolysis, but instead the enzymes may just be present within the water column (Boon 1990). These results indicate that rates of ER in conjunction with EEA need to be measured to gain an accurate understanding of metabolic processes within reaches.

During the low and no-flow periods, there was larger variation in the mean nutrient concentrations and stoichiometry between reaches in comparison to the higher flows. This finding agrees with the predictions of temporary-river ecology, which suggests that reaches will become more heterogeneous as surface water contracts into fragmented pools (Stanley et al. 1998, Dent & Grimm 1999, Larned et al. 2010). It was also predicted that the effects from riparian vegetation would drive the differences in nutrient stoichiometry, and this would be through increased heterotrophic respiration associated with larger quantities of allochthonous organic matter at vegetated reaches. However, from the net C-fixation estimates and P/R

ratios, the dominant metabolic processes within both the vegetated and non-vegetated reaches appeared to be autotrophic production rather than respiration processes relating to allochthonous organic matter dynamics. This was supported by the results of the enzyme analyses, which showed that the dominant sources of carbon were from autochthonous sources. These results suggest that the contribution of coarse organic matter from riparian vegetation is not the dominant process regulating nutrient stoichiometry during periods of surface water fragmentation. Additionally, although there appeared to be no significant effect of riparian vegetation on the rates of GPP and ER, the results did show that riparian vegetation had a significant negative effect on macrophyte biomass. As macrophytes require nutrients for growth and contribute to DOC pools within reaches (Clarke 2002), this suggests that macrophyte communities may be important in regulating C:nutrient stoichiometry in non-vegetated reaches during low and no-flow periods. Furthermore, these results support the suggestion that the dominant biogeochemical processes between reaches will be more heterogeneous and reach-scale parameters such as riparian vegetation will become more important in driving these different processes during the low and no-flow periods.

7.4 Advantages and limitations of study design

There was no consistent pattern in the retention of nutrients or organic matter at reach-scales within and between vegetated and non-vegetated reaches. It is important to acknowledge that patterns of nutrient and organic matter retention may not exist and that the data from this study is accurate. However, there may also be several reasons as to why this study failed to detect any patterns. Firstly, the lack of significant difference in nutrient retention between vegetated and non-vegetated reaches is similar to recent findings for reach scale (approximately 200 m) restoration projects using channel configuration in North America (Filoso & Palmer 2011, Sudduth et al. 2011). From the findings of these studies, it was suggested that reach-scale nutrient retention processes may be masked by larger catchment-scale issues (Bernhardt & Palmer 2011). This suggests that it is difficult to measure and detect an effect of reach scale vegetation in such an altered landscape, and particularly during high-flow periods. However, if restoration projects are going to focus at the reach scale, then sub-

reach scale drivers of retention such as physical entrainment by protruding geomorphic features or biotic uptake also need to be explored. In addition, although the vegetated reaches used in this study were not the direct result of revegetation, extensive surveying of the area did show that these reaches were a true representation of 'vegetated' areas within the area and the length that is commonly fenced and revegetated in restoration projects in the study system and more broadly (Bernhardt et al. 2005, Williams et al. 2011).

Significant differences in nutrient stoichiometry have been found in several studies comparing the effects of vegetation at the catchment (watershed) scales. The seminal study by Likens et al. (1970) found that the nitrogen export was 14 to 15 times higher from a deforested catchment compared to a forested catchment within the Hubbard Brook Experimental Forest and was attributed to an increase in soil nitrification processes. A study of the effects of forest succession after deforestation on in-stream biogeochemical processes was also investigated at catchment scales in North Carolina, USA (Vallet et al. 2002). Although the study used solute injections to quantify rates of nutrient uptake rather than total in-stream retention, the study still found that there were significant differences between catchments related to forest age (Vallet et al. 2002). Studies that have compared the export of nutrients between streams draining pine forests and pasture dominated catchments have also found an increase in nutrient export and has been attributed to a combination of increased input and decreased in-stream retention (Quinn & Stroud 2002, Vink et al. 2007). In combination with the non-significant results of nutrient stoichiometry and retention found in this study, this suggests that revegetation and fencing needs to occur at scales larger than 100 m reaches to achieve significant increases in nutrient retention and other biogeochemical processes. However, it is also important to note that *C. cunninghamiana* trees can be easily fragmented, which may mean that the reaches used in this study may naturally have little capacity to retain nutrients. Additionally, the lack of significant difference in nutrient stoichiometry or retention detected between reaches during the flow periods may also be attributed to the movement of water. When water is transported through reaches, the effects of surrounding environmental parameters such as riparian vegetation may not be detected until further downstream (Thorp & de Long 1994). This suggestion introduces the problem of temporal and spatial scale because it

is unclear as to how far downstream these effects may be detected and the interaction this will play with discharge or the velocity of surface water.

An issue that has previously been noted with stream restoration projects is the lack of available reference conditions to assist in developing targets and outcomes of restoration (Palmer et al. 2005, Mika et al. 2010). This is particularly pertinent to the current study, where it was difficult to locate suitable reaches that were representative of reference conditions in an area that has been widely developed by agriculture. The matched-pairs approach where both a treatment and control reach were present on the same stream was employed to help overcome this issue in this study. Another problem that can arise when selecting reaches is that there will be natural variation in some biogeochemical parameters such as nutrient concentrations between streams within the same catchment (Palmer et al. 2005). The three-factor statistical model that was used to test for differences between treatments, streams and sampling times (attributed to changes in hydrology) showed that there were significant differences in nutrient stoichiometry among streams, and again highlights the advantage of employing a matched-pairs approach and analysing differences between streams. Similarly, some biogeochemical parameters such as chlorophyll *a* concentration and temperature can naturally vary as water is transported downstream (Thorp & de Long 1994). The spatial arrangement of having all non-vegetated reaches located upstream of the vegetated reaches in the treatment streams meant that during periods when surface water was connected the organic matter present in the non-vegetated reaches was not from organic matter exported from the vegetated reaches. However, to overcome the problem natural longitudinal variation, control streams which had a pair of either vegetated or non-vegetated reaches were used. The location tests were then able to show whether any significant effects of riparian vegetation could not be due to natural longitudinal variation. The outcomes of this study suggest that using a matched-pairs approach together with control streams can overcome the problems of dealing with natural variation within and between streams, as well as, a lack of suitable reference conditions when monitoring the outcomes of restoration.

The large variation in nutrient and organic matter retention within and between vegetated and non-vegetated reaches may also suggest that retention is naturally variable or alternatively, that the sampling size is not adequate to detect an effect in this study despite the large sampling effort to collect this amount of data. Post-hoc calculations showed that the sample size of five vegetated and five non-vegetated reaches in this study was only able to provide a statistical power of approximately 25%. This indicates that a sample size of more than 30 streams would be needed in order to detect a significant effect at 0.80 confidence level. However, this is not possible because this number of streams does not exist in this area and the sampling effort associated is already very large for just a small number (i.e. 10) streams. Another limitation with using the mass-balance approach compared to other techniques to estimate retention such as solute injections (Mulholland et al. 2002, Dodds et al. 2004, Fellows et al. 2006b), is that the sampling cannot be increased (i.e. replicated) at the individual reach level to reduce within reach variance, which makes it difficult to predict broad-scale patterns of biogeochemical processes. In the present study, alternative techniques such as nutrient additions and solute injections could not be used as they are expensive and also very difficult to perform in large streams and rivers (Stream Solute Workshop 1990).

The DEMs were able to facilitate transformation of point-concentration and aerial unit scale data to load and whole-reach scales, respectively. The transformation of data was essential for understanding the effects of riparian vegetation and hydrology on biogeochemical processes at the reach-scale. However, in addition to the problem of low sample size, a further limitation of using the mass-balance method is that it is difficult to detect patterns of retention when nutrient concentrations are below detection limits, such as NOx in this study. Volatile tracer and solute injections would not be able to overcome the issue of low nutrient concentration as they are adding to the ambient concentration rather than measuring the change of in-situ nutrients (Stream Solute Workshop 1990). However, the ability of the mass-balance method to estimate the true retention of nutrient during different discharges is necessary to answer the research questions of this thesis. In the current study, the SRP mass-balance estimates were able to show that while the magnitude of nutrients being transported through the reaches can change with the magnitude of discharge, the retention of some nutrients did not. A related

advantage is that while the nutrient point concentration decreased there was actually an increase in the total load of nutrients imported to the reaches, which indicates that increased discharge had a dilution effect. Similarly, the increase in wetted area during high discharge periods showed that there was no consistent pattern between reaches as to whether there was an increase or decrease in CBOM and FBOM mass at aerial unit and whole-reach scales. Solute additions cannot be used to estimate organic matter retention and the inconsistent results of the benthic organic matter surveys show that organic matter mass cannot show whether there is an increase in retention. Therefore, mass-balance estimates are necessary to give an accurate indication of whether both vegetated and non-vegetated reaches can retain organic matter. Additionally, the mass-balance method is able to detect how retention processes will respond to changes in nutrient and organic matter point concentrations, and total loads associated with fluctuations in discharge at the whole-reach scale.

7.5 Implications for restoration

Riparian revegetation is commonly carried out at reach-scales (<200 m) with the aim to restore biogeochemical processes and other ecosystem functions through increased geomorphic complexity (structural habitat) and organic matter inputs (Bernhardt & Palmer 2011). In this study, the presence of riparian vegetation at reach-scales was related to the type of some geomorphic features (i.e. more flood debris accumulation points and less macrophyte beds), reduce surface area:volume ratios and surface water velocities. However, these differences in structural habitat did not lead to a change in the quantity of organic matter retained, as non-vegetated reaches were able to retain organic matter through submerged and emergent macrophytes, which were not present within vegetated reaches. Hydrology also affected organic matter retention and added further complexity to the interaction between retention processes and channel geomorphology. In addition, it was also apparent that high-flow events could lead to channel erosion and move unstable bed substrates, as well as, transporting large amounts of sediment from upstream areas (Stewart & Ryder 2012 – see Appendix 1) that smothered organic matter or potentially reduced organic matter retention. Therefore, the results of this study suggest that if restoration aims to enhance organic matter retention in

unregulated systems, the influence of catchment hydrology and its' interaction with reach geomorphology needs to be considered.

Ecosystem functions such as autotrophic production and heterotrophic decomposition of organic matter are also considered to be important for controlling nutrient stoichiometry and retention within reaches (Aldridge et al. 2009). In this study, the quantity and quality of organic matter did not appear to influence nutrient stoichiometry or retention. During low and no-flow periods, the results of the EEA's and metabolism estimates indicated that GPP was the dominant process controlling nutrient stoichiometry. Although the presence of riparian vegetation did reduce macrophyte biomass, there was no overall effect on whole-system rates of GPP, which also meant that no significant effect of vegetated on nutrient retention was detected. However, the results of this study showed that these macrophytes were important sources of DOC for heterotrophic communities during periods of surface water contraction and fragmentation. The current understanding of the role of riparian vegetation is that it can enhance organic matter retention, which is essential for ecosystem metabolic processes, but the results of this study indicate that the structural habitat created by macrophytes is playing a surrogate role for the functions of riparian vegetation in non-vegetated reaches. In addition, this study has also shown that although there was no effect of riparian vegetation on the overall rates of metabolic processes, there were differences in the main drivers of these processes between vegetated and non-vegetated reaches, with algae the main driver in vegetated reaches and both algae and macrophytes the drivers of processes within non-vegetated reaches.

7.6 Conclusion

Reach-scale riparian vegetation is thought to increase the geomorphic complexity of streams and enhance organic matter inputs, which may lead to an increase in organic matter retention. It is currently understood that the presence of organic matter will play a pivotal role in the biogeochemical cycling of in-stream nutrients and therefore C:nutrient stoichiometry. As such, the potential increase in organic matter quantities in combination with the shading provided by riparian vegetation is expected to shift the metabolic state to heterotrophy in vegetated

reaches, particularly during periods of surface water contraction and fragmentation. During periods of connectivity, the increase in allochthonous organic matter within vegetated reaches will lead to increased nutrient retention at the reach-scale. The results of this study have shown that the presence of riparian vegetation did affect the type of some geomorphic features present within reaches. However, as these geomorphic features were still able to retain organic matter and that there was a complex interaction of hydrology on organic matter retention, there was no overall effect of riparian vegetation on organic matter quantity. This study also showed that the presence of organic matter was not central in controlling rates of nutrient retention or metabolism, and similarly, there was no effect of riparian vegetation on the overall rates of these processes, which contrasts to the current expectations. However, the presence of riparian vegetation did reduce macrophyte biomass, which was shown to be essential in trapping organic matter and controlling nutrient cycling. This indicates that reach-scale riparian vegetation may not alter overall C:nutrient stoichiometry, but vegetation can affect the mechanisms regulating biotic and abiotic processes that are important in stream nutrient cycling.

References

Abeliovich, A. & Weisman, D. (1978) Role of heterotrophic nutrition in growth of the alga *Scenedesmus obliquus* in high-rate oxidation ponds. *Applied and Environmental Microbiology,* **35,** 32-37.

Acuña, V., Giorgi, A., Muñoz, I., Sabater, F. & Sabater, S. (2007) Meteorological and Riparian Influences on Organic Matter Dynamics in a Forested Mediterranean Stream. *Journal of the North American Benthological Society,* **26,** 54-69.

Acuña, V., Giorgi, A., Muñoz, I., Uehlinger, U. & Sabater, S. (2004) Flow extremes and benthic organic matter shape the metabolism of a headwater Mediterranean stream. *Freshwater Biology,* **49,** 960-971.

Acuña, V., Vilches, C. & Giorgi, A. (2011) As productive and slow as a stream can be - the metabolism of a Pampean stream. *Journal of the North American Benthological Society,* **30,** 71-83.

Aldridge, K.T., Brookes, J.D. & Ganf, G.G. (2009) Rehabilitation of Stream Ecosystem Functions through the Reintroduction of Coarse Particulate Organic Matter. *Restoration Ecology,* **17,** 97-106.

Allan, J.D. (2004) Landscapes and Riverscapes: The Influence of Land Use on Stream Ecosystems. *Annual Review of Ecology, Evolution, and Systematics,* **35,** 257-284.

Anderson, M.J., Gorley, R.N. & Clarke, K.R. (2008) *PERMANOVA+ for PRIMER: Guide to software and statistical methods,* PRIMER-E, Plymouth, United Kingdom.

Angradi, T. (1991) Transport of coarse particulate organic matter in an Idaho river, USA. *Hydrobiologia,* **211,** 171-183.

ANZECC. (2000) Australian and New Zealand guidelines for fresh and marine water quality. *National Water Quality Management Strategy*, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

Aristegi, L., Izagirre, O. & Elosegi, A. (2009) Comparison of several methods to calculate reaeration in streams, and their effects on estimation of metabolism. *Hydrobiologia,* **635,** 113-124.

Arnosti, C. (2003) Microbial extracellular enzymes and their role in dissolved organic matter cycling. In: *Aquatic ecosystems: interactivity of dissolved organic matter.* (Eds. Findlay, S.E.G., Sinsabaugh, R.L.), pp. 315-342. Academic Press, Amsterdam.

Artigas, J., Romaní, A.M., Gaudes, A., Muñoz, I. & Sabater, S. (2009) Organic matter availability structures microbial biomass and activity in a Mediterranean stream. *Freshwater Biology,* **54,** 2025-2036.

Atkinson, B.L., Grace, M.R., Hart, B.T. & Vanderkruk, K. (2008) Sediment Instability Affects the Rate and Location of Primary Production and Respiration in a Sand-Bed Stream. *Journal of the North American Benthological Society,* **27,** 581-592.

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.

Banens, R.J. (1987) The Geochemical Character of Upland Waters of Northeast New South Wales. *Limnology and Oceanography,* **32,** 1291-1306.

Bärlocher, F., Stewart, M. & Ryder, D. S. (2011) Analyzing aquatic fungal communities in Australia: impacts of sample incubation and geographic distance of streams. *Czech Mycology,* **63,** 113-132.

Bärlocher, F., Helson, J.E. & Williams, D.D. (2010) Aquatic hyphomycete communities across a land-use gradient of Panamanian streams. *Fundamental and Applied Limnology,* **177,** 209-221.

Bärlocher, F., Stewart, M. & Ryder, D.S. (2012) Processing of *Eucalyptus viminalis* leaves in Australian streams importance of aquatic hyphomycetes and zoosporic fungi. *Fundamental and Applied Limnology,* **179,** 305-319.

Barnese, L. & Schelske, C. (1994) Effects of nitrogen, phosphorus and carbon enrichment on planktonic and periphytic algae in a softwater, oligotrophic lake in Florida, USA. *Hydrobiologia,* **277,** 159-170.

Beechie, T.J., Pess, G., Kennard, P., Bilby, R.E. & Bolton, S. (2000) Modeling Recovery Rates and Pathways for Woody Debris Recruitment in Northwestern Washington Streams. *North American Journal of Fisheries Management,* **20,** 436-452.

Benfield, E.F., Webster, J.R., Tank, J.L. & Hutchens, J.J. (2001) Long-Term Patterns in Leaf Breakdown in Streams in Response to Watershed Logging. *International Review of Hydrobiology,* **86,** 467-474.

Benstead, J.P., Rosemond, A.D., Cross, W.F., Wallace, J.B., Eggert, S.L., Suberkropp, K., Gulis, V., Greenwood, J.L. & Tant, C.J. (2009) Nutrient enrichment alters storage and fluxes of detritus in a headwater stream ecosystem. *Ecology,* **90,** 2556-2566.

Bernal, S., Butturini, A. & Sabater, F. (2005) Seasonal Variations of Dissolved Nitrogen and DOC:DON Ratios in an Intermittent Mediterranean Stream. *Biogeochemistry,* **75,** 351-372.

Bernhardt, E.S., Likens, G.E., Buso, D.C. & Driscoll, C.T. (2003) In-stream uptake dampens effects of major forest disturbance on watershed nitrogen export. *Proceedings of the National Academy of Sciences,* **100,** 10304-10308.

Bernhardt, E.S. & Palmer, M.A. (2011) River restoration: the fuzzy logic of repairing reaches to reverse catchment scale degradation. *Ecological Applications,* **21,** 1926-1931.

Bernhardt, E.S., Palmer, M.A., Allan, J.D., Alexander, G., Barnas, K., Brooks, S., Carr, J., Clayton, S., Dahm, C., Follstad-Shah, J., Galat, D., Gloss, S., Goodwin, P., Hart, D., Hassett, B., Jenkinson, R., Katz, S., Kondolf, G.M., Lake, P.S., Lave, R., Meyer, J.L., O'donnell, T.K., Pagano, L., Powell, B. & Sudduth, E. (2005) Synthesizing U.S. River Restoration Efforts. *Science,* **308,** 636-637.

Bernot, M.J., Sobota, D.J., Hall, R.O., Mulholland, P.J., Dodds, W.K., Webster, J.R., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Gregory, S.V., Grimm, N.B., Hamilton, S.K., Johnson, S.L., Mcdowell, W.H., Meyer, J.L., Peterson, B., Poole, G.C., Valett, H.M., Arango, C., Beaulieu, J.J., Burgin, A.J., Crenshaw, C., Helton, A.M., Johnson, L., Merriam, J., Niederlehner, B.R., O'brien, J.M., Potter, J.D., Sheibley, R.W., Thomas, S.M. & Wilson, K. (2010) Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology,* **55,** 1874-1890.

Bernot, M.J., Tank, J.L., Royer, T.V. & David, M.B. (2006) Nutrient uptake in streams draining agricultural catchments of the midwestern United States. *Freshwater Biology,* **51,** 499-509.

Beschta, R.L. & Platts, W.S. (1986) Morphological features of small streams: significance and function. *JAWRA Journal of the American Water Resources Association,* **22,** 369-379.

Biggs, B.J.F. (1995) The contribution of flood disturbance, catchment geology and land use to the habitat template of periphyton in stream ecosystems. *Freshwater Biology,* **33,** 419-438.

Biggs, B.J.F. (2000) Eutrophication of Streams and Rivers: Dissolved Nutrient-Chlorophyll Relationships for Benthic Algae. *Journal of the North American Benthological Society,* **19,** 17-31.

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.

Bilby, R.E. & Ward, J.W. (1989) Changes in Characteristics and Function of Woody Debris with Increasing Size of Streams in Western Washington. *Transactions of the American Fisheries Society,* **118,** 368-378.

Blanco, A.C., Nadaoka, K., Yamamoto, T. & Kinjo, K. (2010) Dynamic evolution of nutrient discharge under stormflow and baseflow conditions in a coastal agricultural watershed in Ishigaki Island, Okinawa, Japan. *Hydrological Processes,* **24,** 2601-2616.

Boon, P.I. (1990) Organic matter degradation and nutrient regeneration in Australian freshwaters: II. Spatial and temporal variation, and relation with environmental conditions. *Arch. Hydrobiol.,* **117,** 405-436.

Boon, P.I. (1991) Bacterial Assemblages in Rivers and Billabongs of Southeastern Australia. *Microbial Ecology,* **22,** 27-52.

Boon, P.I. & Sorrell, B.K. (1991) Biogeochemistry of billabong sediments. I. The effect of macrophytes. *Freshwater Biology,* **26,** 209-226.

Bott, T., Newbold, J. & Arscott, D. (2006) Ecosystem Metabolism in Piedmont Streams: Reach Geomorphology Modulates the Influence of Riparian Vegetation. *Ecosystems,* **9,** 398-421.

Bott, T.L., Kaplan, L.A. & Kuserk, F.T. (1984) Benthic Bacterial Biomass Supported by Streamwater Dissolved Organic Matter. *Microbial Ecology,* **10,** 335-344.

Bouarab, L., Dauta, A. & Loudiki, M. (2004) Heterotrophic and mixotrophic growth of *Micractinium pusillum* Fresenius in the presence of acetate and glucose: effect of light and acetate gradient concentration. *Water Research,* **38,** 2706-2712.

Boulton, A.J. (1991) Eucalypt leaf decomposition in an intermittent stream in south-eastern Australia. *Hydrobiologia,* **211,** 123-136.

Boulton, A.J. (2007) Hyporheic rehabilitation in rivers: restoring vertical connectivity. *Freshwater Biology,* **52,** 632-650.

Boulton, A.J. & Boon, P.I. (1991) A review of methodology used to measure leaf litter decomposition in lotic environments: Time to turn over an old leaf? *Marine and Freshwater Research,* **42,** 1-43.

Boulton, A.J., Findlay, S., Marmonier, P., Stanley, E.H. & Valett, H.M. (1998) The Functional Significance of the Hyporheic Zone in Streams and Rivers. *Annual Review of Ecology and Systematics,* **29,** 59-81.

Boulton, A.J. & Lake, P.S. (1992) Benthic organic matter and detritivorous macroinvertebrates in two intermittent streams in south-eastern Australia. *Hydrobiologia,* **241,** 107-118.

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.

Brasington, J., Rumsby, B.T. & McVey, R.A. (2000) Monitoring and modelling morphological change in a braided gravel-bed river using high resolution GPS-based survey. *Earth Surface Processes and Landforms,* **25,** 973-990.

Brierley, G., Fryers, K., Boulton, A. & Cullum, C. (2008) *River futures: an integrative scientific approach to river repair,* Island Press, Washington, USA.

Brookes, J., Aldridge, K., Wallace, T., Linden, L. & Ganf, G. (2005) Multiple Interception Pathways for Resource Utilisation and Increased Ecosystem Resilience. *Hydrobiologia,* **552,** 135-146.

Brooks, A.P., Gehrke, P.C., Jansen, J.D. & Abbe, T.B. (2004) Experimental reintroduction of woody debris on the Williams River, NSW: geomorphic and ecological responses. *River Research and Applications,* **20,** 513-536.

Brookshire, E.N.J. & Dwire, K.A. (2003) Controls on Patterns of Coarse Organic Particle Retention in Headwater Streams. *Journal of the North American Benthological Society,* **22,** 17-34.

Bunn, S. (1988) Processing of leaf litter in two northern Jarrah forest streams, Western Australia: II. The role of macroinvertebrates and the influence of soluble polyphenols and inorganic sediment. *Hydrobiologia,* **162,** 211-223.

Bunn, S.E. & Boon, P.I. (1993) What Sources of Organic Carbon Drive Food Webs in Billabongs? A Study Based on Stable Isotope Analysis. *Oecologia,* **96,** 85-94.

Bunn, S.E., Davies, P.M., Kellaway, D.M. & Prosser, I.P. (1998) Influence of invasive macrophytes on channel morphology and hydrology in an open tropical lowland stream, and potential control by riparian shading. *Freshwater Biology,* **39,** 171-178.

Bunn, S.E., Davies, P.M. & Mosisch, T.D. (1999) Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology,* **41,** 333-345.

Burgin, A.J. & Hamilton, S.K. (2007) Have We Overemphasized the Role of Denitrification in Aquatic Ecosystems? A Review of Nitrate Removal Pathways. *Frontiers in Ecology and the Environment,* **5,** 89-96.

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

Butturini, A. & Sabater, F. (2000) Seasonal Variability of Dissolved Organic Carbon in a Mediterranean Stream. *Biogeochemistry,* **51,** 303-321.

Campbell, I.C., James, K.R., Hart, B.T. & Devereaux, A. (1992) Allochthonous coarse particulate organic material in forest and pasture reaches of two south-eastern Australian streams. *Freshwater Biology,* **27,** 341-352.

Canfield, D.E. & Hoyer, M.V. (1988) Influence of Nutrient Enrichment and Light Availability on the Abundance of Aquatic Macrophytes in Florida Streams. *Canadian Journal of Fisheries and Aquatic Sciences,* **45,** 1467-1472.

Cardinale, B.J., Palmer, M.A., Swan, C.M., Brooks, S. & Poff, N.L. (2002) The Influence of Substrate Heterogeneity on Biofilm Metabolism in a Stream Ecosystem. *Ecology,* **83,** 412-422.

Carr, G.V.M., Duthie, H.C. & Taylor, W.D. (1997) Models of aquatic plant productivity: a review of the factors that influence growth. *Aquatic Botany,* **59,** 195-215.

Carvalho, E.M. & Uieda, V.S. (2009) Seasonal leaf mass loss estimated by litter bag technique in two contrasting stretches of a tropical headstream. *Acta Limnol. Bras.,* **21,** 209-215.

Champion, P.D. & Tanner, C.C. (2000) Seasonality of macrophytes and interaction with flow in a New Zealand lowland stream. *Hydrobiologia,* **441,** 1-12.

Chappell, K.R. & Goulder, R. (1995) A Between-River Comparison of Extracellular-Enzyme Activity. *Microbial Ecology,* **29,** 1-17.

Chapra, S.C. & Di Toro, D.M. (1991) Delta method for estimating primary production, respiration, and reaeration in streams. *Journal of Environmental Engineering,* **117,** 640-655.

Chessman, B.C. (1985) Estimates of ecosystem metabolism in the La Trobe River, Victoria. *Marine and Freshwater Research,* **36,** 873-880.

Chessman, B.C., Hutton, P.E. & Burch, J.M. (1992) Limiting nutrients for periphyton growth in sub-alpine, forest, agricultural and urban streams. *Freshwater Biology,* **28,** 349-361.

Chrost, R.J. (1991) Environmental control of the synthesis and activity of aquatic microbial enzymes. In: *Microbial enzymes in aquatic environments.* (Ed. R.J. Chrost), pp. 29-59. Springer-Verlag, New York.

Claessens, L., Tague, C.L., Groffman, P.M. & Melack, J.M. (2010) Longitudinal and seasonal variation of stream N uptake in an urbanizing watershed: effect of organic matter, stream size, transient storage and debris dams. *Biogeochemistry: An International Journal,* **98,** 45-62.

Clapcott, J.E. & Barmuta, L.A. (2010) Forest clearance increases metabolism and organic matter processes in small headwater streams. *Journal of the North American Benthological Society,* **29,** 546-561.

Claret, C. & Boulton, A.J. (2003) Diel variation in surface and subsurface microbial activity along a gradient of drying in an Australian sand-bed stream. *Freshwater Biology,* **48,** 1739-1755.

Clarke, S.J. (2002) Vegetation growth in rivers: influences upon sediment and nutrient dynamics. *Progress in Physical Geography,* **26,** 159-172.

Clément, J. C., Holmes, R.M., Peterson, B.J. & Pinay, G. (2003) Isotopic investigation of denitrification in a riparian ecosystem in western France. *Journal of Applied Ecology,* **40,** 1035-1048.

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

Colón-Gaud, C., Peterson, S., Whiles, M., Kilham, S., Lips, K. & Pringle, C. (2008) Allochthonous litter inputs, organic matter standing stocks, and organic seston dynamics in upland Panamanian streams: potential effects of larval amphibians on organic matter dynamics. *Hydrobiologia,* **603,** 301-312.

Copeland, B.J. & Duffer, W.R. (1964) Use of a Clear Plastic Dome to Measure Gaseous Diffusion Rates in Natural Waters. *Limnology and Oceanography,* **9,** 494-499.

Cordova, J.M., Rosi-Marshall, E.J., Tank, J.L. & Lamberti, G.A. (2008) Coarse particulate organic matter transport in low-gradient streams of the Upper Peninsula of Michigan. *Journal of the North American Benthological Society,* **27,** 760-771.

Coser, P.R. (1989) Nutrient concentration-Flow relationships and loads in the South Pine River, South-eastern Queensland. I. Phosphorus loads. *Marine and Freshwater Research,* **40,** 613-630.

Cox, B.A. (2003) A review of dissolved oxygen modelling techniques for lowland rivers. *The Science of The Total Environment,* **314-316,** 303-334.

Cross, W.F., Benstead, J.P., Frost, P.C. & Thomas, S.A. (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshwater Biology,* **50,** 1895-1912.

Cummins, K.W. (1974) Structure and Function of Stream Ecosystems. *BioScience,* **24,** 631-641.

Cummins, K.W., Wilzbach, M.A., Gates, D.M., Perry, J.B. & Taliaferro, W.B. (1989) Shredders and riparian vegetation. *BioScience,* **39,** 24-30.

Danger, A.R. & Robson, B.J. (2004) The effects of land use on leaf-litter processing by macroinvertebrates in an Australian temperate coastal stream. *Aquatic Sciences - Research Across Boundaries,* **66,** 296-304.

Dawson, F.H. (1976) The annual production of the aquatic macrophyte *Ranunculus penicillatus* var. *calcareus* (R.W. Butcher) C.D.K. Cook. *Aquatic Botany,* **2,** 51-73.

Deegan, L., Neill, C., Haupert, C., Ballester, M., Krusche, A., Victoria, R., Thomas, S. & De Moor, E. (2011) Amazon deforestation alters small stream structure, nitrogen biogeochemistry and connectivity to larger rivers. *Biogeochemistry,* **105,** 53-74.

Demars, B.O.L., Russell Manson, J., Ólafsson, J.S., Gíslason, G.M., Gudmundsdóttir, R., Woodward, G., Reiss, J., Pichler, D.E., Rasmussen, J.J. & Friberg, N. (2011) Temperature and the metabolic balance of streams. *Freshwater Biology,* **56,** 1106-1121.

Dent, C.L. & Grimm, N.B. (1999) Spatial Heterogeneity of Stream Water Nutrient Concentrations over Successional Time. *Ecology,* **80,** 2283-2298.

Dewson, Z.S., James, A.B.W. & Death, R.G. (2007) A Review of the Consequences of Decreased Flow for Instream Habitat and Macroinvertebrates. *Journal of the North American Benthological Society,* **26,** 401-415.

Dieter, D., von Schiller, D., García-Roger, E., Sánchez-Montoya, M., Gómez, R., Mora-Gómez, J., Sangiorgio, F., Gelbrecht, J. & Tockner, K. (2011) Preconditioning effects of intermittent stream flow on leaf litter decomposition. *Aquatic Sciences - Research Across Boundaries,* **73,** 599-609.

Dodds, W.K. (2003) Misuse of Inorganic N and Soluble Reactive P Concentrations to Indicate Nutrient Status of Surface Waters. *Journal of the North American Benthological Society,* **22,** 171-181.

Dodds, W.K., Martí, E., Tank, J.L., Pontius, J., Hamilton, S.K., Grimm, N.B., Bowden, W.B., Mcdowell, W.H., Peterson, B.J., Valett, H.M., Webster, J.R. & Gregory, S. (2004) Carbon and nitrogen stoichiometry and nitrogen cycling rates in streams. *Oecologia,* **140,** 458-467.

Dodds, W.K. & Welch, E.B. (2000) Establishing Nutrient Criteria in Streams. *Journal of the North American Benthological Society,* **19,** 186-196.

Drewry, J.J., Newham, L.T.H., Greene, R.S.B., Jakeman, A.J. & Croke, B.F.W. (2006) A review of nitrogen and phosphorus export to waterways: context for catchment modelling. *Marine and Freshwater Research,* **57,** 757-774.

Duff, J.H., Tesoriero, A.J., Richardson, W.B., Strauss, E.A. & Munn, M.D. (2008) Whole-Stream Response to Nitrate Loading in Three Streams Draining Agricultural Landscapes. *Journal of Environmental Quality,* **37,** 1133-1133-1144.

Ehrman, T.P. & Lamberti, G.A. (1992) Hydraulic and Particulate Matter Retention in a 3rd-Order Indiana Stream. *Journal of the North American Benthological Society,* **11,** 341-349.

Ellis, B.K. & Stanford, J.A. (1982) Comparative Photoheterotrophy, Chemoheterotrophy, and Photolithotrophy in a Eutrophic Reservoir and an Oligotrophic Lake. *Limnology and Oceanography,* **27,** 440-454.

Elosegi, A., Díez, J. & Mutz, M. (2010) Effects of hydromorphological integrity on biodiversity and functioning of river ecosystems. *Hydrobiologia,* **657,** 199-215.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B. & Smith, J.E. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters,* **10,** 1135-1142.

Enríquez, S., Duarte, C.M. & Sand-Jensen, K. (1993) Patterns in Decomposition Rates among Photosynthetic Organisms: The Importance of Detritus C:N:P Content. *Oecologia,* **94,** 457-471.

Erskine, W.D. & Webb, A.A. (2003) Desnagging to resnagging: new directions in river rehabilitation in southeastern Australia. *River Research and Applications,* **19,** 233-249.

Esslemont, G., Maher, W., Ford, P. & Lawrence, I. (2007) Riparian Plant Material Inputs to the Murray River, Australia: Composition, Reactivity, and Role of Nutrients. *Journal of Environmental Quality,* **36,** 963-974.

Fairchild, G.W. & Everett, A.C. (1988) Effects of nutrient (N, P, C) enrichment upon periphyton standing crop, species composition and primary production in an oligotrophic softwater lake. *Freshwater Biology,* **19,** 57-70.

Fellows, C., Clapcott, J., Udy, J., Bunn, S., Harch, B., Smith, M. & Davies, P. (2006) Benthic Metabolism as an Indicator of Stream Ecosystem Health. *Hydrobiologia,* **572,** 71-87.

Fellows, C., Wos, M., Pollard, P. & Bunn, S. (2007) Ecosystem metabolism in a dryland river waterhole. *Marine and Freshwater Research,* **58,** 250-262.

Fellows, C.S., Valett, H.M., Dahm, C.N., Mulholland, P.J., Thomas, S.A. (2006) Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems,* **9,** 788-804.

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.

Ferreira, V. & Chauvet, E. (2011) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. *Global Change Biology,* **17,** 551-564.

Figueroa-Nieves, D.B., Royer, T. & David, M. (2006) Controls on chlorophyll-*a* in nutrient-rich agricultural streams in Illinois, USA. *Hydrobiologia,* **568,** 287-298.

Filoso, S. & Palmer, M.A. (2011) Assessing stream restoration effectiveness at reducing nitrogen export to downstream waters. *Ecological Applications,* **21,** 1989-2006.

Findlay, S., Hickey, C.W. & Quinn, J.M. (1997) Microbial enzymatic response to catchmentâ€• scale variations in supply of dissolved organic carbon. *New Zealand Journal of Marine and Freshwater Research,* **31,** 701-706.

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,** 345-355.

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.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. (2003) The role of biofilms in the uptake and transformation of dissolved organic matter, In: *Aquatic ecosystems: interactivity of dissolved organic matter.* (Eds. Findlay, S.E.G., Sinsabaugh, R.L.). pp 285-315, Academic Press, Amsterdam.

Fisher, S.G., Gray, L.J., Grimm, N.B. & Busch, D.E. (1982) Temporal Succession in a Desert Stream Ecosystem Following Flash Flooding. *Ecological Monographs,* **52,** 93-110.

Fisher, S.G., Grimm, N.B., Marti, E., Holmes, R.M. & Jones, J.B., Jr. (1998) Material Spiraling in Stream Corridors: A Telescoping Ecosystem Model. *Ecosystems,* **1,** 19-34.

Fisher, S.G. & Likens, G.E. (1973) Energy Flow in Bear Brook, New Hampshire: An Integrative Approach to Stream Ecosystem Metabolism. *Ecological Monographs,* **43,** 421-439.

Fletcher, D.E., Wilkins, S.D., Mcarthur, J.V. & Meffe, G.K. (2000) Influence of riparian alteration on canopy coverage and macrophyte abundance in Southeastern USA blackwater streams. *Ecological Engineering,* **15,** S67-S78.

Flores, L., Larrañaga, A., Díez, J. & Elosegi, A. (2011) Experimental wood addition in streams: effects on organic matter storage and breakdown. *Freshwater Biology,* **56,** 2156-2167.

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.

França, J.S., Gregorio, R.S., De Paula, J.D., Goncalves, J.F., Ferreira, F.A. & Callisto, M. (2009) Composition and dynamics of allochthonous organic matter inputs and benthic stock in a Brazilian stream. *Marine and Freshwater Research,* **60,** 990-998.

Francoeur, S.N., Biggs, B.J.F., Smith, R.A. & Lowe, R.L. (1999) Nutrient Limitation of Algal Biomass Accrual in Streams: Seasonal Patterns and a Comparison of Methods. *Journal of the North American Benthological Society,* **18,** 242-260.

Frankforter, J., Weyers, H., Bales, J., Moran, P. & Calhoun, D. (2009) The relative influence of nutrients and habitat on stream metabolism in agricultural streams. *Environmental Monitoring and Assessment,* **168,** 461-479.

Frazier, P., Ryder, D., Mcintyre, E. & Stewart, M. (2011) Understanding Riverine Habitat Inundation Patterns: Remote Sensing Tools and Techniques. *Wetlands*, **32,** 225-237.

Freeman, C., Liska, G., Ostle, N., Jones, S. & Lock, M. (1995) The use of fluorogenic substrates for measuring enzyme activity in peatlands. *Plant and Soil,* **175,** 147-152.

Gasith, A. & Resh, V.H. (1999) Streams in Mediterranean Climate Regions: Abiotic Influences and Biotic Responses to Predictable Seasonal Events. *Annual Review of Ecology and Systematics,* **30,** 51-81.

Gawne, B., Merrick, C., Williams, D.G., Rees, G., Oliver, R., Bowen, P.M., Treadwell, S., Beattie, G., Ellis, I., Frankenberg, J. & Lorenz, Z. (2007) Patterns of primary and heterotrophic productivity in an arid lowland river. *River Research and Applications,* **23,** 1070-1087.

Gessner, M.O. & Chauvet, E. (1994) Importance of Stream Microfungi in Controlling Breakdown Rates of Leaf Litter. *Ecology,* **75,** 1807-1817.

Gessner, M.O. & Chauvet, E. (2002) A Case for Using Litter Breakdown to Assess Functional Stream Integrity. *Ecological Applications,* **12,** 498-510.

Gessner, M.O., Chauvet, E. & Dobson, M. (1999) A Perspective on Leaf Litter Breakdown in Streams. *Oikos,* **85,** 377-384.

Gibson, C.A. & O'Reilly, C.M. (2012) Organic matter stoichiometry influences nitrogen and phosphorus uptake in a headwater stream. *Freshwater Science,* **35,** 395-407.

Giling, D., Reich, P. & Thompson, R.M. (2009) Loss of riparian vegetation alters the ecosystem role of a freshwater crayfish (*Cherax destructor*) in an Australian intermittent lowland stream. *Journal of the North American Benthological Society,* **28,** 626-637.

Gómez, R., García, V., Vidal-Abarca, R. & Suárez, L. (2009) Effect of intermittency on N spatial variability in an arid Mediterranean stream. *Journal of the North American Benthological Society,* **28,** 572-583.

González, E. & Pozo, J. (1996) Longitudinal and temporal patterns of benthic coarse particulate organic matter in the Agüera stream (northern Spain). *Aquatic Sciences - Research Across Boundaries,* **58,** 355-366.

Gooseff, M.N., Hall, R.O., Jr. & Tank, J.L. (2007) Relating transient storage to channel complexity in streams of varying land use in Jackson Hole, Wyoming. *Water Resour. Res.,* **43,** W01417.

Gordon, N.D., Mcmahon, T. A., Finlayson, B.L., Gippel, C.J. & Nathan, R. J. (2004) *Stream Hydrology: An introduction for Ecologists,* John Wiley & Sons Ltd, Chichester, England.

Grace, M.R. & Imberger, S.J. (2006) Stream metabolism: performing and interpreting measurements. p. 204. Water Studies Centre Monash University, Murray Darling Basin Commission and New South Wales Department of Environment and Climate Change.

Graham, J.M., Lembi, C.A., Adrian, H.L. & Spencer, D.F. (1995) Physiological responses to temperature and irradiance in *Spirogyra* (Zygnematales, Charophyceae). *Journal of Phycology,* **31,** 531-540.

Gregory, S.V., Swanson, F.J., Mckee, W.A. & Cummins, K.W. (1991) An Ecosystem Perspective of Riparian Zones. *BioScience,* **41,** 540-551.

Guasch, H., Martí, E. & Sabater, S. (1995) Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology,* **33,** 373-383.

Gulis, V. & Suberkropp, K. (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater Biology,* **48,** 123-134.

Gurnell, A.M. & Midgley, P. (1994) Aquatic weed growth and flow resistance: Influence on the relationship between discharge and stage over a 25 year river gauging station record. *Hydrological Processes,* **8,** 63-73.

Gurtz, M.E., Marzolf, G.R., Killingbeck, K.T., Smith, D.L. & Mcarthur, J.V. (1988) Hydrologic and Riparian Influences on the Import and Storage of Coarse Particulate Organic Matter in a Prairie Stream. *Canadian Journal of Fisheries and Aquatic Sciences,* **45,** 655-665.

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.

Hagen, E., McTammany, M., Webster, J. & Benfield, E. (2010) Shifts in allochthonous input and autochthonous production in streams along an agricultural land-use gradient. *Hydrobiologia,* **655,** 61-77.

Hagen, E.M., Webster, J.R. & Benfield, E.F. (2006) Are Leaf Breakdown Rates a Useful Measure of Stream Integrity along an Agricultural Landuse Gradient? *Journal of the North American Benthological Society,* **25,** 330-343.

Hall, R.O., Jr. & Tank, J.L. (2003) Ecosystem Metabolism Controls Nitrogen Uptake in Streams in Grand Teton National Park, Wyoming. *Limnology and Oceanography,* **48,** 1120-1128.

Hanson, B., Cummins, K., Barnes, J. & Carter, M. (1984) Leaf litter processing in aquatic systems: A two variable model. *Hydrobiologia,* **111,** 21-29.

Harbott, E.L. & Grace, M.R. (2005) Extracellular enzyme response to bioavailability of dissolved organic C in streams of varying catchment urbanization. *Journal of the North American Benthological Society,* **14,** 588-601.

Harms, T.K. & Grimm, N.B. (2010) Influence of the hydrologic regime on resource availability in a semi-arid stream-riparian corridor. *Ecohydrology,* **3,** 349-359.

Harper, D. & Everard, M. (1998) Why should the habitat-level approach underpin holistic river survey and management? *Aquatic Conservation: Marine and Freshwater Ecosystems,* **8,** 395-413.

Harris, G.P. (2001) Biogeochemistry of nitrogen and phosphorus in Australian catchments, rivers and estuaries: effects of land use and flow regulation and comparisons with global patterns. *Marine and Freshwater Research,* **52,** 139-149.

Heard, S.B., Schultz, G.A., Ogden, C.B. & Griesel, T.C. (1999) Mechanical abrasion and organic matter processing in an Iowa stream. *Hydrobiologia,* **400,** 179-186.

Hendry, K., Cragg-Hine, D., O'Grady, M., Sambrook, H. & Stephen, A. (2003) Management of habitat for rehabilitation and enhancement of salmonid stocks. *Fisheries Research,* **62,** 171-192.

Hester, E.T. & Doyle, M.W. (2008) In-stream geomorphic structures as drivers of hyporheic exchange. *Water Resources Research,* **44,** W03417.

Hill, B., Elonen, C., Jicha, T., Bolgrien, D. & Moffett, M. (2010) Sediment microbial enzyme activity as an indicator of nutrient limitation in the great rivers of the Upper Mississippi River basin. *Biogeochemistry,* **97,** 195-209.

Hill, B.H., Elonen, C.M., Jicha, T.M., Cotter, A.M., Trebitz, A.S. & Danz, N.P. (2006) Sediment microbial enzyme activity as an indicator of nutrient limitation in Great Lakes coastal wetlands. *Freshwater Biology,* **51,** 1670-1683.

Hill, B.H., Mccormick, F.H., Harvey, B.C., Johnson, S.L., Warren, M.L. & Elonen, C.M. (2010) Microbial enzyme activity, nutrient uptake and nutrient limitation in forested streams. *Freshwater Biology,* **55,** 1005-1019.

Hinton, M.J., Schiff, S.L. & English, M.C. (1998) Sources and flowpaths of dissolved organic carbon during storms in two forested watersheds of the Precambrian Shield. *Biogeochemistry,* **41,** 175-197.

Hladyz, S., Gessner, M.O., Giller, P.S., Pozo, J. & Woodward, G. (2009) Resource quality and stoichiometric constraints on stream ecosystem functioning. *Freshwater Biology,* **54,** 957-970.

Hladyz, S., Tiegs, S.D., Gessner, M.O., Giller, P.S., Rîşnoveanu, G., Preda, E., Nistorescu, M., Schindler, M. & Woodward, G. (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. *Freshwater Biology,* **55,** 1916-1929.

Hoellein, T.J., Tank, J.L., Rosi-Marshall, E.J. & Entrekin, S.A. (2009) Temporal variation in substratum-specific rates of N uptake and metabolism and their contribution at the stream-reach scale. *Journal of the North American Benthological Society,* **28,** 305-318.

Hoffman, A., Armstrong, D., Lathrop, R. & Penn, M. (2009) Characteristics and Influence of Phosphorus Accumulated in the Bed Sediments of a Stream Located in an Agricultural Watershed. *Aquatic Geochemistry,* **15,** 371-389.

Hoffmann, C.C., Kjaergaard, C., Uusi-Kämppä, J., Hansen, H.C.B. & Kronvang, B. (2009) Phosphorus Retention in Riparian Buffers: Review of Their Efficiency. *Journal Environmental Quality,* **38,** 1942-1955.

Hoover, T.M., Marczak, L.B., Richardson, J.S. & Yonemitsu, N. (2010) Transport and settlement of organic matter in small streams. *Freshwater Biology,* **55,** 436-449.

Hoover, T.M., Richardson, J.S. & Yonemitsu, N. (2006) Flow-substrate interactions create and mediate leaf litter resource patches in streams. *Freshwater Biology,* **51,** 435-447.

Hoppe, H.G. (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl substrates. *Marine Ecology Progress Series,* **11,** 299-308.

Hoppe, H.G. (1993) Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. In: *Handbook of methods in aquatic microbial ecology.* (Eds P.F. Kemp, Sherr, B.F., Sherr, E.B., Cole, J.J.), pp. 423-431. Lewis Publishers, Boca Raton, Florida.

Horvath, T.G. (2004) Retention of particulate matter by macrophytes in a first-order stream. *Aquatic Botany,* **78,** 27-36.

Hosomi, M. & Sudo, R. (1986) Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion. *International Journal of Environmental Studies,* **27,** 267-275.

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.

Hoyer, M., Frazer, T. & Notestein, S. (2004) Vegetative characteristics of three low-lying Florida coastal rivers in relation to flow, light, salinity and nutrients. *Hydrobiologia,* **528,** 31-43.

Hutchinson, M.F. (1989) A new procedure for gridding elevation and stream line data with automatic removal of spurious pits. *Journal of Hydrology,* **106,** 211-232.

Imberger, S.J., Thompson, R.M. & Grace, M.R. (2011) Urban catchment hydrology overwhelms reach scale effects of riparian vegetation on organic matter dynamics. *Freshwater Biology*, **56,** 1370-1389.

Inwood, S.E., Tank, J.L. & Bernot, M.J. (2005) Patterns of Denitrification Associated with Land Use in 9 Midwestern Headwater Streams. *Journal of the North American Benthological Society,* **24,** 227-245.

Iwata, T., Takahashi, T., Kazama, F., Hiraga, Y., Fukuda, N., Honda, M., Kimura, Y., Kota, K., Kubota, D., Nakagawa, S., Nakamura, T., Shimura, M., Yanagida, S. & Xeu, L. (2007) Metabolic balance of streams draining urban and agricultural watersheds in central Japan. *Limnology,* **8,** 243-250.

James, A.B.W. & Henderson, I.M. (2005) Comparison of coarse particulate organic matter retention in meandering and straightened sections of a third-order New Zealand stream. *River Research and Applications,* **21,** 641-650.

Johnson, L.T., Tank, J.L. & Dodds, W.K. (2009) The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. *Canadian Journal of Fisheries and Aquatic Sciences,* **66,** 1081-1094.

Johnson, S. & Covich, A. (1997) Scales of observation of riparian forests and distributions of suspended detritus in a prairie river. *Freshwater Biology,* **37,** 163-175.

Jones, A.F., Brewer, P.A., Johnstone, E. & Macklin M.G. (2007) High-resolution interpretative geomorphological mapping of river valley environments using airborne LIDAR data. *Earth Surface Processes and Landforms,* **32,** 1574-1592.

Jones, J.B. (1997) Benthic Organic Matter Storage in Streams: Influence of Detrital Import and Export, Retention Mechanisms, and Climate. *Journal of the North American Benthological Society,* **16,** 109-119.

Jones, J.B., Fisher, S.G. & Grimm, N.B. (1995) Vertical Hydrologic Exchange and Ecosystem Metabolism in a Sonoran Desert Stream. *Ecology,* **76,** 942-952.

Jones, J.B., Jr. & Smock, L.A. (1991) Transport and Retention of Particulate Organic Matter in Two Low-Gradient Headwater Streams. *Journal of the North American Benthological Society,* **10,** 115-126.

Jones Jr, J.B. & Holmes, R.M. (1996) Surface-subsurface interactions in stream ecosystems. *Trends in Ecology & Evolution,* **11,** 239-242.

Jones, R.I., Shaw, P.J. & De Haan, H. (1993) Effects of dissolved humic substances on the speciation of iron and phosphate at different pH and ionic strength. *Environmental Science & Technology,* **27,** 1052-1059.

Jones, S.E. & Lock, M.A. (1989) Hydrolytic extracellular enzyme activity in heterotrophic biofilms from two contrasting streams. *Freshwater Biology,* **22,** 289-296.

Julian, J., Stanley, E. & Doyle, M. (2008) Basin-Scale Consequences of Agricultural Land Use on Benthic Light Availability and Primary Production Along a Sixth-Order Temperate River. *Ecosystems,* **11,** 1091-1105.

Julian, J.P., Seegert, S.Z., Powers, S.M., Stanley, E.H. & Doyle, M.W. (2010) Light as a first-order control on ecosystem structure in a temperate stream. *Ecohydrology,* **4,** 422-432.

Kaplan, L.A., Larson, R.A. & Bott, T.L. (1980) Patterns of Dissolved Organic Carbon in Transport. *Limnology and Oceanography,* **25,** 1034-1043.

Kleeberg, A., Köhler, J.A.N., Sukhodolova, T. & Sukhodolov, A. (2010) Effects of aquatic macrophytes on organic matter deposition, resuspension and phosphorus entrainment in a lowland river. *Freshwater Biology,* **55,** 326-345.

Koetsier, P. & McArthur, J.V. (2000) Organic Matter Retention by Macrophyte Beds in 2 Southeastern USA, Low-Gradient, Headwater Streams. *Journal of the North American Benthological Society,* **19,** 633-647.

Köhler, S., Buffam, I., Jonsson, A. & Bishop, K. (2002) Photochemical and microbial processing of stream and soil water dissolved organic matter in a boreal forested catchment in northern Sweden. *Aquatic Sciences - Research Across Boundaries,* **64,** 269-281.

Kosinski, R.J. (1984) A comparison of the accuracy and precision of several open-water oxygen productivity techniques. *Hydrobiologia,* **119,** 139-148.

Lake, P.S. (2000) Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society,* **19,** 573-592.

Lake, P.S. (2003) Ecological effects of perturbation by drought in flowing waters. *Freshwater Biology,* **48,** 1161-1172.

Lake, P.S., Bond, N. & Reich, P. (2007) Linking ecological theory with stream restoration. *Freshwater Biology,* **52,** 597-615.

Langhans, S.D. & Tockner, K. (2006) The Role of Timing, Duration, and Frequency of Inundation in Controlling Leaf Litter Decomposition in a River-Floodplain Ecosystem (Tagliamento, Northeastern Italy). *Oecologia,* **147,** 501-509.

Larned, S., Datry, T. & Robinson, C. (2007) Invertebrate and microbial responses to inundation in an ephemeral river reach in New Zealand: effects of preceding dry periods. *Aquatic Sciences - Research Across Boundaries,* **69,** 554-567.

Larned, S.T., Datry, T., Arscott, D.B. & Tockner, K. (2010) Emerging concepts in temporary-river ecology. *Freshwater Biology,* **55,** 717-738.

Laubel, A., Kronvang, B., Hald, A.B. & Jensen, C. (2003) Hydromorphological and biological factors influencing sediment and phosphorus loss via bank erosion in small lowland rural streams in Denmark. *Hydrological Processes,* **17,** 3443-3463.

Leckie, D.G., Cloney, E., Jay, C. & Paradine, D. (2005) Automated mapping of stream features with high-resolution multispectral imagery: an example of the capabilities. *Photogrammetric Engineering & Remote Sensing,* **71,** 145-155.

Lefebvre, S., Clement, J.C., Pinay, G., Thenail, C., Durand, P. & Marmonier, P. (2007) ^{15}N-Nitrate signature in low-order streams: effects of land cover and agricultural practices. *Ecological Applications,* **17,** 2333-2346.

Legendre, P. & Anderson, M.J. (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs,* **69,** 1-24.

Lepori, F., Palm, D. & Malmqvist, B. (2005) Effects of stream restoration on ecosystem functioning: detritus retentiveness and decomposition. *Journal of Applied Ecology,* **42,** 228-238.

Lester, R. & Boulton, A. (2008) Rehabilitating Agricultural Streams in Australia with Wood: A Review. *Environmental Management,* **42,** 310-326.

Lundström, T., Stoffel, M. & Stöckli, V. (2008) Fresh-stem bending of silver fir and Norway spruce. *Tree Physiology,* **28,** 355-366.

Lunt, I.D., Eldridge, D.J., Morgan, J.W. & Witt, G.B. (2007) Turner Review No. 13 A framework to predict the effects of livestock grazing and grazing exclusion on conservation values in natural ecosystems in Australia. *Australian Journal of Botany,* **55,** 401-415.

Maloney, K., Mulholland, P. & Feminella, J. (2005) Influence of Catchment-Scale Military Land Use on Stream Physical and Organic Matter Variables in Small Southeastern Plains Catchments (USA). *Environmental Management,* **35,** 677-691.

Mansfield, S.D., Mooney, C. & Saddler, J.N. (1999) Substrate and Enzyme Characteristics that Limit Cellulose Hydrolysis. *Biotechnology Progress,* **15,** 804-816.

Marcus, W.A. & Fonstad, M.A. (2007) Remote sensing of rivers: the emergence of a subdiscipline in the river sciences. *Earth Surface Processes and Landforms,* **35,** 1867-1872.

Marcus, W.A. & Fonstad, M.A. (2008) Optical remote mapping of rivers at sub-meter resolutions and watershed extents. *Earth Surface Processes and Landforms,* **33,** 4-24.

Marcus, W.A., Legleiter, C.J., Aspinall, R.J., Boardman, J.W. & Crabtree, R.L. (2003) High spatial resolution hyperspectral mapping of in-stream habitats, depths, and woody debris in mountain streams. *Geomorphology,* **55,** 363-380.

Marzolf, E.R., Mulholland, P.J. & Steinman, A.D. (1994) Improvements to the diurnal upstream-downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. *Canadian Journal of Fisheries and Aquatic Sciences,* **51,** 1591-1599.

Mayo, A.W. & Noike, T. (1994) Effect of glucose loading on the growth behavior of Chlorella vulgaris and heterotrophic bacteria in mixed culture. *Water Research,* **28,** 1001-1008.

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, S., Bishop, A.G., Prenzler, P.D. & Robards, K. (2004) Analytical chemistry of freshwater humic substances. *Analytica Chimica Acta,* **527,** 105-124.

McIntyre, R.E.S., Adams, M.A., Ford, D.J. & Grierson, P.F. (2009) Rewetting and litter addition influence mineralisation and microbial communities in soils from a semi-arid intermittent stream. *Soil Biology and Biochemistry,* **41,** 92-101.

McKee, L.J., Eyre, B.D. & Hossain, S. (2000) Transport and Retention of Nitrogen and Phosphorus in the Sub-Tropical Richmond River Estuary, Australia: A Budget Approach. *Biogeochemistry,* **50,** 241-278.

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.

McTammany, M.E., Benfield, E.F. & Webster, J.R. (2007) Recovery of Stream Ecosystem Metabolism from Historical Agriculture. *Journal of the North American Benthological Society,* **26,** 532-545.

McTammany, M.E., Webster, J.R., Benfield, E.F. & Neatrour, M.A. (2003) Longitudinal Patterns of Metabolism in a Southern Appalachian River. *Journal of the North American Benthological Society,* **22,** 359-370.

Melillo, J.M., Aber, J.D. & Muratore, J.F. (1982) Nitrogen and Lignin Control of Hardwood Leaf Litter Decomposition Dynamics. *Ecology,* **63,** 621-626.

Menéndez, M., Hernández, O. & Comín, F.A. (2003) Seasonal comparisons of leaf processing rates in two Mediterranean rivers with different nutrient availability. *Hydrobiologia,* **495,** 159-169.

Meyer, J.L. (1989) Can P/R Ratio Be Used to Assess the Food Base of Stream Ecosystems? A Comment on Rosenfeld and Mackay (1987). *Oikos,* **54,** 119-121.

Meyer, J.L. & Tate, C.M. (1983) The Effects of Watershed Disturbance on Dissolved Organic Carbon Dynamics of a Stream. *Ecology,* **64,** 33-44.

Mika, S., Hoyle, J., Kyle, G., Howell, T., Wolfenden, B., Ryder, D., Keating, D., Boulton, A., Brierley, G., Brooks, A. P., Fryirs, K., Leishman, M., Sanders, M., Arthington, A., Creese, R., Dahm, M., Miller, C., Pusey, B., & Spink, A. (2010) Inside the "black box" of river restoration: using catchment history to identify disturbance and response mechanisms to set targets for process-based restoration. *Ecology and Society,* **15**.

Miller, W. & Boulton, A. (2005) Managing and Rehabilitating Ecosystem Processes in Regional Urban Streams in Australia. *Hydrobiologia,* **552,** 121-133.

Minshall, G.W., Thomas, S.A., Newbold, J.D., Monaghan, M.T. & Cushing, C.E. (2000) Physical factors influencing fine organic particle transport and deposition in streams. *Journal of the North American Benthological Society,* **19,** 1-16.

Mitrovic, S.M., Hardwick, L. & Dorani, F. (2011) Use of flow management to mitigate cyanobacterial blooms in the Lower Darling River, Australia. *Journal of Plankton Research,* **33,** 229-241.

Mitrovic, S.M., Oliver, R.L., Rees, C., Bowling, L.C. & Buckney, R.T. (2003) Critical flow velocities for the growth and dominance of Anabaena circinalis in some turbid freshwater rivers. *Freshwater Biology,* **48,** 164-174.

Mollá, S., Robles, S. & Casado, C. (2006) Seasonal Variability of Particulate Organic Matter in a Mountain Stream in Central Spain. *International Review of Hydrobiology,* **91,** 406-422.

Monaghan, M.T., Thomas, S.A., Minshall, G.W., Newbold, J.D. & Cushing, C.E. (2001) The Influence of Filter-Feeding Benthic Macroinvertebrates on the Transport and Deposition of Particulate Organic Matter and Diatoms in Two Streams. *Limnology and Oceanography,* **46,** 1091-1099.

Mosisch, T.D., Bunn, S.E. & Davies, P.M. (2001) The relative importance of shading and nutrients on algal production in subtropical streams. *Freshwater Biology,* **46,** 1269-1278.

Moulin, B., Schenk, E.R. & Hupp, C.R. (2011) Distribution and characterization of in-channel large wood in relation to geomorphic patterns on a low-gradient river. *Earth Surface Processes and Landforms,* **36,** 1137-1151.

Mulholland, P.J., Fellows, C.S., Tank, J.L., Grimm, N.B., Webster, J.R., Hamilton, S.K., Martí, E., Ashkenas, L., Bowden, W.B., Dodds, W.K., Mcdowell, W.H., Paul, M.J. & Peterson, B.J. (2001) Inter-biome comparison of factors controlling stream metabolism. *Freshwater Biology,* **46,** 1503-1517.

Mulholland, P.J., Tank, J.L., Webster, J.R., Bowden, W.B., Dodds, W.K., Gregory, S.V., Grimm, N.B., Hamilton, S.K., Johnson, S.L., Martí, E., Mcdowell, W.H., Merriam, J.L., Meyer, J.L., Peterson, B.J., Valett, H.M. & Wollheim, W.M. (2002) Can uptake length in streams be determined by nutrient addition experiments? Results from an interbiome comparison study. *Journal of the North American Benthological Society,* **21,** 544-560.

Mulholland, P.J. & Webster, J.R. (2010) Nutrient dynamics in streams and the role of J-NABS. *Journal of the North American Benthological Society,* **29,** 100-117.

Munn, M., Osborne, L. & Wiley, M. (1989) Factors influencing periphyton growth in agricultural streams of central Illinois. *Hydrobiologia,* **174,** 89-97.

Muotka, T. & Laasonen, P. (2002) Ecosystem recovery in restored headwater streams: the role of enhanced leaf retention. *Journal of Applied Ecology,* **39,** 145-156.

Murphy, J. & Riley, J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytical Chimica Acta,* **27,** 31-36.

Myers, T.J. & Swanson, S. (1991) Aquatic habitat condition index, stream type, and livestock bank damage in northern Nevada. *JAWRA Journal of the American Water Resources Association,* **27,** 667-677.

Naegeli, M.W. & Uehlinger, U. (1997) Contribution of the Hyporheic Zone to Ecosystem Metabolism in a Prealpine Gravel-Bed-River. *Journal of the North American Benthological Society,* **16,** 794-804.

Neilson, A.H. & Lewin, R.A. (1974) The uptake and utilization of organic carbon by algae: an essay in comparative biochemistry. *Phycologia,* **13,** 227-264.

Newbold, J.D., Elwood, J. W., O'Neill, R. V. & Van Winkle, W. (1981) Measuring nutrient spiralling in streams. *Canadian Journal of Fisheries and Aquatic Sciences,* **38,** 860-863.

Newbold, J.D., Herbert, S., Sweeney, B.W., Kiry, P. & Alberts, S.J. (2010) Water Quality Functions of a 15-Year-Old Riparian Forest Buffer System1. *JAWRA Journal of the American Water Resources Association,* **46,** 299-310.

Newham, M., Fellows, C. & Sheldon, F. (2011) Functions of riparian forest in urban catchments: a case study from sub-tropical Brisbane, Australia. *Urban Ecosystems,* **14,** 165-180.

O'Brien, J., Dodds, W., Wilson, K., Murdock, J. & Eichmiller, J. (2007) The saturation of N cycling in Central Plains streams: ^{13}N experiments across a broad gradient of nitrate concentrations. *Biogeochemistry,* **84,** 31-49.

Olander, L.P. & Vitousek, P.M. (2000) Regulation of soil phosphatase and chitinase activityby N and P availability. *Biogeochemistry,* **49,** 175-191.

Oliver, R.L. & Merrick, C.J. (2006) Partitioning of river metabolism identifies phytoplankton as a major contributor in the regulated Murray River (Australia). *Freshwater Biology,* **51,** 1131-1148.

Ortiz-Zayas, J.R., Lewis, W.M., Jr., Saunders, J.F., iii, McCutchan, J.H., Jr. & Scatena, F.N. (2005) Metabolism of a Tropical Rainforest Stream. *Journal of the North American Benthological Society,* **24,** 769-783.

Osborne, L.L., Kovacic, D.A. (1993) Riparian vegetated buffer strips in water quality restoration and stream management. *Freshwater Biology,* **29,** 243-258.

Palmer, M.A., Ambrose, R.F. & Poff, N.L. (1997) Ecological Theory and Community Restoration Ecology. *Restoration Ecology,* **5,** 291-300.

Palmer, M.A., Bernhardt, E.S., Allan, J.D., Lake, P.S., Alexander, G., Brooks, S., Carr, J., Clayton, S., Dahm, C.N., Follstad Shah, J., Galat, D.L., Loss, S.G., Goodwin, P., Hart, D.D., Hassett, B., Jenkinson, R., Kondolf, G.M., Lave, R., Meyer, J.L., O'donnell, T.K., Pagano, L. & Sudduth, E. (2005) Standards for ecologically successful river restoration. *Journal of Applied Ecology,* **42,** 208-217.

Parsons, T.R., Maita, Y. & Lalli, C.M. (1984) Plant pigments. In: *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, Oxford, UK.

Petersen, R.C. & Cummins, K.W. (1974) Leaf processing in a woodland stream. *Freshwater Biology,* **4,** 343-368.

Peterson, B.J., Wollheim, W.M., Mulholland, P.J., Jackson, R.W., Meyer, J.L., Tank, J.L., Marti, E.N., Bowden, W.B., Valett, H.M., Hershey, A.E., Mcdowell, W.H., Dodds, W.K., Hamilton, S.K., Gregory, S. & Morrall, D.D. (2001) Control of Nitrogen Export from Watersheds by Headwater Streams. *Science,* **292,** 86-90.

Petrone, K., Richards, J. & Grierson, P. (2009) Bioavailability and composition of dissolved organic carbon and nitrogen in a near coastal catchment of south-western Australia. *Biogeochemistry,* **92,** 27-40.

Pettit, N.E. & Naiman, R.J. (2005) Flood-Deposited Wood Debris and Its Contribution to Heterogeneity and Regeneration in a Semi-Arid Riparian Landscape. *Oecologia,* **145,** 434-444.

Pettit, N.E., Naiman, R.J., Rogers, K.H. & Little, J.E. (2005) Post-flooding distribution and characteristics of large woody debris piles along the semi-arid Sabie River, South Africa. *River Research and Applications,* **21,** 27-38.

Phlips, E.J., Cichra, M., Aldridge, F.J., Jembeck, J., Hendrickson, J. & Brody, R. (2000) Light Availability and Variations in Phytoplankton Standing Crops in a Nutrient-Rich Blackwater River. *Limnology and Oceanography,* **45,** 916-929.

Pidgeon, R. & Cairns, S. (1981) Decomposition and colonisation by invertebrates of native and exotic leaf material in a small stream in New England (Australia). *Hydrobiologia,* **77,** 113-127.

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.

Poole, G.C., Stanford, J.A., Running, S.W. & Frissell, C.A. (2006) Multiscale geomorphic drivers of groundwater flow paths: subsurface hydrologic dynamics and hyporheic habitat diversity. *Journal of the North American Benthological Society,* **25,** 288-303.

Prosser, I.P., Rutherfurd, I.D., Olley, J.M., Young, W.J., Wallbrink, P.J. & Moran, C.J. (2001) Large-scale patterns of erosion and sediment transport in river networks, with examples from Australia. *Marine and Freshwater Research,* **52,** 817-817.

Pusey, B.J. & Arthington, A.H. (2003) Importance of the riparian zone to the conservation and management of freshwater fish: a review. *Marine and Freshwater Research,* **54,** 1-16.

Quinn, G.P., Keough, M.J. (2002) *Experimental design and data analysis for biologists,* Cambridge University Press, Cambridge, United Kingdom.

Quinn, J.M., Cooper, A.B., Davies-Colley, R.J., Rutherford, J.C. & Williamson, R.B. (1997) Land use effects on habitat, water quality, periphyton, and benthic invertebrates in Waikato, New Zealand, hill-country streams. *New Zealand Journal of Marine and Freshwater Research,* **31,** 579-597.

Quinn, J.M., Phillips, N.R. & Parkyn, S.M. (2007) Factors influencing retention of coarse particulate organic matter in streams. *Earth Surface Processes and Landforms,* **32,** 1186-1203.

Quinn, J.M. & Stroud, M.J. (2002) Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use. *New Zealand Journal of Marine and Freshwater Research,* **36,** 409-429.

Rabalais, N.N., Turner, R.E. & Scavia, D. (2002) Beyond Science into Policy: Gulf of Mexico Hypoxia and the Mississippi River. *BioScience,* **52,** 129-142.

Raymond, P. & Saiers, J. (2010) Event controlled DOC export from forested watersheds. *Biogeochemistry,* **100,** 197-209.

Reddy, K.R., Kadlec, R.H., Flaig, E. & Gale, P.M. (1999) Phosphorus Retention in Streams and Wetlands: A Review. *Critical Reviews in Environmental Science and Technology,* **29,** 83-146.

Redfield, A.C. (1958) The biological control of chemical factors in the environment. *American Scientist,* **46,** 205-221.

Reid, D.J., Lake, P.S., Quinn, G.P. & Reich, P. (2008) Association of reduced riparian vegetation cover in agricultural landscapes with coarse detritus dynamics in lowland streams. *Marine and Freshwater Research,* **59,** 998-1014.

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.

Rier, S. & King, D. (1996) Effects of inorganic sedimentation and riparian clearing on benthic community metabolism in an agriculturally-disturbed stream. *Hydrobiologia,* **339,** 111-121.

Rier, S., Nawrocki, K. & Whitley, J. (2011) Response of biofilm extracellular enzymes along a stream nutrient enrichment gradient in an agricultural region of north central Pennsylvania, USA. *Hydrobiologia,* **669,** 119-131.

Rier, S.T., Kuehn, K.A. & Francoeur, S.N. (2007) Algal Regulation of Extracellular Enzyme Activity in Stream Microbial Communities Associated with Inert Substrata and Detritus. *Journal of the North American Benthological Society,* **26,** 439-449.

Rier, S.T. & Stevenson, R.J. (2002) Effects of light, dissolved organic carbon, and inorganic nutrients [2pt] on the relationship between algae and heterotrophic bacteria in stream periphyton. *Hydrobiologia,* **489,** 179-184.

Roberts, B.J., Mulholland, P.J. & Houser, J.N. (2007) Effects of Upland Disturbance and Instream Restoration on Hydrodynamics and Ammonium Uptake in Headwater Streams. *Journal of the North American Benthological Society,* **26,** 38-53.

Roberts, W.M., Stutter, M.I. & Haygarth, P.M. (2012) Phosphorus Retention and Remobilization in Vegetated Buffer Strips: A Review. *Journal Environmental Quality,* **41,** 389-399.

Robertson, A.I., Bunn, S.E., Walker, K.F. & Boon, P.I. (1999) Sources, sinks and transformations of organic carbon in Australian floodplain rivers. *Marine and Freshwater Research,* **50,** 813-829.

Rodríguez-Blanco, M.L., Taboada-Castro, M.M. & Taboada-Castro, M.T. (2010) Sediment and phosphorus loss in runoff from an agroforestry catchment, NW Spain. *Land Degradation & Development,* **21,** 161-170.

Romaní, A., Vázquez, E. & Butturini, A. (2006) Microbial Availability and Size Fractionation of Dissolved Organic Carbon After Drought in an Intermittent Stream: Biogeochemical Link Across the Stream–Riparian Interface. *Microbial Ecology,* **52,** 501-512.

Romaní, A.M. & Sabater, S. (2000) Influence of Algal Biomass on Extracellular Enzyme Activity in River Biofilms. *Microbial Ecology,* **40,** 16-24.

Rosenfeld, J.S. & Mackay, R.J. (1987) Assessing the Food Base of Stream Ecosystems: Alternatives to the P/R Ratio. *Oikos,* **50,** 141-147.

Royer, T.V. & Minshall, G.W. (2001) Effects of nutrient enrichment and leaf quality on the breakdown of leaves in a hardwater stream. *Freshwater Biology,* **46,** 603-610.

Rulík, M. & Spáčil, R. (1999) Assessment of extracellular enzyme activities of alpha- and beta-glucosidase in sediments of a small lowland stream (Sitka stream, Czech Republic). *Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium,* **37,** 99-105.

Ryder, D. & Miller, W. (2005) Setting Goals and Measuring Success: Linking Patterns and Processes in Stream Restoration. *Hydrobiologia,* **552,** 147-158.

Ryder, D.S. (2004) Response of epixylic biofilm metabolism to water level variability in a regulated floodplain river. *Journal of the North American Benthological Society,* **23,** 214-223.

Ryder, D.S. & Vink, S. (2007) Managing regulated flows and contaminant cycles in floodplain rivers. In: *Salt, nutrient, sediment and interactions: findings from the national river contaminants program*. (Eds Lovett, S., Price, P. & Edgar, B.) Land and Water Australia, Canberra, ACT, Australia.

Ryder, D.S., Watts, R.J., Nye, E. & Burns, A. (2006) Can flow velocity regulate epixylic biofilm structure in a regulated floodplain river? *Marine and Freshwater Research,* **57,** 29-36.

Sabater, F., Butturini, A., Martí, E.N., Muñoz, I., Romaní, A., Wray, J. & Sabater, S. (2000) Effects of Riparian Vegetation Removal on Nutrient Retention in a Mediterranean Stream. *Journal of the North American Benthological Society,* **19,** 609-620.

Sabater, S., Bernal, S., Butturini, A., Nin, E. & Sabater, F. (2001) Wood and leaf debris input in a Mediterranean stream: The influence of riparian vegetation. *Archiv. Hydrobiologie,* **153,** 91-102.

Sabater, S., Artigas, J., Gaudes, A., Muñoz, I., Urrea, G. & Romaní, A.M. (2011) Long-term moderate nutrient inputs enhance autotrophy in a forested Mediterranean stream. *Freshwater Biology,* **56,** 1266-1280.

Saraceno, J.F., Pellerin, B.A., Downing, B.D., Boss, E., M. Bachand, P.A. & Bergamaschi, B.A. (2009) High-frequency in situ optical measurements during a storm event: Assessing relationships between dissolved organic matter, sediment concentrations, and hydrologic processes. *Journal of Geophysical Research. Biogeosciences,* **114,** G00F09.

Scarsbrook, M.R. & Halliday, J. (1999) Transition from pasture to native forest land-use along stream continua: Effects on stream ecosystems and implications for restoration. *New Zealand Journal of Marine and Freshwater Research,* **33,** 293-310.

Schade, J.D. & Fisher, S.G. (1997) Leaf Litter in a Sonoran Desert Stream Ecosystem. *Journal of the North American Benthological Society,* **16,** 612-626.

Schulz, M., Kozerski, H. P., Pluntke, T. & Rinke, K. (2003) The influence of macrophytes on sedimentation and nutrient retention in the lower River Spree (Germany). *Water Research,* **37,** 569-578.

Schulze, D.J. & Walker, K.F. (1997) Riparian eucalypts and willows and their significance for aquatic invertebrates in the River Murray, South Australia. *Regulated Rivers: Research & Management,* **13,** 557-577.

Shields, J.F.D., Morin, N. & Cooper, C.M. (2004) Large Woody Debris Structures for Sand-Bed Channels. *Journal of Hydraulic Engineering,* **130,** 208-217.

Simon, K.S., Townsend, C.R., Biggs, B.J.F. & Bowden, W.B. (2005) Temporal Variation of N and P Uptake in 2 New Zealand Streams. *Journal of the North American Benthological Society,* **24,** 1-18.

Sinsabaugh, R., Van Horn, D., Follstad Shah, J.J. & Findlay, S. (2010) Ecoenzymatic Stoichiometry in Relation to Productivity for Freshwater Biofilm and Plankton Communities. *Microbial Ecology,* **60,** 885-893.

Sinsabaugh, R.L. (1997) Large-Scale Trends for Stream Benthic Respiration. *Journal of the North American Benthological Society,* **16,** 119-122.

Sinsabaugh, R.L., Carreiro, M.M., Alvarez, S. (2002) Enzyme and microbial dynamics of litter decomposition In: *Enzymes in the environment.* (Eds R.G. Burns, Dick, R.P.), p. 614. Marcel Dekker, New York.

Sinsabaugh, R.L., Benfield, E.F. & Linkins, A.E., Iii (1981) Cellulase Activity Associated with the Decomposition of Leaf Litter in a Woodland Stream. *Oikos,* **36,** 184-190.

Sinsabaugh, R.L. & Findlay, S. (1995) Microbial Production, Enzyme Activity, and Carbon Turnover in Surface Sediments of the Hudson River Estuary. *Microbial Ecology,* **30,** 127-141.

Sinsabaugh, R.L., Findlay, S., Franchini, P. & Fischer, D. (1997) Enzymatic Analysis of Riverine Bacterioplankton Production. *Limnology and Oceanography,* **42,** 29-38.

Sinsabaugh, R.L. & Moorhead, D.L. (1994) Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. *Soil Biology and Biochemistry,* **26,** 1305-1311.

Somville, M. (1984) Measurement and study of substrate specificity of exoglucosidase activity in eutrophic water. *Applied Environmental Microbiology,* **48,** 1181-1185.

Sovell, L.A., Vondracek, B., Frost, J.A. & Mumford, K.G. (2000) Impacts of Rotational Grazing and Riparian Buffers on Physicochemical and Biological Characteristics of Southeastern Minnesota, USA, Streams. *Environmental Management,* **26,** 629-641.

Sponseller, R.A. & Benfield, E.F. (2001) Influences of land use on leaf breakdown in southern Appalachian headwater streams: a multiple-scale analysis. *Journal of the North American Benthological Society,* **20,** 44-59.

Sridhar, K.R. & Bärlocher, F. (2000) Initial Colonization, Nutrient Supply, and Fungal Activity on Leaves Decaying in Streams. *Applied Environmental Microbiology,* **66,** 1114-1119.

Stanley, E.H., Fisher, S.G. & Grimm, N.B. (1997) Ecosystem Expansion and Contraction in Streams. *BioScience,* **47,** 427-435.

Stanley, E.H., Powers, S.M. & Lottig, N.R. (2010) The evolving legacy of disturbance in stream ecology: concepts, contributions, and coming challenges. *Journal of the North American Benthological Society,* **29,** 67-83.

Stanley, E.H., Powers, S.M., Lottig, N.R., Buffam, I. & Crawford, J.T. (2010) Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: is there a role for DOC management? *Freshwater Biology*, **57** (s1), 26-42.

Steinberg, C.E.W., Kamara, S., Prokhotskaya, V.Y., Manusadžianas, L., Karasyova, T.A., Timofeyev, M.A., Jie, Z., Paul, A., Meinelt, T., Farjalla, V.F., Matsuo, A.Y.O., Kent Burnison, B. & Menzel, R. (2006) Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? *Freshwater Biology,* **51,** 1189-1210.

Steinman, A.D., Mcintire, C.D., Gregory, S.V. & Lamberti, G.A. (1989) Effects of irradiance and grazing on lotic algal assemblages. *Journal of Phycology,* **25,** 478-485.

Stelzer, R.S., Heffernan, J. & Likens, G.E. (2003) The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshwater Biology,* **48,** 1925-1937.

Sterner, R.W., Elser, J.J. (2002) *Ecological stoichiometry: the biology of elements from molecules to the bioshpere,* Princeton University Press, New Jersey, USA.

Stevenson, R., Rier, S., Riseng, C., Schultz, R. & Wiley, M. (2006) Comparing Effects of Nutrients on Algal Biomass in Streams in Two Regions with Different Disturbance Regimes and with Applications for Developing Nutrient Criteria. *Hydrobiologia,* **561,** 149-165.

Stewart, B.A. & Davies, B.R. (1990) Allochthonous input and retention in a small mountain stream, South Africa. *Hydrobiologia,* **202,** 135-146.

Stewart, M. & Ryder, D. (2012) Does restoring riparian vegetation affect nutrient and sediment retention in agricultural streams? In: *6th Australian Stream Management Conference.* (Eds Grove, J. & Rutherford, I.), pp. 381 - 387. River Basin Management Society.

Stoffel, M. & Wilford, D.J. (2012) Hydrogeomorphic processes and vegetation: disturbance, process histories, dependencies and interactions. *Earth Surface Processes and Landforms,* **37,** 9-22.

Strauss, E.A. & Lamberti, G. A. (2002) Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. *Freshwater Biology,* **47,** 65±74.

Stream, S., Workshop (1990) Concepts and Methods for Assessing Solute Dynamics in Stream Ecosystems. *Journal of the North American Benthological Society,* **9,** 95-119.

Sudduth, E.B., Hassett, B.A., Cada, P. & Bernhardt, E.S. (2011) Testing the Field of Dreams Hypothesis: functional responses to urbanization and restoration in stream ecosystems. *Ecological Applications,* **21,** 1972-1988.

Tank, J.L. & Dodds, W.K. (2003) Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology,* **48,** 1031-1049.

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.

Tank, J.L. & Webster, J.R. (1998) Interaction of Substrate and Nutrient Availability on Wood Biofilm Processes in Streams. *Ecology,* **79,** 2168-2179.

Tank, J.L., Webster, J.R., Benfield, E.F. & Sinsabaugh, R.L. (1998) Effect of Leaf Litter Exclusion on Microbial Enzyme Activity Associated with Wood Biofilms in Streams. *Journal of the North American Benthological Society,* **17,** 95-103.

Tank, J.L. & Winterbourn, M.J. (1995) Biofilm development and invertebrate colonization of wood in four New Zealand streams of contrasting pH. *Freshwater Biology,* **34,** 303-315.

Thomas, S.A., Newbold, J.D., Monaghan, M.T., Minshall, G.W., Georgian, T. & Cushing, C.E. (2001) The Influence of Particle Size on Seston Deposition in Streams. *Limnology and Oceanography,* **46,** 1415-1424.

Thoms, M.C. & Parsons, M. (2002) Eco-geomorphology: an interdisciplinary approach to river science. In: *IAHS Publication No. 276.* pp. 113-119. IAHS Press, Wallingford.

Thorp, J.H. & Delong, M.D. (1994) The Riverine Productivity Model: An Heuristic View of Carbon Sources and Organic Processing in Large River Ecosystems. *Oikos,* **70,** 305-308.

Tietjen, T., Vähätalo, A.V. & Wetzel, R.G. (2005) Effects of clay mineral turbidity on dissolved organic carbon and bacterial production. *Aquatic Sciences - Research Across Boundaries,* **67,** 51-60.

Tietjen, T. & Wetzel, R.G. (2003) Extracellular enzyme-clay mineral complexes: Enzyme adsorption, alteration of enzyme activity, and protection from photodegradation. *Aquatic Ecology,* **37,** 331-339.

Tiquia, S. (2011) Extracellular Hydrolytic Enzyme Activities of the Heterotrophic Microbial Communities of the Rouge River: An Approach to Evaluate Ecosystem Response to Urbanization. *Microbial Ecology,* **62,** 679-689.

Townsend, C.R. & Riley, R.H. (1999) Assessment of river health: accounting for perturbation pathways in physical and ecological space. *Freshwater Biology,* **41,** 393-405.

Tranvik, L.J. & Bertilsson, S. (2001) Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecology Letters,* **4,** 458-463.

Treadwell, S.A., Campbell, I.C. & Edwards, R.T. (1997) Organic Matter Dynamics in Keppel Creek, Southeastern Australia. *Journal of the North American Benthological Society,* **16,** 58-61.

Triska, F.J., Kennedy, V.C., Aranzino, R.J., Zellweger, G.W. & Bencala, K.E. (1990) In situ retention-transport response to nitrate loading and storm discharge in a third-order stream. *Journal of the North American Benthological Society,* **9,** 229-239.

Triska, F.J., Sedell, J.R., Cromack, K., Jr., Gregory, S.V. & McCorison, F.M. (1984) Nitrogen Budget for a Small Coniferous Forest Stream. *Ecological Monographs,* **54,** 119-140.

Tuchman, N., Schollett, M., Rier, S. & Geddes, P. (2006) Differential Heterotrophic Utilization of Organic Compounds by Diatoms and Bacteria under Light and Dark Conditions. *Hydrobiologia,* **561,** 167-177.

Tulonen, T., Salonen, K. & Arvola, L. (1992) Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. *Hydrobiologia,* **229,** 239-252.

Tzoraki, O., Nikolaidis, N.P., Amaxidis, Y. & Skoulikidis, N.T. (2007) In-Stream Biogeochemical Processes of a Temporary River. *Environmental Science & Technology,* **41,** 1225-1231.

Uehlinger, U. (2000) Resistance and resilience of ecosystem metabolism in a flood-prone river system. *Freshwater Biology,* **45,** 319-332.

Uehlinger, U. & Naegeli, M.W. (1998) Ecosystem Metabolism, Disturbance, and Stability in a Prealpine Gravel Bed River. *Journal of the North American Benthological Society,* **17,** 165-178.

Uehlinger, U. (2006) Annual cycle and inter-annual variability of gross primary production and ecosystem respiration in a floodprone river during a 15-year period. *Freshwater Biology,* **51,** 938-950.

Underwood, A.J. (1997) *Experiments in ecology: their logical design and interpretation using analysis of variance,* Cambridge University Press, Cambridge, United Kingdom.

Üveges, V. & Padisák, J. (2011) Photosynthetic activity of epilithic algal communities in sections of the Torna stream (Hungary) with natural and modified riparian shading. *Hydrobiologia,* **679,** 267-281.

Vähätalo, A.V., Wetzel, R.G. & Paerl, H.W. (2005) Light absorption by phytoplankton and chromophoric dissolved organic matter in the drainage basin and estuary of the Neuse River, North Carolina (U.S.A.). *Freshwater Biology,* **50,** 477-493.

Valett, H.M., Crenshaw, C.L. & Wagner, P.F. (2002) Stream Nutrient Uptake, Forest Succession, and Biogeochemical Theory. *Ecology,* **83,** 2888-2901.

Valett, H.M., Thomas, S.A., Mulholland, P.J., Webster, J.R., Dahm, C.N., Fellows, C.S., Crenshaw, C.L. & Peterson, C.G. (2008) Endogenous and Exogenous Control of Ecosystem Function: N Cycling in Headwater Streams. *Ecology,* **89,** 3515-3527.

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

Vidon, P., Allan, C., Burns, D., Duval, T.P., Gurwick, N., Inamdar, S., Lowrance, R., Okay, J., Scott, D. & Sebestyen, S. (2010) Hot Spots and Hot Moments in Riparian Zones: Potential for Improved Water Quality Management. *JAWRA Journal of the American Water Resources Association,* **46,** 278-298.

Vilches, C. & Giorgi, A. (2010) Metabolism in a macrophyte-rich stream exposed to flooding. *Hydrobiologia,* **654,** 57-65.

Vink, S., Ford, P., Bormans, M., Kelly, C. & Turley, C. (2007) Contrasting nutrient exports from a forested and an agricultural catchment in south-eastern Australia. *Biogeochemistry,* **84,** 247-264.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H. & Tilman, D.G. (1997) Technical Report: Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. *Ecological Applications,* **7,** 737-750.

von Schiller, D., Acuña, V., Graeber, D., Martí, E., Ribot, M., Sabater, S., Timoner, X. & Tockner, K. (2011) Contraction, fragmentation and expansion dynamics determine nutrient availability in a Mediterranean forest stream. *Aquatic Sciences - Research Across Boundaries*, **73,** 485-497.

von Schiller, D., Bernal, S. & Martí, E. (2011) Technical Note: A comparison of two empirical approaches to estimate in-stream net nutrient uptake. *Biogeosciences,* **8,** 875-882.

von Schiller, D., Martí, E. & Riera, J.L. (2009) Nitrate retention and removal in Mediterranean streams bordered by contrasting land uses: a ^{15}N tracer study. *Biogeosciences,* **6,** 181-196.

von Schiller, D., Martí, E., Riera, J.L., Ribot, M., Marks, J.C. & Sabater, F. (2008) Influence of land use on stream ecosystem function in a Mediterranean catchment. *Freshwater Biology,* **53,** 2600-2612.

von Schiller, D., Martí, E., Riera, J.L. & Sabater, F. (2007) Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology,* **52,** 891-906.

Wallace, J.B., Whiles, M.R., Eggert, S., Cuffney, T.F., Lugthart, G.J. & Chung, K. (1995) Long-Term Dynamics of Coarse Particulate Organic Matter in Three Appalachian Mountain Streams. *Journal of the North American Benthological Society,* **14,** 217-232.

Ward, J.V., Tockner, K., Uehlinger, U. & Malard, F. (2001) Understanding natural patterns and processes in river corridors as the basis for effective river restoration. *Regulated Rivers: Research & Management,* **17,** 311-323.

Warren, D.R., Bernhardt, E.S., Hall, R.O. & Likens, G.E. (2007) Forest age, wood and nutrient dynamics in headwater streams of the Hubbard Brook Experimental Forest, NH. *Earth Surface Processes and Landforms,* **32,** 1154-1163.

Watkins, S.C., Quinn, G.P. & Gawne, B. (2010) Changes in organic-matter dynamics and physicochemistry, associated with riparian vegetation loss and river regulation in floodplain wetlands of the Murray River, Australia. *Marine and Freshwater Research,* **61,** 1207-1217.

Watson, A. & Barmuta, L. (2010) Litter retention in Tasmanian headwater streams after clear-fell logging. *Hydrobiologia,* **637,** 197-206.

Webster, J.R. & Benfield, E.F. (1986) Vascular Plant Breakdown in Freshwater Ecosystems. *Annual Review of Ecology and Systematics,* **17,** 567-594.

Webster, J.R. & Meyer, J.L. (1997) Organic Matter Budgets for Streams: A Synthesis. *Journal of the North American Benthological Society,* **16,** 141-161.

Webster, J.R., Benfield, E.F., Ehrman, T. P., Schaeffer, M. A., Tank, J. L., Hutchens J. J. & D'Angelo, D. J. (1999) What happens to allochthonous material that falls into streams? A synthesis of new and published information from Coweeta. *Freshwater Biology,* **41,** 687-705.

Weiss, M.S., Abele, U., Weckesser, J., Welte, W., Schiltz, E. & Schulz, G.E. (1991) Molecular Architecture and Electrostatic Properties of a Bacterial Porin. *Science,* **254,** 1627-1630.

Westhorpe, D., Mitrovic, S., Ryan, D. & Kobayashi, T. (2010) Limitation of lowland riverine bacterioplankton by dissolved organic carbon and inorganic nutrients. *Hydrobiologia,* **652,** 101-117.

Wetzel, R.G., Hatcher, P.G. & Bianchi, T.S. (1995) Natural Photolysis by Ultraviolet Irradiance of Recalcitrant Dissolved Organic Matter to Simple Substrates for Rapid Bacterial Metabolism. *Limnology and Oceanography,* **40,** 1369-1380.

Wiegner, T., N. & Seitzinger, S., P. (2001) Photochemical and microbial degradation of external dissolved organic matter inputs to rivers. *Aquatic Microbial Ecology,* **24,** 27-40.

Wiegner, T.N., Seitzinger, Sybil P., Glibert, P. M. & Bronk, D. A. (2006) Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. *Aquatic Microbial Ecology,* **43,** 277-287.

Wiens, J.A. (2002) Riverine landscapes: taking landscape ecology into the water. *Freshwater Biology,* **47,** 501-515.

Willey, J.D., Kieber, R.J., Eyman, M.S. & Avery, G.B., Jr. (2000) Rainwater dissolved organic carbon: Concentrations and global flux. *Global Biogeochemical Cycles,* **14,** 139-148.

Williams, L., Hale, R., Cavagnaro, T., Reich, P. & Lake, P.S. (2011) Developing a vision for improved monitoring and reporting of riparian restoration projects. *Ecological Management & Restoration,* **12,** e11-e16.

Wilson, H.F. & Xenopoulos, M.A. (2009) Effects of agricultural land use on the composition of fluvial dissolved organic matter. *Nature Geosciences,* **2,** 37-41.

Wilson, J.S., Baldwin, D.S., Rees, G.N. & Wilson, B.P. (2011) The effects of short-term inundation on carbon dynamics, microbial community structure and microbial activity in floodplain soil. *River Research and Applications,* **27,** 213-225.

Wohl, E. & Jaeger, K. (2009) A conceptual model for the longitudinal distribution of wood in mountain streams. *Earth Surface Processes and Landforms,* **34,** 329-344.

Wold, A.P. & Hershey, A.E. (1999) Spatial and Temporal Variability of Nutrient Limitation in 6 North Shore Tributaries to Lake Superior. *Journal of the North American Benthological Society,* **18,** 2-14.

Wolfenden, B. (2009) *Leaf litter dynamics and the rehabilitation of degraded coastal rivers in NSW, Australia.* Doctor of Philosophy, University of New England, Armidale.

Wood, E.D., Armstrong, F.A.J. & Richards, F.A. (1967) Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *Journal of the Marine Biological Association of the United Kingdom,* **47,** 23-31.

Wood, P.J. & Armitage, P.D. (1997) Biological Effects of Fine Sediment in the Lotic Environment. *Environmental Management,* **21,** 203-217.

Wood, P.J. & Petts, G.E. (1999) The influence of drought on chalk stream macroinvertebrates. *Hydrological Processes,* **13,** 387-399.

Ylla, I., Sanpera-Calbet, I., Muã±Oz, I., Romanã, A. & Sabater, S. (2011) Organic matter characteristics in a Mediterranean stream through amino acid composition: changes driven by intermittency. *Aquatic Sciences - Research Across Boundaries,* **73,** 523-535.

Ylla, I., Sanpera-Calbet, I., Vázquez, E., Romaní, A., Muñoz, I., Butturini, A. & Sabater, S. (2010) Organic matter availability during pre- and post-drought periods in a Mediterranean stream. *Hydrobiologia,* **657,** 217-232.

Young, R.G. & Huryn, A.D. (1996) Interannual variation in discharge controls ecosystem metabolism along a grassland river continuum. *Canadian Journal of Fisheries and Aquatic Sciences,* **53,** 2199-2211.

Young, R.G. & Huryn, A.D. (1998) Comment: Improvements to the diurnal upstream-downstream dissolved oxygen technique for determining whole-stream metabolism in small streams. *Canadian Journal of Fisheries and Aquatic Sciences,* **55,** 1784-1785.

Young, R.G. & Huryn, A.D. (1999) Effects of Land Use on Stream Metabolism and Organic Matter Turnover. *Ecological Applications,* **9,** 1359-1376.

Young, R.G., Huryn, A.D. & Townsend, C.R. (1994) Effects of agricultural development on processing of tussock leaf litter in high country New Zealand streams. *Freshwater Biology,* **32,** 413-427.

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.

Zander, A., Bishop, A., Prenzler, P. & Ryder, D. (2007) Allochthonous DOC in floodplain rivers: identifying sources using solid phase microextraction with gas chromatography. *Aquatic Sciences - Research Across Boundaries,* **69,** 472-483.

Zotina, T., Köster, O. & Jüttner, F. (2003) Photoheterotrophy and light-dependent uptake of organic and organic nitrogenous compounds by *Planktothrix rubescens* under low irradiance. *Freshwater Biology,* **48,** 1859-1872.

Zweig, L.D. & Rabeni, C.F. (2001) Biomonitoring for Deposited Sediment Using Benthic Invertebrates: A Test on 4 Missouri Streams. *Journal of the North American Benthological Society,* **20,** 643-657.

Appendix 2: Summary of physico-chemical parameters

Table A2.1: Summary of mean physico-chemical parameters (n=3) from February and May sampling periods including: pH, conductivity (µS), total suspended solids (TSS, mg L^{-1}) and dissolved oxygen (DO, mg L^{-1}). Data are missing for the Moredun vegetated and Booralong CS reaches during May as no surface water was present.

Reach	pH	Conductivity	TSS	DO
February				
Moredun Vegetated	8.10	275.7	2.94	4.37
Gwydir Vegetated	8.77	235.5	9.04	6.15
Roumalla Vegetated	7.95	194.2	11.27	1.13
Booralong CS	8.20	192.7	8.42	1.13
Booralong Bridge	8.66	243.2	8.56	0.97
Moredun Non-vegetated	9.14	195.0	9.94	5.25
Gwydir Non-vegetated	7.85	226.0	12.83	5.84
Roumalla Non-vegetated	8.27	184.8	8.88	4.95
Laura Homestead	7.91	207.1	13.00	5.09
Laura Bridge	8.55	249.8	16.17	2.46
May				
Moredun Vegetated				
Gwydir Vegetated	7.91	311.0	1066.46	0.39
Roumalla Vegetated	7.61	244.8	1052.00	4.44
Booralong CS				
Booralong Bridge	7.81	273.7	1670.40	1.94
Moredun Non-vegetated	8.29	404.8	1798.73	0.79
Gwydir Non-vegetated	8.48	214.2	1107.08	7.00
Roumalla Non-vegetated	9.22	241.2	1080.83	6.63
Laura Homestead	8.16	212.3	1672.13	2.64
Laura Bridge	7.87	705.8	3534.67	1.27

Table A2.2: Summary of mean physico-chemical parameters (n=3) from September and November sampling periods including: pH, conductivity (µS), total suspended solids (TSS, mg L^{-1}) and dissolved oxygen (DO, mg L^{-1}).

Reach	pH	Conductivity	TSS	DO
September				
Moredun Vegetated	6.83	90.8	4.83	8.97
Gwydir Vegetated	7.02	135.7	4.75	8.88
Roumalla Vegetated	6.79	93.6	7.29	7.55
Booralong CS	7.22	154.2	4.02	7.96
Booralong Bridge	7.11	152.0	3.96	7.77
Moredun Non-vegetated	6.75	88.9	5.38	8.68
Gwydir Non-vegetated	7.09	135.6	4.38	7.18
Roumalla Non-vegetated	6.84	92.3	6.83	7.08
Laura Homestead	7.01	112.6	3.79	9.69
Laura Bridge	7.08	109.4	5.21	9.09
November				
Moredun Vegetated	6.85	104.2	2.46	7.69
Gwydir Vegetated	7.04	151.6	5.71	7.09
Roumalla Vegetated	6.58	128.4	7.42	6.43
Booralong CS	7.04	157.0	3.13	8.64
Booralong Bridge	7.27	157.1	2.92	6.84
Moredun Non-vegetated	7.01	97.5	2.04	8.55
Gwydir Non-vegetated	6.92	154.6	6.83	5.23
Roumalla Non-vegetated	6.59	122.8	6.33	6.01
Laura Homestead	7.05	126.8	3.13	7.71
Laura Bridge	6.88	128.0	4.63	8.74

Appendix 3: Tables of formula for unbalanced PERMANOVA location tests

Chapter 3

Table A3.1: Statistical formula of unbalanced PERMANOVA tests for significant differences in nutrient concentration (mg L^{-1}) and chlorophyll *a* mass (nutrient enrichment experiment, g cm^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$10.3\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	19	σ^2_e		
	Total	20			
2	Location = Lo	1	$12\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	22	σ^2_e		
	Total	23			
3	Location = Lo	1	$22.4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	43	σ^2_e		
	Total	44			

Table A3.2: Statistical formula of unbalanced PERMANOVA tests for significant differences in dissolved and particulate nutrient stoichiometry between locations on (1) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$7.5\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	13	σ^2_e		
	Total	14			

Table A3.3: Statistical formula of unbalanced PERMANOVA tests for significant differences in nutrient retention (g day^{-1}) between locations on (1) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$6\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	10	σ^2_e		
	Total	11			

Chapter 4

Table A3.4: Statistical formula of unbalanced PERMANOVA tests for significant differences in FBOM mass (g cm^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$44.2\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	88	σ^2_e		
	Total	89			
2	Location = Lo	1	$48.8\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	96	σ^2_e		
	Total	97			
3	Location = Lo	1	$93.9\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	186	σ^2_e		
	Total	187			

Table A3.5: Statistical formula of unbalanced PERMANOVA tests for significant differences in CBOM mass (g cm^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$44.2\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	88	σ^2_e		
	Total	89			
2	Location = Lo	1	$52\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	102	σ^2_e		
	Total	103			
3	Location = Lo	1	$96.6\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	192	σ^2_e		
	Total	193			

Table A3.6: Statistical formula of unbalanced PERMANOVA tests for significant differences in FBOM and CBOM retention (g day^{-1}) between locations on (1) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$6\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	10	σ^2_e		
	Total	11			

Table A3.7: Statistical formula of unbalanced PERMANOVA tests for significant differences in organic matter breakdown (% total mass lost) and total inorganic matter accumulation (g) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$8.8\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	17	σ^2_e		
	Total	18			
2	Location = Lo	1	$11\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	20	σ^2_e		
	Total	21			
3	Location = Lo	1	$20.2\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	39	σ^2_e		
	Total	40			

Table A3.8: Statistical formula of unbalanced PERMANOVA tests for significant differences in average daily surface water temperature (°C) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$3.4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	5	σ^2_e		
	Total	6			
2	Location = Lo	1	$4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	6	σ^2_e		
	Total	7			
3	Location = Lo	1	$7.4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	13	σ^2_e		
	Total	14			

Chapter 5

Table A3.9: Statistical formula of unbalanced PERMANOVA tests for significant differences in mean EEA (µM 4-MUB L^{-1} h^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$3.4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	5	σ^2_e		
	Total	6			
2	Location = Lo	1	$4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	6	σ^2_e		
	Total	7			
3	Location = Lo	1	$7.4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	13	σ^2_e		
	Total	14			

Chapter 6

Table A3.10: Statistical formula of unbalanced PERMANOVA tests for significant differences in rates of GPP, ER, NEP (mg L^{-1} day^{-1}) and P/R ratios between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$3^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	4	σ^2_e		
	Total	5			
2	Location = Lo	1	$4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	6	σ^2_e		
	Total	7			
3	Location = Lo	1	$7\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	11	σ^2_e		
	Total	13			

Table A3.11: Statistical formula of unbalanced PERMANOVA tests for significant differences in mean daily light values (lumens m^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$2.4^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	4	σ^2_e		
	Total	5			
2	Location = Lo	1	$3\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	5	σ^2_e		
	Total	6			
3	Location = Lo	1	$5.5\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	10	σ^2_e		
	Total	11			

Table A3.12: Statistical formula of unbalanced PERMANOVA tests for significant differences in water column chlorophyll *a* concentration (mg L^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$9\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	16	σ^2_e		
	Total	17			
2	Location = Lo	1	$12\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	22	σ^2_e		
	Total	23			
3	Location = Lo	1	$20\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	40	σ^2_e		
	Total	41			

Table A3.13: Statistical formula of unbalanced PERMANOVA tests for significant differences in total macrophyte biomass (kg) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Location = Lo	1	$3.4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	5	σ^2_e		
	Total	6			
2	Location = Lo	1	$4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	6	σ^2_e		
	Total	7			
3	Location = Lo	1	$7.4\sigma^2_{Lo} + \sigma^2_e$	1*Lo	1*Res
	Residual = e	13	σ^2_e		
	Total	14			

Appendix 4: Tables of location tests

Chapter 3

Table A4.1: Results of PERMANOVA tests for significant differences in NOx concentration (mg L^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	21493.000	21493.000	$1.876e^{-3}$	0.975
	Res	19	$2.177e^{+8}$	$1.146e^{+7}$		
	Total	20	$2.177e^{+8}$			
2	Lo	1	$2.226e^{+6}$	$2.226e^{+6}$	0.240	0.643
	Res	22	$2.038e^{+8}$	$9.265e^{+6}$		
	Total	23	$2.061e^{+8}$			
3	Lo	1	$6.669e^{+5}$	$6.669e^{+5}$	$6.682e^{-2}$	0.809
	Res	43	$4.292e^{+8}$	$9.981e^{+6}$		
	Total	44	$4.298Ee^{+8}$			

Table A4.2: Results of PERMANOVA tests for significant differences in SRP concentration (mg L^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	1239.500	1239.500	0.384	0.535
	Res	19	61303.000	3226.500		
	Total	20	62543.000			
2	Lo	1	43.323	43.323	$3.338e^{-2}$	0.843
	Res	22	2855.000	1297.700		
	Total	23	28593.000			
3	Lo	1	178.180	178.180	$8.164e^{-2}$	0.773
	Res	43	93848.000	2182.500		
	Total	44	94027.000			

Table A4.3: Results of PERMANOVA tests for significant differences in TN concentration (mg L^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined. Significant results are in bold type.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	$6.384e^{-2}$	$6.384e^{-2}$	4.465	**0.024**
	Total	20	0.336			
	Res	19	0.272	$1.430e^{-2}$		
2	Lo	1	$2.750e^{-2}$	$2.750e^{-2}$	0.428	0.555
	Res	22	1.418	$6.444e^{-2}$		
	Total	23	1.445			
3	Lo	1	$3.569e^{-4}$	$3.569e^{-4}$	$7.797e^{-3}$	0.916
	Res	43	1.968	$4.577e^{-2}$		
	Total	44	1.968			

Table A4.4: Results of PERMANOVA tests for significant differences in TP concentration (mg L^{-1}) between locations in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	94.291	94.291	2.038	0.182
	Res	19	879.140	46.270		
	Total	20	973.430			
2	Lo	1	2.254	2.254	$3.935e^{-2}$	0.831
	Res	22	1260.000	57.274		
	Total	23	1262.300			
3	Lo	1	22.248	22.248	0.411	0.524
	Res	43	2328.000	54.140		
	Total	44	2350.300			

Table A4.5: Results of PERMANOVA tests for significant differences in DOC concentration (mg L^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined. Significant results are in bold type.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	11.754	11.754	3.474	0.093
	Res	19	64.281	3.383		
	Total	20	76.035			
2	Lo	1	45.637	45.637	18.329	**0.002**
	Res	22	54.776	2.490		
	Total	23	100.410			
3	Lo	1	3.693	3.693	0.591	0.453
	Res	43	268.650	6.248		
	Total	44	272.340			

Table A4.6: Results of PERMANOVA tests for significant differences in dissolved (1) and particulate (2) nutrient stoichiometry between locations (Lo) in both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	0.135	0.135	$4.203e^{-2}$	0.990
	Res	13	41.865	3.220		
	Total	14	42.000			
2	Lo	1	0.229	0.229	$7.118e^{-2}$	0.969
	Res	13	41.771	3.213		
	Total	14	42.000			

Table A4.7: Results of PERMANOVA tests for significant differences in chlorophyll *a* mass (g m^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	9.934	9.934	1.258	0.224
	Res	19	150.070	7.898		
	Total	20	160.000			
2	Lo	1	3.771	3.771	0.460	0.666
	Res	22	180.230	8.192		
	Total	23	184.000			
3	Lo	1	7.068	7.068	0.881	0.374
	Res	43	344.930	8.022		
	Total	44	352.000			

Table A4.8: Results of PERMANOVA test for significant differences in nutrient retention (g day^{-1}) between locations. Data from both Booralong Creek and Laura Creek were used in the analyses.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
NOx	Lo	1	14.691	14.691	0.119	0.734
	Res	10	1233.000	123.300		
	Total	11	1247.700			
SRP	Lo	1	77.192	77.192	0.362	0.558
	Res	10	2133.200	213.320		
	Total	11	2210.400			
TN	Lo	1	261.070	261.070	1.255	0.300
	Res	10	2079.500	207.950		
	Total	11	2340.600			
TP	Lo	1	188.010	188.010	0.403	0.543
	Res	10	4670.900	467.090		
	Total	11	4858.900			
DOC	Lo	1	0.107	0.107	$5.900e^{-6}$	0.999
	Res	10	$1.821e^{+5}$	18213.000		
	Total	11	$1.821e^{+5}$			

Chapter 4

Table A4.9: Results of PERMANOVA tests for significant differences in FBOM (g m^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined. Significant results are in bold type.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	2.890	2.890	7.695	**0.009**
	Res	88	33.055	0.376		
	Total	89	35.945			
2	Lo	1	8.544	8.544	9.034	**0.004**
	Res	96	90.794	0.946		
	Total	97	99.338			
3	Lo	1	1786.600	1786.600	2.188	0.133
	Res	186	$1.519e^{+5}$	816.430		
	Total	187	$1.536e^{+5}$			

Table A4.10: Results of PERMANOVA tests for significant differences in total CBOM (g m^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined. Significant results are in bold type.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	4.134	4.138	8.721	**0.002**
	Res	88	41.753	0.475		
	Total	89	45.890			
2	Lo	1	2.926	2.926	2.991	0.091
	Res	102	99.792	0.978		
	Total	103	102.720			
3	Lo	1	$3.254e^{-2}$	$3.254e^{-2}$	$4.183e^{-2}$	0.826
	Res	192	149.370	0.780		
	Total	193	149.410			

Table A4.11: Results of PERMANOVA tests for significant differences in CBOM composition (g m^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined. The multivariate dataset included masses (g m^{-2}) of: needles, wood, *Casuarina* reproductive structures, leaf/bark, macrophytes, and unidentified fractions. Significant results are in bold type.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	2.890	2.890	7.695	**0.009**
	Res	88	33.055	0.376		
	Total	89	35.945			
2	Lo	1	8.544	8.544	9.034	**0.004**
	Res	96	90.794	0.946		
	Total	97	99.338			
3	Lo	1	1786.600	1786.600	2.188	
	Res	186	$1.519e^{+5}$	816.430		
	Total	187	$1.536e^{+5}$			0.133

Table A4.12: Results of PERMANOVA test for significant differences in organic matter retention (g day^{-1}) between locations. Data from both Booralong Creek and Laura Creek was used in the analyses.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
Total FOM	Lo	1	51.022	51.022	0.989	0.359
	Res	10	515.740	51.574		
	Total	11	566.760			
Total COM	Lo	1	$8.724e^{-2}$	$8.724e^{-2}$	$7.997e^{-2}$	0.753
	Res	10	10.913	1.091		
	Total	11	11.000			
Multi-variate COM	Lo	1	2.737	2.737	0.524	0.618
	Res	10	52.263	5.226		
	Total	11	55.000			

Table A4.13: Results of PERMANOVA tests for significant differences in litterbag decomposition (% mass lost, determined from area under the curve) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	324.240	324.240	1.760	0.241
	Res	20	3683.600	184.180		
	Total	21	4007.800			
2	Lo	1	1.344	1.344	$4.952e^{-2}$	0.831
	Res	17	461.310	27.136		
	Total	18	462.660			
3	Lo	1	188.900	188.900	1.687	0.207
	Res	39	4367.000	111.970		
	Total	40	4555.900			

Table A5.14: Results of PERMANOVA tests for significant differences in mean daily surface water temperature (°C) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	15.228	15.228	0.19808	0.784
	Res	5	384.38	76.876		
	Total	6	399.61			
2	Lo	1	1.0513	1.0513	1.103E-2	0.831
	Res	6	571.85	95.309		
	Total	7	572.9			
3	Lo	1	14.036	14.036	0.18198	0.784
	Res	13	1002.7	77.131		
	Total	14	1016.7			

Table A4.15: Results of PERMANOVA tests for significant differences in inorganic matter mass accumulated (g) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	0.38431	0.38431	2.2906	0.225
	Res	5	0.83888	0.16778		
	Total	6	1.2232			
2	Lo	1	1.134	1.134	5.3	0.062
	Res	6	1.2838	0.21396		
	Total	7	2.4178			
3	Lo	1	14.036	14.036	0.18198	0.784
	Res	13	1002.7	77.131		
	Total	14	1016.7			

Chapter 5

Table A4.16: Results of PERMANOVA tests for significant differences in AP activity (µM 4-MUB L^{-1} h^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	0.720	0.720	0.174	0.736
	Res	5	20.686	4.137		
	Total	6	21.406			
2	Lo	1	1.036	1.036	1.075	0.334
	Res	6	5.785	0.964		
	Total	7	6.821			
3	Lo	1	1.585	1.585	0.752	0.399
	Res	13	27.377	2.106		
	Total	14	28.961			

Table A4.17: Results of PERMANOVA tests for significant differences in LAP activity (µM 4-MUB L^{-1} h^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	$8.443e^{-4}$	$8.443e^{-4}$	$2.039e^{-3}$	0.970
	Res	5	2.070	0.414		
	Total	6	2.071			
2	Lo	1	$3.444e^{-2}$	$3.444e^{-2}$	0.3425	0.605
	Res	6	0.603	0.101		
	Total	7	0.638			
3	Lo	1	$3.477e^{-2}$	$3.477e^{-2}$	0.124	0.723
	Res	13	3.643	0.280		
	Total	14	3.678			

Table A4.18: Results of PERMANOVA tests for significant differences in β-glu activity (µM 4-MUB L^{-1} h^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	0.363	0.363	0.491	0.601
	Res	5	3.691	0.738		
	Total	6	4.053			
2	Lo	1	$8.280e^{-2}$	$8.280e^{-2}$	$5.294e^{-2}$	0.819
	Res	6	9.385	1.564		
	Total	7	9.467			
3	Lo	1	$1.706e^{-2}$	$1.706e^{-2}$	$1.543e^{-2}$	0.913
	Res	13	14.377	1.106		
	Total	14	14.394			

Table A4.19: Results of PERMANOVA tests for significant differences in α-glu activity (µM 4-MUB L^{-1} h^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	0.452	0.452	0.260	0.729
	Res	5	8.693	1.739		
	Total	6	9.145			
2	Lo	1	$5.687e^{-2}$	$5.687e^{-2}$	$9.424e^{-2}$	0.771
	Res	6	3.621	0.603		
	Total	7	3.678			
3	Lo	1	0.395	0.395	0.413	0.553
	Res	13	12.429	0.956		
	Total	14	12.823			

Table A4.20: Results of PERMANOVA tests for significant differences in xylo activity (µM 4-MUB L^{-1} h^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	0.187	0.187	0.297	0.613
	Res	5	3.144	0.629		
	Total	6	3.331			
2	Lo	1	$1.002e^{-2}$	$1.002e^{-2}$	$2.433e^{-2}$	0.846
	Res	6	2.470	0.412		
	Total	7	2.480			
3	Lo	1	$6.487e^{-2}$	$6.487e^{-2}$	0.141	0.709
	Res	13	5.987	0.461		
	Total	14	6.052			

Chapter 6

Table A4.21: Results of PERMANOVA tests for significant differences in metabolic parameters including rates of GPP, ER, net DO change (mg L^{-1}) and P/R ratios between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	3.560	3.560	0.866	0.728
	Res	4	16.440	4.110		
	Total	5	20.000			
2	Lo	1	1.362	1.362	0.307	0.829
	Res	6	26.638	4.440		
	Total	7	28.000			
3	Lo	1	2.717	2.717	0.662	0.614
	Res	12	49.283	4.107		
	Total	13	52.000			

Table A4.22: Results of PERMANOVA tests for significant differences in mean daily light values (lumens m^{-2}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	$9.276e^{6}$	$9.276e^{6}$	$3.056e^{-3}$	1
	Res	3	$9.106e^{9}$	$3.035e^{9}$		
	Total	4	$9.115e^{9}$			
2	Lo	1	$3.226e^{9}$	$3.226e^{9}$	$4.021e^{-2}$	0.526
	Res	4	$3.209e^{11}$	$8.023e^{10}$		
	Total	5	$3.241e^{11}$			
3	Lo	1	$4.735e^{9}$	$4.735e^{9}$	0.109	0.575
	Res	9	$3.894e^{11}$	$4.326e^{10}$		
	Total	10	$3.941e^{11}$			

Table A5.23: Results of PERMANOVA tests for significant differences in chlorophyll *a* concentration (mg L^{-1}) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	$3.744e^{-4}$	$3.744e^{-4}$	0.198	0.611
	Res	16	$3.020e^{-2}$	$1.888e^{-3}$		
	Total	17	$3.058e^{-2}$			
2	Lo	1	$4.371e^{-3}$	$4.371e^{-3}$	0.223	0.624
	Res	22	0.431	$1.959e^{-2}$		
	Total	23	0.435			
3	Lo	1	$3.925e^{-3}$	$3.924e^{-3}$	0.279	0.607
	Res	40	0.562	$1.406e^{-2}$		
	Total	41	0.566			

Table A4.24: Results of PERMANOVA tests for significant differences in total macrophyte biomass (kg) between locations (Lo) in (1) Booralong Creek only, (2) Laura Creek only, and (3) both control streams combined. Data was Box-Cox transformed prior to analysis.

Test	Source	df	SS	MS	Pseudo-F	P(perm)
1	Lo	1	$2.176e^{-3}$	$2.176e^{-3}$	1.350	0.426
	Res	5	$8.058e^{-3}$	$1.612e^{-3}$		
	Total	6	$1.023e^{-2}$			
2	Lo	1	0.637	0.637	0.494	0.472
	Res	6	7.729	1.288		
	Total	7	8.365			
3	Lo	1	0.181	0.181	0.153	0.686
	Res	13	15.312	1.178		
	Total	14	15.493			

Appendix 5: Tables of formula for unbalanced PERMANOVA main factor tests

Chapter 3

Table A5.1: Statistical formula of unbalanced PERMANOVA tests for significant differences in nutrient concentrations (mg L^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + 3\sigma^2_{TrStTi} + 7.7\sigma^2_{TrTi} + 10.3\sigma^2_{TrSt} + 30.9\sigma^2_{Tr}$	0.943*TrxStxTi + 1*Tr	0.964*TrxTi + 0.980*TrxSt
2	Stream = St	4	$\sigma^2_e + 4.8\sigma^2_{StTi} + 19.3\sigma^2_{St}$	1*St	0.959*StxTi + 0.040*Res
3	Time = Ti	3	$\sigma^2_e + 5.4\sigma^2_{StTi} + 26.9\sigma^2_{Ti}$	0.080*Res + 1*Ti	1.071*StxTi
4	Tr x St	2	$\sigma^2_e + 3\sigma^2_{TrStTi} + 10.5\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + 3\sigma^2_{TrStTi} + 8\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	12	$\sigma^2_e + 5\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	5	$\sigma^2_e + 3\sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	83	σ^2_e		
	Total	113			

Table A5.2: Statistical formula of unbalanced PERMANOVA tests for significant differences in dissolved and particulate nutrient stoichiometry between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 2.6\sigma^2_{TrTi} + 3.4\sigma^2_{TrSt} + 10.3\sigma^2_{Tr}$	0.944*TrxStxTi + 1*Tr	0.964*TrxTi + 0.980*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.6\sigma^2_{StTi} + 6.4\sigma^2_{St}$	1*St	0.959*StxTi + 0.040*Res
3	Time = Ti	3	$\sigma^2_e + 1.8\sigma^2_{StTi} + 9.0\sigma^2_{Ti}$	0.080*Res + 1*Ti	1.071*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3.5\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + \sigma^2_{TrStTi} + 2.7\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	12	$\sigma^2_e + 1.7\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	5	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	7	σ^2_e		
	Total	37			

Table A5.3: Statistical formula of unbalanced PERMANOVA tests for significant differences in chlorophyll *a* mass (g cm^{-2}) from nutrient enrichment experiment between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + 3\sigma^2_{TrStTi} + 5.8\sigma^2_{TrTi} + 7.7\sigma^2_{TrSt} + 23.3\sigma^2_{Tr}$	1.006*TrxStxTi + 1*Tr	0.971*TrxTi + 1.035*TrxSt
2	Stream = St	4	$\sigma^2_e + 4.0\sigma^2_{StTi} + 15.9\sigma^2_{St}$	1*St	0.889*StxTi + 0.111*Res
3	Time = Ti	3	$\sigma^2_e + 4.4\sigma^2_{StTi} + 22.2\sigma^2_{Ti}$	1*Ti	0.990*StxTi+ 0.010*Res
4	Tr x St	2	$\sigma^2_e + 3\sigma^2_{TrStTi} + 7.5\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + 3\sigma^2_{TrStTi} + 6\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	12	$\sigma^2_e + 4.5\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	3	$\sigma^2_e + 3\sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	79	σ^2_e		
	Total	107			

Table A5.4: Statistical formula of unbalanced PERMANOVA tests for significant differences in nutrient retention (g day^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 3\sigma^2_{TrTi} + 3\sigma^2_{TrSt} + 9\sigma^2_{Tr}$	1*TrxStxTi + 1*Tr	1*TrxTi + 1*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.8\sigma^2_{StTi} + 5.4\sigma^2_{St}$	1*St	1*StxTi
3	Time = Ti	2	$\sigma^2_e + 2\sigma^2_{StTi} + 10\sigma^2_{Ti}$	0.111*Res + 1*Ti	1.111*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	8	$\sigma^2_e + 1.8\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	4	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	6	σ^2_e		
	Total	29			

Chapter 4

Table A5.5: Statistical formula of unbalanced PERMANOVA tests for significant differences in FBOM mass (g m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present. Nine other samples also had to be excluded due to sample bags splitting.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + 8.6\sigma^2_{TrStTi} + 25.9\sigma^2_{TrTi} + 34.5\sigma^2_{TrSt} + 103.5\sigma^2_{Tr}$	0.763*TrxStxTi + 1*Tr	0.833*TrxTi + 0.889*TrxSt + 0.004*Res
2	Stream = St	4	$\sigma^2_e + 18.9\sigma^2_{StTi} + 75.7\sigma^2_{St}$	1*St	0.903*StxTi + 0.010*Res
3	Time = Ti	3	$\sigma^2_e + 21.2\sigma^2_{StTi} + 106.0\sigma^2_{Ti}$	1*Ti + 0.001*Res	1.012*StxTi
4	Tr x St	2	$\sigma^2_e + 9.7\sigma^2_{TrStTi} + 38.8\sigma^2_{TrSt}$	1*TrxSt	0.858*TrxStxTi + 0.142*Res
5	Tr x Ti	3	$\sigma^2_e + 10.3\sigma^2_{TrStTi} + 31.0\sigma^2_{TrTi}$	1*TrxTi	0.916*TrxStxTi + 0.008*Res
6	St x Ti	12	$\sigma^2_e + 21.0\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	6	$\sigma^2_e + 11.3\sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	454	σ^2_e		
	Total	485			

Table A5.6: Statistical formula of unbalanced PERMANOVA tests for significant differences in CBOM mass (g m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present. Four other samples also had to be excluded due to sample bags splitting.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + 8.6\sigma^2_{TrStTi} + 25.9\sigma^2_{TrTi} + 34.6\sigma^2_{TrSt} + 103.7\sigma^2_{Tr}$	0.762*TrxStxTi + 1*Tr	0.832*TrxTi + 0.890*TrxSt + 0.004*Res
2	Stream = St	4	$\sigma^2_e + 19.2\sigma^2_{StTi} + 76.8\sigma^2_{St}$	1*St	0.905*StxTi + 0.010*Res
3	Time = Ti	3	$\sigma^2_e + 21.5\sigma^2_{StTi} + 107.5\sigma^2_{Ti}$	1*Ti + 0.001*Res	1.013*StxTi
4	Tr x St	2	$\sigma^2_e + 9.7\sigma^2_{TrStTi} + 38.8\sigma^2_{TrSt}$	1*TrxSt	0.856*TrxStxTi + 0.144*Res
5	Tr x Ti	3	$\sigma^2_e + 10.4\sigma^2_{TrStTi} + 31.2\sigma^2_{TrTi}$	1*TrxTi	0.916*TrxStxTi + 0.008*Res
6	St x Ti	12	$\sigma^2_e + 21.2\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	6	$\sigma^2_e + 11.3\sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	461	σ^2_e		
	Total	492			

Table A5.7: Statistical formula of unbalanced PERMANOVA tests for significant differences in CBOM composition (g m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present. Four other samples also had to be excluded due to sample bags splitting.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + 8.6\sigma^2_{TrStTi} + 25.9\sigma^2_{TrTi} + 34.6\sigma^2_{TrSt} + 103.7\sigma^2_{Tr}$	0.762*TrxStxTi + 1*Tr	0.832*TrxTi + 0.890*TrxSt + 0.004*Res
2	Stream = St	4	$\sigma^2_e + 19.2\sigma^2_{StTi} + 76.8\sigma^2_{St}$	1*St	0.905*StxTi + 0.010*Res
3	Time = Ti	3	$\sigma^2_e + 21.5\sigma^2_{StTi} + 107.5\sigma^2_{Ti}$	1*Ti + 0.001*Res	1.013*StxTi
4	Tr x St	2	$\sigma^2_e + 9.7\sigma^2_{TrStTi} + 38.8\sigma^2_{TrSt}$	1*TrxSt	0.856*TrxStxTi + 0.144*Res
5	Tr x Ti	3	$\sigma^2_e + 10.4\sigma^2_{TrStTi} + 31.2\sigma^2_{TrTi}$	1*TrxTi	0.916*TrxStxTi + 0.008*Res
6	St x Ti	12	$\sigma^2_e + 21.2\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	6	$\sigma^2_e + 11.3\sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	461	σ^2_e		
	Total	492			

Table A5.8: Statistical formula of unbalanced PERMANOVA tests for significant differences in FBOM and CBOM mass, and CBOM composition (g m^{-2}) between treatment (Tr), streams (St), time (Ti), and their significant interactions.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 3\sigma^2_{TrTi} + 3\sigma^2_{TrSt} + 9\sigma^2_{Tr}$	1*TrxStxTi + 1*Tr	1*TrxTi + 1*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.8\sigma^2_{StTi} + 5.4\sigma^2_{St}$	1*St	1*StxTi
3	Time = Ti	2	$\sigma^2_e + 2\sigma^2_{StTi} + 10\sigma^2_{Ti}$	0.111*Res + 1*Ti	1.111*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	8	$\sigma^2_e + 1.8\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	4	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	6	σ^2_e		
	Total	29			

Table A5.9: Statistical formula of unbalanced PERMANOVA tests for significant differences in organic matter breakdown (% mass loss) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present. Sixteen other samples were lost when strong surface water velocities removed the litterbags from reaches.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + 3.0\sigma^2_{TrStTi} + 4.5\sigma^2_{TrTi} + 5.9\sigma^2_{TrSt} + 17.8\sigma^2_{Tr}$	0.591*TrxStxTi + 1*Tr + 0.037*Res	0.783*TrxTi + 0.844*TrxSt
2	Stream = St	4	$\sigma^2_e + 3.9\sigma^2_{StTi} + 14.6\sigma^2_{St}$	1*St	0.894*StxTi + 0.106*Res
3	Time = Ti	3	$\sigma^2_e + 4.8\sigma^2_{StTi} + 22.4\sigma^2_{Ti}$	0.116*Res + 1*Ti	1.116*StxTi
4	Tr x St	2	$\sigma^2_e + 2.8\sigma^2_{TrStTi} + 7.0\sigma^2_{TrSt}$	1*TrxSt+ 0.003*Res	1.003*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + 2.9\sigma^2_{TrStTi} + 5.7\sigma^2_{TrTi}$	1*TrxTi+ 0.019*Res	1.019*TrxStxTi
6	St x Ti	11	$\sigma^2_e + 4.3\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	3	$\sigma^2_e + 2.8\sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	71	σ^2_e		
	Total	98			

Table A5.10: Statistical formula of unbalanced PERMANOVA tests for significant differences in mean organic matter breakdown (% mass loss), total inorganic matter accumulation (g), and average daily surface water temperature (°C) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from Booralong CS and Moredun vegetated reaches during May as no surface water was present. Data are also missing from Roumalla non-vegetated during, Moredun non-vegetated and Moredun vegetated reaches during November when strong surface water velocities removed the litterbags.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 1.5\sigma^2_{TrTi} + 2\sigma^2_{TrSt} + 6\sigma^2_{Tr}$	0.550*TrxStxTi + 1*Tr	0.750*TrxTi + 0.800*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.4\sigma^2_{StTi} + 5.2\sigma^2_{St}$	1*St	0.894*StxTi + 0.106*Res
3	Time = Ti	3	$\sigma^2_e + 1.7\sigma^2_{StTi} + 8.0\,\sigma^2_{Ti}$	0.136*Res + 1*Ti	1.136*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 2.5\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + \sigma^2_{TrStTi} + 2\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	11	$\sigma^2_e + 1.5\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	3	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	7	σ^2_e		
	Total	34			

Chapter 5

Table A5.11: Statistical formula of unbalanced PERMANOVA tests for significant differences in mean EEA (µM 4-MUB L^{-1} h^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from two reaches (Booralong CS and Moredun vegetated) during May as no surface water was present.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 2.6\sigma^2_{TrTi} + 3.4\sigma^2_{TrSt} + 10.3\sigma^2_{Tr}$	0.944*TrxStxTi + 1*Tr	0.964*TrxTi + 0.980*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.6\sigma^2_{StTi} + 6.4\sigma^2_{St}$	1*St	0.959*StxTi + 0.040*Res
3	Time = Ti	3	$\sigma^2_e + 1.8\sigma^2_{StTi} + 9.0\sigma^2_{Ti}$	0.080*Res + 1*Ti	1.071*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3.5\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + \sigma^2_{TrStTi} + 2.7\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	12	$\sigma^2_e + 1.7\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	5	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	7	σ^2_e		
	Total	37			

Chapter 6

Table A5.12: Statistical formula of unbalanced PERMANOVA tests for significant differences in rates of GPP, ER, NEP (mg L^{-1} day^{-1}) and P/R ratios between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from Booralong CS and Moredun vegetated during May as no surface water was present, and Gwydir non-vegetated reach during September when no rate could be determined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 2.3\sigma^2_{TrTi} + 3.1\sigma^2_{TrSt} + 9.3\sigma^2_{Tr}$	1.04*TrxStxTi + 1*Tr	1*TrxTi + 1.037*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.5\sigma^2_{StTi} + 5.9\sigma^2_{St}$	1*St	0.924*StxTi + 0.076*Res
3	Time = Ti	3	$\sigma^2_e + 1.6\sigma^2_{StTi} + 8.2\sigma^2_{Ti}$	0.041*Res + 1*Ti	1.041*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + \sigma^2_{TrStTi} + 2.3\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	12	$\sigma^2_e + 1.6\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	4	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	7	σ^2_e		
	Total	36			

Table A5.13: Statistical formula of unbalanced PERMANOVA tests for significant differences in mean daily light values (lumens m^2) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from all reaches during February due to technical issues; Booralong CS and Moredun vegetated during May as no surface water was present, and Gwydir non-vegetated reach during September when no rate could be determined.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 2.1\sigma^2_{TrTi} + 2.1\sigma^2_{TrSt} + 6.4\sigma^2_{Tr}$	1.143*TrxStxTi + 1*Tr	1.071*TrxTi + 1.071*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.4\sigma^2_{StTi} + 4.1\sigma^2_{St}$	1*St	0.921*StxTi + 0.079*Res
3	Time = Ti	2	$\sigma^2_e + 1.5\sigma^2_{StTi} + 7.5\sigma^2_{Ti}$	0.014*Res + 1*Ti	1.014*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 2\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	2	$\sigma^2_e + \sigma^2_{TrStTi} + 2\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	8	$\sigma^2_e + 1.5\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	2	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	5	σ^2_e		
	Total	26			

Table A5.14: Statistical formula of unbalanced PERMANOVA tests for significant differences in water column chlorophyll *a* concentration (mg L^{-1}) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from Booralong CS and Moredun vegetated during May as no surface water was present.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + 3\sigma^2_{TrStTi} + 7.7\sigma^2_{TrTi} + 10.3\sigma^2_{TrSt} + 30.9\sigma^2_{Tr}$	0.943*TrxStxTi + 1*Tr	0.964*TrxTi + 0.980*TrxSt
2	Stream = St	4	$\sigma^2_e + 4.8\sigma^2_{StTi} + 19.3\sigma^2_{St}$	1*St	0.959*StxTi + 0.040*Res
3	Time = Ti	3	$\sigma^2_e + 5.4\sigma^2_{StTi} + 26.9\sigma^2_{Ti}$	0.080*Res + 1*Ti	1.071*StxTi
4	Tr x St	2	$\sigma^2_e + 3\sigma^2_{TrStTi} + 10.5\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + 3\sigma^2_{TrStTi} + 8\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	12	$\sigma^2_e + 5\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	5	$\sigma^2_e + 3\sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	83	σ^2_e		
	Total	113			

Table A5.15: Statistical formula of unbalanced PERMANOVA tests for significant differences in total macrophyte biomass (kg) between treatment (Tr), streams (St), time (Ti), and their significant interactions. Data are missing from Booralong CS and Moredun vegetated during May as no surface water was present.

	Source	Degrees of freedom	Expected mean square	F-ratio Numerator	F-ratio Denominator
1	Treatment = Tr	1	$\sigma^2_e + \sigma^2_{TrStTi} + 2.6\sigma^2_{TrTi} + 3.4\sigma^2_{TrSt} + 10.3\sigma^2_{Tr}$	0.944*TrxStxTi + 1*Tr	0.964*TrxTi + 0.980*TrxSt
2	Stream = St	4	$\sigma^2_e + 1.6\sigma^2_{StTi} + 6.4\sigma^2_{St}$	1*St	0.959*StxTi + 0.040*Res
3	Time = Ti	3	$\sigma^2_e + 1.8\sigma^2_{StTi} + 9.0\sigma^2_{Ti}$	0.080*Res + 1*Ti	1.071*StxTi
4	Tr x St	2	$\sigma^2_e + \sigma^2_{TrStTi} + 3.5\sigma^2_{TrSt}$	1*TrxSt	1*TrxStxTi
5	Tr x Ti	3	$\sigma^2_e + \sigma^2_{TrStTi} + 2.7\sigma^2_{TrTi}$	1*TrxTi	1*TrxStxTi
6	St x Ti	12	$\sigma^2_e + 1.7\sigma^2_{StTi}$	1*StxTi	1*Res
7	Tr x St x Ti	5	$\sigma^2_e + \sigma^2_{TrStTi}$	1*TrxStxTi	1*Res
8	Residual = e	7	σ^2_e		
	Total	37			

Appendix 6: Macrophyte biomass coefficient of correlations

Table A6.1: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at Moredun non-vegetated reach during all four sampling periods.

Species	February	May	September	November
Vallisneria gigantea	0.83	0.89		
Scirpus validus	0.81			0.89
Rumex crispus			0.85	0.86
Juncus usitatus			0.82	

Table A6.2: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at Moredun vegetated reach during all four sampling periods.

Species	February	May	September	November
Triglochin procera				0.85
Carex spp.				0.80
Juncus spp.				0.80

Table A6.3: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at Gwydir non-vegetated reach during all four sampling periods.

Species	February	May	September	November
Vallisneria gigantea	0.90	0.85		
Ranunculus trichophyllus	0.88	0.90		0.79
Hydrilla verticillata		0.88		
Scirpus validus	0.85			0.83
Cyperus spp.	0.85			0.83
Carex spp.				0.87

Table A6.4: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at Gwydir vegetated reach during all four sampling periods.

Species	February	May	September	November
Vallisneria gigantea	0.90			

Table A6.5: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at Roumalla non-vegetated reach during all four sampling periods.

Species	February	May	September	November
Vallisneria gigantea		0.93		0.81
Eleocharis sphacelata			0.83	0.76
Eleocharis acuta				0.80
Nitella spp.		0.94		
Chara spp.		0.93		

Table A6.6: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at upstream vegetated control reach, Booralong CS, during all four sampling periods.

Species	February	May	September	November
Carex spp.			0.81	0.82
Juncus spp.			0.83	

Table A6.7: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at downstream vegetated control reach, Booralong Bridge, during all four sampling periods.

Species	February	May	September	November
Scirpus validus			0.87	
Carex spp.			0.81	
Juncus spp.			0.82	0.81

Table A6.8: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at upstream non-vegetated control reach, Laura HD, during all four sampling periods.

Species	February	May	September	November
Vallisneria gigantea	0.86	0.80		0.87
Eleocharis sphacelata				
Scirpus validus				0.87
Rumex crispus			0.85	0.86

Table A6.9: Coefficient of correlation (r^2) values from percent cover-biomass regressions for macrophyte species recorded at downstream non-vegetated control reach, Laura Bridge, during all four sampling periods.

Species	February	May	September	November
Vallisneria gigantea	0.93	0.91		
Eleocharis sphacelata	0.77	0.90		
Scirpus validus	0.70			0.92
Cyperus exaltus	0.82			
Carex fasicularis				0.82
Juncus usitatus			0.81	
Rumex crispus			0.87	0.88