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Longitudinal trends in river functioning: Patterns of nutrient and carbon processing in three Australian rivers.

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Abstract

Understanding longitudinal trends in the processing of carbon in rivers represents a much conceptualised, but infrequently tested, issue in aquatic ecology. In this study, we conducted concurrent longitudinal examinations of three very different rivers in eastern Australia to determine whether general principles in river functioning exist across broad geographic and hydrologic scales. Specifically, we examined trends in ambient basic water chemistry, nutrient concentrations, dissolved organic carbon (DOC), extracellular enzymes and food web structure and functioning and conducted bioassays to examine the degree to which DOC and nutrients limit heterotrophic bacterial respiration. These parameters revealed striking similarities across all sites. For metazoan communities, stable isotope analysis showed that algal carbon was the dominant basal resource utilised by consumers in all three rivers, suggesting that in-stream primary producers strongly underpin trophic pathways regardless of the position within a catchment or catchment condition. Analyses of extracellular enzymes revealed that microbial communities are actively utilising DOC at all sites. In fact, heterotrophic microbial respiration was strongly limited by DOC at all sites, with nutrient additions resulting in only relatively minor increases in respiration. Ultimately, this study demonstrates that DOC and algal carbon are critically important drivers of ecosystem processes in Australian riverine ecosystems. Furthermore, across all of our sites and rivers, ambient nutrient concentrations did not influence carbon processing. The consistent longitudinal trends in river function identified in this study provide useful insights for catchment managers and modellers with respect to identifying key principles that underpin ecosystem functioning in Australian rivers.

Introduction

Longitudinal trends in the source, concentration and composition of carbon and nutrient pools, and how they influence river function, represent an important yet often overlooked component of river management and understanding (Gawne *et al.*, 2007). This is despite the fact that longitudinal connectivity and transport of both nutrients and carbon within river systems have received considerable conceptual attention. For example, the River Continuum Concept (RCC) of Vannote *et al.* (1980) discusses the capacity of upstream river conditions to influence both the sources of carbon and the ecological processes in downstream reaches. Despite recent attempts to examine food web dynamics and carbon processing at different sections of rivers (e.g. middle and lower reaches of large rivers - Thorp and Delong, 1994), very few studies have sought to examine concurrent aspects of carbon and nitrogen sources, forms and processes in headwater, middle and lower reaches of a single river system. This absence of whole-of-river studies is particularly surprising given that most conceptual models of river functioning predict fundamental changes in carbon processing from headwater to middle and lower reaches of rivers (Vannote *et al.*, 1980; Junk *et al.*, 1989; Thorp and Delong, 1994).

Whilst catchment-wide or whole-of-river examinations of carbon dynamics are few, ambient nutrients (especially nitrogen and phosphorus) and dissolved organic carbon (DOC) concentrations are often measured and interpreted at these broader spatial scales. Indeed, the development of catchment models for sediment and nutrient loads has been based on the understanding gained from catchment, or at least sub-basin, assessments of the delivery, transport and processing of carbon, nitrogen and phosphorus through river networks (Williams and Melack, 1997; Harris, 2001; Biggs *et al.*, 2004; Clark *et al.*, 2004; Smith *et al.*, 2005; Drewry *et al.*, 2006; Dodds and Oates, 2006). In general, this understanding suggests that nutrient and DOC concentrations are likely to increase longitudinally (i.e. from upstream to downstream) along the river. Whilst modifications to these general patterns can be observed due to point source, land use and/or hydrological influences (Kelly, 2001; Jarvie *et al.*, 2008; Neal *et al.*, 2008), there is general agreement that downstream sites are likely to have higher nutrient concentrations than upstream sites.

Based on the knowledge available for Australian river ecosystems, we suggest that whilst nutrient concentrations are likely to increase somewhat predictably from headwater to lower reaches (Harris, 2001), riverine functioning, and carbon processes in particular, may be substantially more robust to change. To this end, these processes may be more consistent both within and between catchments. For example, we predict that Australian riverine metazoan food webs are likely to be predominately driven by algal sources of carbon irrespective of position in catchment. Although this prediction runs counter to some of the prevailing riverine conceptual models (the RCC in particular), recent research in Australian aquatic ecosystems has revealed that algal sources of carbon, particularly benthic algae, tend to fuel food webs (Bunn *et al.*, 2003; Douglas *et al.*, 2005; Spears *et al.*, submitted). Why benthic algae should be such an important source of organic matter to consumers in Australian river systems is a topic of considerable debate, although one possible explanation was put forward by Roberston *et al.* (1999). They suggested that reductions in the delivery of floodplain and riparian carbon to inland rivers, largely due to modifications of flow and physical alterations to catchments and floodplains, might explain why many Australian river food webs rely on benthic algal carbon as the dominant basal resource. While human modification of catchments and rivers may have played a role, Douglas *et al.* (2005) have also noted the disproportionate importance of benthic algae as a food resource in tropical rivers, including those in catchments with few or no modifications. Regardless of the underlying mechanisms involved in establishing (and maintaining) benthic algae as the dominant food source in Australian rivers, it does appear that this is the case in a wide range of systems, from rivers and streams (Bunn *et al.*, 2003; Spears *et al.*, submitted) to wetlands and lakes (Hadwen and Bunn, 2004). Furthermore, benthic algae seems to play this role more or less independently of the delivery and transport of nutrient and carbon sources at particular sites.

Just as longitudinal studies of river function are lacking, so too are studies that concurrently examine hydrologically and geographically distinct rivers. As noted by Bunn and Arthington (2002), this absence of studies of contrasting rivers has limited our capacity to identify general principles in river function. To address this knowledge gap and to examine broad patterns in river function, we adopted a multi-catchment

approach in this study. Specifically, we examined how aspects of nutrient and carbon processing change along the lengths of three rivers in geographically distinct regions in eastern Australia. Our approach was to simultaneously characterise carbon and nutrient pools, heterotrophic microbial activity and metazoan foodwebs under low flow conditions in all three catchments. We also sought to examine how ecological conceptual models and our knowledge of ecosystem function, which tend to focus on carbon and energy flows, might relate to longitudinal patterns in nutrient concentrations. Finally, we hoped to ascertain how carbon and nutrient processing might be linked to improve our understanding of catchment and water quality monitoring and management.

Materials and Methods

Site descriptions

The three rivers used for this study are all located in eastern Australia (Figure 1). The Logan River (Queensland) flows east out of the McPherson Range, eventually discharging into southern Moreton Bay. Both the Gwydir River (New South Wales) and the Ovens River (Victoria) flow inland from the Great Dividing Range and form part of the Murray-Darling Basin, Australia's largest river system. Rainfall and flow regimes differ in each area, with highest flow events in summer in the Logan and Gwydir Rivers and in spring in the Ovens River. Although peak flow periods in the Gwydir and Logan are both in summer, winter rainfall can also stimulate substantial flows in the Gwydir.

The Logan River is part of the Logan-Albert catchment which covers an area of 3740 km² in southeast Queensland. The Logan River sub-catchment is significantly larger than that of the Albert River and the main stem of the Logan River has a total length of 185 km. Headwaters of the main stem of Logan River exit the McPherson Range at an altitude of 400 m, however several of the major tributaries drain areas above 800 m. Annual rainfall across the catchment is variable, ranging from 700 mm in the southwest to 2000 mm in the southeastern headwater areas. Headwater streams throughout the catchment lie in forest reserves, while the mid to lower reaches have

been cleared with cattle grazing as the major landuse. In addition, urban and rural residential developments are also located throughout the lower catchment.

The Gwydir River catchment (26500 km²) is located in northern New South Wales. Draining west from the New England Tablelands (elevation – 1050 m), the river travels approximately 310 km before becoming a system of braided channels over an extensive floodplain. It is also regulated and diverted between the main stem and the Mehi River at a weir near the town of Moree. Mean annual rainfall declines from a high of 750 mm in the east, to a low of 450 mm at the western edge of the catchment. Flows in the catchment are also affected by a significant water storage in the upper reaches (Copeton Dam – 1360 GL capacity). The landscape has been extensively modified in all areas of the catchment with the major landuses being agriculture (irrigated and dry-land) and cattle grazing.

The Ovens River drains the Victorian highlands and travels northwest, eventually flowing into the Murray River. The main stem of the river is approximately 150 km long and the Ovens catchment has an area of 7780 km². Mean annual rainfall is highest in the upper catchment (1500 mm), declining to 600 mm in the north. The Ovens River is unregulated and is considered to be one of the last rivers in Victoria with a relatively natural flow regime (Rees *et al.*, 2005). In addition, the lower floodplain has been declared a Heritage River by virtue of its unique environmental values (Department of Natural Resources and Environment 1991). A relatively large proportion (48%) of the catchment consists of native vegetation although middle reaches of the river have relatively poor (< 40%) riparian woody cover (De Rose *et al.*, 2005). Land use in the middle reaches of the river includes grazing and agriculture (plantation and cropping).

Four sites were selected along the length of each river to capture major changes in geomorphology. As a result, at least one site from each of upland, middle and lowland reaches of each river were sampled. Sites are hereafter referred to using the following notation; Logan River sites LR, Gwydir River sites GR and Ovens Rivers sites OR. Within each river, sites were also numbered numerically from most upstream sites to

most downstream sites, resulting in LR1, LR2, LR3 and LR4 in the Logan River and the equivalent codes in the other two catchments.

For each site, a representative 100 m long reach was selected such that all major channel morphological features (run, riffles and pools) and local landuses were represented (Table 1). The physical characteristics of each site (specifically the riparian zone, stream channel and adjacent land use) were assessed using a standard protocol adopted from that used routinely by the New South Wales Department of Water and Energy (NSW DWE). In addition, stream discharge was determined either using flow meters along two transects at each site or from data from nearby gauging stations. Fieldwork was carried out concurrently in all rivers, during the week of December 4 to December 8, 2006. For all three catchments, this period fell within one of the worst droughts observed in eastern Australia in the last 200 years (Bond *et al.*, 2008), so despite the historical differences in the seasonality and magnitude of rainfall and associated flows across these catchments, all measures were collected during a very low flow period.

Field and laboratory methods

Physico-chemical measurements

A well-mixed part of the reach was selected at each site for all water chemistry readings. Dissolved oxygen (DO), water temperature, electrical conductivity (EC) and pH were measured using standard field meters. Turbidity was measured in triplicate, either using a meter in the field (Gwydir - Hach 2100P turbidimeter) or in the laboratory (Logan - Hach 2100AN turbidimeter). No DO or turbidity measures were recorded for the Ovens River sites.

Water and chlorophyll-a samples

Composite water column samples were collected in triplicate, from a well-mixed area of each study site, using 10 L buckets. Each water samples was subsampled for nutrient, DOC and chlorophyll-*a* concentration determination, resulting in three replicate measures per site. All samples were put into polyethylene containers and

stored on ice until frozen in the laboratory, unless otherwise noted. For inorganic nutrients (ammonium, nitrogen oxides and filterable reactive phosphorus (FRP)) two 10 ml subsamples were filtered using 0.45 μm cellulose acetate membranes. For total nitrogen (TN) and total phosphorus (TP), a 100 ml subsample of unfiltered water was collected. Nutrient samples were analysed at the Murray –Darling Freshwater Research Centre (MDFRC) laboratory in Wodonga, Victoria. Nutrient concentrations were measured using standard methods (American Public Health Association, 1998) in an analytical laboratory operating to national guidelines of quality control and quality assurance (National Association of Testing Authorities, Australia).

For DOC samples, 50 ml of water was filtered through a 0.45 μm cellulose acetate membrane that had been pre-washed with 20 ml of sample water. The filtered sample was stored in a pre-ashed 100-ml amber glass bottle and acidified with 2-3 drops of concentrated HCl. Refrigerated DOC samples were sent to the NSW DWE laboratory (Wolli Creek, NSW) for analysis using the High Temperature Combustion Method, 5310 B (American Public Health Association, 1998), as outlined in Wetzel and Likens (2000).

One 50 ml subsample from each composite water column sample was collected and analysed for exoenzyme activities at the MDFRC laboratory. The activities of esterase, leucine aminopeptidase, phosphatase, β -glucosidase, α -glucosidase and β -xylosidase were measured. Activities were derived from the release of fluorochromes from fluorogenic substrates, supplied at saturation concentrations (Findlay *et al.*, 1998). Fluorescent products were detected on a Fluoroskan II fluorescence plate reader (Labsystems) and enzyme activities were corrected for quenching.

For the determination of water column chlorophyll-*a* concentrations, a measured volume of water from each composite sample was filtered through a 0.7 μm glass fibre filter using a Mitivac vacuum hand pump. In addition to the water column chlorophyll-*a* analyses, we collected, where possible, five samples from each major benthic substrate type for benthic chlorophyll-*a* determination from each site (none were collected from Ovens River sites). For hard surfaces (cobbles), a recorded area

of substrate was scrubbed for biofilm. The slurry was then filtered through a 0.7 μm glass fibre filter using a Mitivac vacuum hand pump. For soft sediments (gravel, sand and silt), a fixed area of the top 20 mm of substrate was removed using a cut-off 60 ml syringe. All chlorophyll samples were wrapped in aluminum foil to prevent exposure to light, transported on ice, and stored frozen in the laboratory prior to analysis. In the laboratory, chlorophyll pigments were extracted by adding 90% ethanol and heating in a water bath (5 mins at 75 °C) according to standard procedures (ISO, 1994). Chlorophyll-*a* was determined by spectrophotometric absorption and concentrations were calculated as $\mu\text{g L}^{-2}$ for water column samples and $\mu\text{g m}^{-2}$ for benthic samples.

Food webs

At each site, we collected replicate (n=3 wherever possible) samples for stable isotope analyses of the food web. Samples of new growth of the dominant riparian vegetation was collected by hand, as were emergent and submerged macrophytes, with care taken to remove any attached algae/detritus. Algal samples (including filamentous, biofilms, epiphytes and epilithon) were collected using a scalpel. Benthic organic matter was collected using a series of graded sieves, whereby particulates were rinsed, using site water, to wash away inorganic sediment. The retained organic matter fractions were graded according to sieve mesh size as follows: coarse particulate organic matter (CPOM) - 1 mm; fine particulate organic matter (FPOM) – 250 μm ; and ultra fine particulate organic matter (UFPOM) – 100 μm . Water column organic matter fractions were collected using a plankton tow net (65 μm or 75 μm mesh) in open water sections of each site. All source materials were stored separately in labelled zip-lock bags or plastic containers and were stored on ice prior to being frozen in the laboratory.

In-stream macroinvertebrates were gathered by hand, or using dip (250 μm mesh) or seine nets (1 mm mesh). Some crustaceans and small fish were sampled using baited fish-traps. All consumer groups were stored in labelled zip-lock bags and immediately placed on ice. This procedure allowed the specimens to void their guts, removing unassimilated material and thereby aiding laboratory processing (*sensu* Hadwen and

Bunn, 2005). Samples were frozen upon return to the laboratory prior to further processing.

All stable isotope samples were processed and analysed at Griffith University using standard procedures. Briefly, all samples were initially dried in an oven at 60°C for a minimum of 48 h. Dried riparian vegetation and CPOM were pulverised in a puck and mill grinder for approximately 1 minute, whereas algae, FPOM and UFPOM samples were pulverised using a Retsch MM200 ring grinder for 30 seconds or until they had been ground to a fine powder. Macroinvertebrate samples were ground to a fine powder using a mortar and pestle. Bivalves and gastropods were removed from their shells, and where necessary, trichoptera larvae were removed from their cases before processing. Exoskeletons of crustacean taxa (*Macrobrachium* and *Paratya*) were removed by hand prior to processing to ensure that exoskeleton calcium carbonate did not affect carbon isotope values (*sensu* Bunn *et al.*, 1995). Macroinvertebrates were ground individually wherever possible, however smaller taxa often had to be pooled to ensure sufficient material for successful isotopic analyses. Muscle tissue of larger fish specimens was removed with a scalpel, while small fish specimens (< 30 mm) were processed whole. Fish samples were subsequently prepared for analysis as per macroinvertebrate samples. Samples were analysed using a continuous flow-isotope mass spectrometer (GV Isoprime Eurovector EA 3000, Manchester, UK). Isotope ratios are expressed as $\delta^{13}\text{C}$ (ratio of $^{13}\text{C}:^{12}\text{C}$) and $\delta^{15}\text{N}$ (ratio of $^{15}\text{N}:^{14}\text{N}$) and are determined against laboratory standard reference materials (ANU sucrose for $\delta^{13}\text{C}$ and ambient N_2 for $\delta^{15}\text{N}$).

Laboratory Bioassays

A laboratory bioassay was carried out to examine the ability of the heterotrophic microbial community to process DOC and nutrients along the length of each study river. The bioassay was designed to determine whether organic carbon, inorganic nutrients or both were limiting rates of microbial respiration and at the same time assess how labile the ambient DOC was. Site water was incubated for 48 h with four treatments, namely: control (untreated); + DOC from leaf leachate; + inorganic nutrients; and + DOC + inorganic nutrients. Activity of the microbial community was

assessed through measurements of DO and DOC concentrations at the beginning and end of the bioassay.

Standard solutions of DOC leaf leachate and inorganic nutrient stock were prepared for use across all sites. DOC leaf leachate was prepared using River Redgum (*Eucalyptus camaldulensis*) leaves based on the protocol described by Ward and Johnson (1996). The mean (\pm SE) DOC concentration for the stock red-gum leachate was 3367 (\pm 26) mg L⁻¹ based on triplicate samples analysed by the DWE laboratory. The bioassay treatments with the added DOC stock solution had 1.95 mLs of the leachate added to raise DOC concentrations by 10 mg C L⁻¹ over ambient site concentrations. Nutrients added in the treatments with inorganic nutrient sources aimed to raise N and P levels by 500 and 100 μ g L⁻¹ respectively (250 μ g L⁻¹ NH₄-N, 250 μ g L⁻¹ NO₃-N, and 100 μ g L⁻¹ P) by adding 0.5 mL of a stock solution.

Approximately 15 L of water was collected from between 0.25 and 0.5m depth at a midstream point from all four study sites in each river on December 8, 2006. Large particles were removed onsite by passing the water through a 65 μ m sieve. Sieved water was then stored in a 15 L washed and sealed plastic container and transported back to the laboratory, with care taken to preserve water temperature as close as possible to that at the time of sampling.

In the laboratory, 16 x 500 ml plastic bottles were filled with site water for each site. Treatments were assigned randomly to give four replicates per treatment, three for incubation and one for separate analysis of initial water chemistry. DO concentrations were immediately measured for replicates assigned to bioassays using a DO meter. Nutrient and/or DOC solutions were added for treatments, and the bottles were topped up with site water and sealed with no air space. Three replicates from each treatment (12 bottles x 4 sites) were then incubated at room temperature (20 - 22°C) in darkness for 48 h. Filtered water samples (0.45 μ m membrane filter) were taken from the fourth replicate of each treatment for water chemistry analysis of initial starting concentrations for each treatment. At the end of the 48 h incubation period, DO concentrations were measured and filtered water samples were taken for analyses of

end treatment DOC concentrations. All nutrient and DOC water samples were processed as described previously.

Statistical analyses

Enzymes

A fourth root transformation was carried out on the final corrected enzyme activities to down weight the relatively high esterase activities. Comparisons between samples were carried out on a similarity matrix derived on Euclidean distances of the transformed enzyme activities. Visualisation of this dissimilarity was achieved through non metric multidimensional scaling (Clarke and Warwick, 2001) and Analysis of Similarity (ANOSIM) was used to test for relationships between groups. To determine which enzyme activities were responsible for the patterns observed in multivariate space, a similarity percentage analysis (SIMPER) procedure was run. Initially, each river was analysed separately to examine differences among sites. This was followed by an analysis of all sites from all rivers to examine differences among rivers. All multivariate analyses were carried out within the Primer 6 software package (ePrimer, Plymouth, UK).

Bioassays

Significant changes in DO and DOC concentrations across treatments were determined using ANOVA after the Shapiro-Wilk test for normality. For the DOC results, two separate generalised linear model ANOVAs were run as differences in DOC concentration were introduced through the amendments. One ANOVA included the initial, control and inorganic nutrients additions, and the other the initial DOC amended, DOC and DOC + nutrients treatments. Tukey's mean separation technique was used to determine where significant differences existed among treatments.

Food webs

The primary aim of our food web analyses was to examine the degree to which consumers at each site were utilising autochthonous (in-stream autotrophs) or allochthonous (external) sources of carbon. To do this and to compare results across

all sites, we calculated the percent contribution of autochthonous and allochthonous sources for each consumer individually, before calculating the mean contribution of these source groups across all of the sampled consumers at each site (*sensu* Hadwen and Bunn, 2004). These analyses were predominantly conducted using the IsoSource mixing model software developed by Phillips and Gregg (2003). This model calculates feasible combinations (in 1% increments) of autotroph isotope signatures that explain observed consumer isotope signatures. In our analyses, combinations of end member signatures that summed to within 0.01% of the consumer signature were considered feasible. Trophic fractionations of carbon are generally low (less than 1%) and we used no correction in these analyses in light of values reported in the literature (Peterson and Fry, 1987; McCutchan *et al.*, 2003). Nitrogen stable isotope signatures were not included in these analyses due to unknown levels of fractionation in the study organisms (*sensu* Connolly *et al.*, 2005), particularly in sites with elevated $\delta^{15}\text{N}$ signatures (Hadwen *et al.*, 2007).

To facilitate comparisons across sites and to focus on the degree to which food webs were driven by allochthonous and autochthonous sources of carbon, we only used 'pure' sources as end members in the IsoSource mixing model (*sensu* Hadwen and Bunn, 2004; Hadwen and Arthington, 2007). Specifically, the end members that were used in the analyses were riparian vegetation, seston and benthic algal values (including epilithon, epiphyton, filamentous algae and biofilm). CPOM, FPOM and UFPOM were not used on the basis that these samples represent a mixture of other end members (Hadwen and Bunn, 2004). While aquatic submerged and/or emergent macrophytes were present at most sites, these sources were not included in the mixing model analyses on the basis of previous studies that have shown that aquatic macrophytes do not directly contribute to the diets of consumers (Hamilton *et al.*, 1992; Bunn and Boon; 1993, Boon and Bunn, 1994; France, 1995). For one site, GR3, an insufficient number of end members were collected to be able to use the IsoSource software. In this instance, we used the two-source mixing model presented in Bunn and Boon (1993) to determine the degree to which consumers were relying on riparian vegetation (allochthonous) and seston (autochthonous) sources of carbon.

All measures

To examine broad patterns in the data, across all measures and all sites simultaneously, we conducted a multi-dimensional scaling (MDS) multivariate analysis in Primer 6 (ePrimer, Plymouth, UK). The data matrix included physicochemical (temperature, pH and conductivity), nutrient (TN, TP, FRP, nitrogen oxides and ammonium), enzyme activity (esterase, phosphatase, leucine amino peptidase), food web (% autochthonous contribution to entire food web) and bioassay values (DO and DOC responses in control and nutrient addition treatments).

Because there were no bioassay data for OR1 and OR2, analyses were conducted once for all sites without the bioassay data and then again for all sites except OR1 and OR2, this time with the bioassay data included. This approach enabled us to include OR1 and OR2 in a general MDS, but also to examine the degree to which, if at all, the MDS pattern was influenced by the bioassay results. For both sets of ordinations, we also used the BIOENV routine in Primer to investigate which measures were predominantly responsible for the observed patterns.

Results

Water Chemistry

The water chemistry characteristics of the study sites tended to vary more across the three catchments than within each catchment (Table 2). In the Logan River, EC was generally low, but increased downstream. Water temperature was always above 21 °C across all sites (Table 2). Flow at the time of sampling was variable but generally low, with no flow at LR2, some flow at LR1 and higher and roughly equivalent flows at LR3 and LR4. The influence of flow conditions on DO is reflected by the low DO at LR2, with the remaining sites with measurable flow all having high DO concentrations (above 8 mg L⁻¹).

In the Gwydir River, summer irrigation releases resulted in flows several orders of magnitude greater at GR2 (downstream of Copeton Dam) compared to the other sites (Table 2). At GR2, both pH and temperature were lower than at the other three sites due to flow releases from Copeton Dam (Table 2). DO concentrations for the first

three sites (GR1 – 3) were similar to the DO concentrations reported for the Logan River sites with flow. However, GR4 had a comparatively low DO of 6.65 mg L⁻¹.

In the Owens River, no flow, turbidity or DO readings were taken (Table 2). Across all four sites pH was stable, with all values around 7.4. In contrast, temperature and EC both increased from the upland to lowland reaches (Table 2).

Ambient Nutrient and DOC Concentrations

Logan River

The major trends in nutrient concentrations in the Logan River were largely driven by the high concentrations measured at LR3 (Figure 2). The concentrations of TP and FRP recorded at this site were higher than those measured at all other sites across all three catchments. For nitrogen species, ammonium concentrations were relatively low at all four sites, but nitrogen oxides varied appreciably - with particularly high concentrations (> 150 µL⁻¹) recorded at LR3. As was the case for all analytes, concentrations at LR4 were lower than those recorded at LR3. DOC concentrations in the Logan River were relatively low, ranging from 2 to 6 mg L⁻¹, and tended to increase with distance downstream (Figure 2).

Gwydir River

Along the continuum of sites sampled on the Gwydir River, different trends were observed across the measured nutrient analytes (Figure 2). For example, FRP was low at GR1, rose significantly at GR2, fell again at GR3 and then rose to levels like those observed at GR2 at GR4. For TP, concentrations were moderate and roughly equivalent from GR1 to GR3. However, TP at GR4 was very high and equivalent to the values observed at LR4. For TN, concentrations at all four sites in the Gwydir River were high, with the highest concentrations in this study recorded at GR4. For the species of nitrogen examined, converse patterns in concentrations were observed along the four Gwydir River sites, with nitrogen oxide concentrations low at GR1 and GR4 and high at GR2 and GR3 and the reverse trend being observed for ammonium (Figure 2).

Ovens River

In the Ovens River, concentrations of DOC and most forms of nutrients were substantially lower than those measured in the other two rivers (Figure 2).

Ammonium concentrations were the exception, with consistently low values across all four sites, similar to the Logan River. There was a downstream trend of decreasing concentrations of nitrogen oxides and FRP, with highest values in OR1 and lowest in OR4. Total nitrogen and total phosphorus were lowest in upstream sites (OR1 and OR2), with highest concentrations for both of these analytes recorded in OR4. The Ovens River had the lowest levels of DOC of the three rivers studied, with the three upper sites having concentrations below 2 mg L^{-1} . Similar to the Logan River, DOC increased with distance downstream.

Chlorophyll-a concentrations

Water column chlorophyll-*a* concentrations revealed that all three river systems were similarly productive, with mean chlorophyll-*a* concentrations as high as $20 \text{ } \mu\text{g L}^{-1}$ in the Logan River, $15 \text{ } \mu\text{g L}^{-1}$ in the Gwydir River and $6 \text{ } \mu\text{g L}^{-1}$ in the Ovens River (Figure 3A). In all three rivers there was a trend of increasing water column algal biomass with distance downstream from the headwaters, although the highest chlorophyll-*a* concentrations in the Logan River were observed at LR3. The Ovens River had the lowest water column chlorophyll-*a* concentrations, with only the concentrations from the most downstream site (OR4) falling within the range of concentrations measured in the other two rivers (Figure 3A).

Benthic chlorophyll-*a* concentrations tended to be highest at upstream sites in the Logan and Gwydir Rivers (Figure 3B), where depth and turbidity were generally low (Table 1, Table 2). Cobble chlorophyll-*a* concentrations were typically much higher and more variable than those observed for the sediment core samples, with concentrations in excess of $300 \text{ } \mu\text{g L}^{-1}$ recorded from LR1. In contrast, sediment core chlorophyll-*a* concentrations tended to be relatively constant across sites, with most values falling within the range of $15\text{-}30 \text{ } \mu\text{g L}^{-1}$.

Enzymes

Within-river patterns in enzyme activity differed among the three rivers. The ordination of enzyme activities showed that sites LR1, LR3 and LR4 in the Logan River were different from one another (Figure 4A). However, replicates at LR2 were highly variable, and therefore did not form a discrete cluster in the ordination. This result contrasted with that from the Gwydir River, where samples from GR1 and GR4 clustered together, and samples from GR2 and GR3 clustered together, with the two individual clusters occurring as quite distinct groupings (Figure 4B). Enzyme activities were different at each site of the Ovens River (Figure 4C). When enzyme activities were examined from all sites as a global dataset, the Gwydir River showed some differences from both of the other rivers (Figure 5, pairwise comparison with Ovens River, $R = 0.353$, $p = 0.001$; pairwise comparison with Logan River $R = 0.115$, $p = 0.045$). In this global dataset, the enzyme activities measured in the Logan and Ovens Rivers were not different from one another (Figure 5).

The combined esterase, phosphatase, amino peptidase and alpha-glucosidase activities explained 85.4% of the Logan River ordination. The individual contributions of esterase, phosphatase, amino peptidase and alpha-glucosidase activities were 33.8, 24.9, 14.9 and 11.8% respectively. Three enzymes explained 92.7% of the ordination in the Gwydir River, with esterase, amino peptidase and phosphatase activities contributing 48.5, 31.1 and 13.1% respectively. The patterns of enzyme contributions in the Ovens River were very similar to those observed in the Logan River. The individual contributions of esterase, phosphatase, amino peptidase and alpha-glucosidase activities were 30.1, 22.6, 20.1 and 14.8% respectively, which combined, explained 87.6% of the ordination.

Bioassays - DOC and inorganic nutrient addition

Across all sites and all rivers, DO concentrations were significantly lower in treatments that received DOC as leaf leachate compared to those that didn't. Similarly, all sites showed utilisation of the added DOC with reduction in DOC concentrations over the 48 h incubation. Overall, inorganic nutrients had little effect on DO or DOC utilisation, but the response varied among sites and rivers.

Logan River

There were reductions in DO between the initial and control for all sites (Figure 6), and the difference was significant ($p < 0.05$) at all sites except LR1. DOC addition resulted in a significant ($p < 0.05$) reduction in DO concentration, relative to that in controls, for all sites. LR3 and LR4 showed the greatest response to added DOC, and for these sites it is likely that all the DO was used before the completion of the experiment. LR1 and LR2 showed a smaller response to added DOC, with 0.5 to 1.5 mg/L of DO remaining after 48 h (Figure 6). DOC addition in combination with inorganic nutrients led to complete utilisation of DO at all sites, and concentrations were significantly lower than those measured in controls ($p < 0.05$). Inorganic nutrients alone did not decrease DO greatly, although LR2 was significantly lower than the control. DOC utilisation data revealed that for all sites, the control treatment did not significantly change ($p < 0.05$) in DOC concentration from the nutrient and initial concentration over the 48 h of the experiment ($p < 0.05$) (Figure 7). Treatments with added DOC, both with and without inorganic nutrients, had significantly ($p < 0.05$) lower DOC concentrations at the end of the incubation compared to the initial + DOC concentrations at all sites, indicating utilisation of DOC. The addition of inorganic nutrients along with DOC appeared to lead to a greater reduction in DOC concentration for all sites, with DOC + nutrients treatments significantly lower than both the DOC and initial + DOC treatments ($p < 0.05$) (Figure 7).

Gwydir River

Over the course of the bioassay experiment, the Mehi River site (GR4) was the only site to show a reduction in DO concentrations relative to the initial concentration in the controls, although the difference was not significant ($p > 0.05$) (Figure 6). Addition of DOC alone resulted in a significant ($p < 0.05$) reduction in DO concentration for all sites. GR2 and GR3, which are downstream of Copeton Dam, showed the greatest response to added DOC, with all DO utilised (Figure 6). DOC addition with inorganic nutrients led to all DO being utilised at all sites. The DO concentrations in the nutrients alone treatment was significantly lower than the control for all sites except GR4. DOC utilisation data revealed that for all sites the control treatment did not

significantly change ($p < 0.05$) in DOC concentration from the initial concentration or from the inorganic nutrients treatment (Figure 7). The DOC and the DOC + nutrients treatments had significantly ($p < 0.05$) reduced DOC concentrations from that of initial + DOC at all sites. Addition of DOC with inorganic nutrients led to a further significant ($p < 0.05$) reduction in DOC concentration from the DOC treatment at all sites except GR3.

Ovens River

Due to site access difficulties associated with wildfires, only two sites were able to be sampled for this experiment, namely OR3 (Bowmans) and OR4 (Peechelba). A significant ($p < 0.05$) increase in DO was witnessed in the control from the initial concentrations at both sites (Figure 6). The addition of DOC resulted in less of a drop in DO concentrations in the two Ovens River sites than for the other rivers (approximately 1 mg L^{-1}), but the DOC treatments were significantly lower than the controls ($p < 0.05$). DOC added with inorganic nutrients led to a greater decrease in DO than in the DOC alone treatment, and concentrations were significantly lower than those measured in the controls ($p < 0.05$). Inorganic nutrient alone treatments were not significantly different from the control. DOC utilisation data was limited as only duplicate or single values were recorded. However, the data still showed that the control treatment did not change in DOC concentration from initial over the 48 h of the experiment for OR3, but did slightly decrease for OR4 (Figure 7). Treatments with DOC added showed reductions in DOC concentrations relative to the initial. Addition of DOC with inorganic nutrients led to a greater reduction in DOC concentration from that of DOC addition alone at both OR3 and OR4. Inorganic nutrients alone did not lead to a difference in DOC concentration.

Food Webs

Stable isotope signatures

Mean riparian vegetation $\delta^{13}\text{C}$ signatures for all three rivers were around -29.6‰ (-29.58 ± 0.36 for the Logan River, -29.56 ± 0.25 for the Gwydir River and -29.67 ± 0.34 for the Ovens River) and showed very little variability among sites within rivers. In contrast, algal $\delta^{13}\text{C}$ signatures were variable both within and between sites. Site mean

algal $\delta^{13}\text{C}$ signatures ranged from -18.68‰ (± 0.07) to -31.45‰ (± 0.89) in the Logan River, -22.04‰ (± 0.98) to -30.05‰ (± 0.40) in the Gwydir River and -15.41‰ (± 0.20) to -28.56‰ (± 0.00) in the Ovens River.

Nitrogen stable isotope signatures were low in most sites across all three rivers, with the notable exceptions being sites LR3 and LR4 on the Logan River. At these sites, both source and consumer $\delta^{15}\text{N}$ signatures were substantially ^{15}N -enriched relative to those at the upstream sites. Source and consumer signatures had mean $\delta^{15}\text{N}$ signatures of 11.09‰ (± 2.34) and 17.90‰ (± 0.92) respectively, at LR3, and 11.63‰ (± 2.89) and 16.08‰ (± 1.34), respectively, at LR4.

Mixing model analyses of food webs

Food web analyses conducted across all sites in all three river systems revealed that consumers had a strong reliance on algal sources of carbon (Table 3). In the Logan River, autochthonous sources contributed between 78% (LR2) and 98% (LR3) of the carbon in consumer tissues. For sites on the Gwydir River, this range was from 45% (GR3), where a different mixing model was used, to 88% (GR2) and for the Ovens River, autochthonous sources contributed from 60% (OR3) to 85% (OR1).

Our data revealed that even in the upstream sites, all of which tended to be reasonably well shaded (Table 1), algal sources contributed between 81% and 97% of the carbon in consumer tissues across the three catchments. In sites further downstream, the degree to which autochthonous sources of carbon contributed to consumer diets was more variable, but was still largely indicative of a dominance of autochthonous carbon (Table 3). The highest average riparian zone contribution of carbon was 55% at GR3, but this result was from one taxon only (Ephemeroptera) and was determined using a different mixing model to the rest of the analyses (see Methods). Excluding this result, contributions of riparian zone carbon were highest at OR3 (40%). At the remaining ten sites, riparian contributions were all less than 25% (Table 3), further suggesting that in-stream sources of carbon were the most important food resources for consumers at the time of sampling.

Ordination integrating all measures across all sites

Results of the multi-dimensional scaling (MDS) ordination of all common data generated in this study revealed two distinct groups in multidimensional space, with sites from the upper Logan (LR1 and LR2) clustering with sites from the Ovens River and sites from the lower Logan (LR3 and LR4) clustering with sites from the Gwydir River (Figure 8). This pattern held true in both ordinations (with and without the bioassay data and data from sites OR1 and OR2, respectively), suggesting that the bioassay results did not strongly influence the observed groupings from the ordination. This supposition was further supported by the BIOENV output, which, for the ordination without bioassay data (Figure 8A), identified phosphatase, TN and FRP as the three most influential input variables. Together, these variables explained 94% of the variation in the data. For the ordination without data from sites OR1 and OR2, the BIOENV output indicated that leucine amino peptidase, FRP and DOC explained more than 92% of the variation in the dataset. These results indicate that nutrient concentrations and enzyme activities separate the sites in multidimensional space, with the Gwydir and lower Logan River (LR3 and LR4) sites being characterised by higher nutrient concentrations and associated enzymatic activities than the upper Logan (LR1 and LR2) and Ovens River sites.

Discussion

Comparisons across catchments

Water chemistry and ambient nutrient, DOC and chlorophyll-a concentrations

In each catchment, there were interesting longitudinal trends in nutrient concentrations that reflect both in-stream processing and diffuse and point source nutrient inputs. In the Logan River, the overwhelming finding in terms of nutrient concentrations, was the extremely high concentrations of phosphorus and nitrogen at LR3. Coupled with the stable isotope data which show extreme ^{15}N -enrichment of all food web components at this site, these high nutrient concentrations are indicative of a ^{15}N -enriched point source input (*sensu* Hadwen and Arthington, 2007) immediately upstream of this site. Whilst there are no sewage treatment plants in the area, enriched

effluent may be derived from the large number of chicken farms and dairies in the region.

In the Gwydir River, the operational procedures of Copeton Dam releases clearly have a strong influence on ambient nutrient concentrations (and DOC – see below). Sites GR2 and GR3, which are downstream of the dam, had nutrient concentrations that were either lower (ammonium) or higher (nitrogen oxides) than GR1 and GR4, suggesting that the type and concentration of nutrients in released water are different from those in the unregulated reaches. Furthermore, the physicochemical conditions at GR2 and GR3 also suggest that Copeton Dam has both an immediate local and a downstream effect on the middle reaches of the Gwydir River. Specifically, the hypolimnetic off-take strategy led to substantial reductions in water temperature, EC and pH at GR2. Whilst most of these measures had risen by GR3, the longitudinal effect of this reservoir was still evidenced in the nutrient data. Similar changes in downstream nutrients and physicochemical characteristics in response to river regulation, and reservoirs in particular, have been reported in Australia and overseas (Harris, 2001; Kelly, 2001; Davis and Koop, 2006).

Nutrient data from the Ovens River indicated that the relatively less impacted condition of the lower catchment, especially around the protected River Red Gum floodplain and riparian forest at OR4, might explain the nitrogen and phosphorus species concentrations at this site. Specifically, inorganic forms of nitrogen (nitrogen oxides) and phosphorus (FRP) were observed to have the lowest concentrations at OR4, and there was a general trend of decreasing concentrations with distance downstream from OR1. In contrast to this pattern, the totals (TN and TP) tended to increase along this river system, suggesting that inorganic forms contribute less to TN and TP concentrations in the lower reaches of the Ovens River. The increasing importance of organic forms with distance downstream is also supported by the increasing DOC concentrations. These trends in total and dissolved inorganic nutrient concentrations along the longitudinal gradient in this largely unregulated river system deserve further research attention to elucidate the relationships between nutrient species and the bioavailability of dissolved organic forms.

Across all twelve sites, the ambient DOC concentrations during the course of this study fell within the range found in most lotic systems (e.g. Mulholland, 2003). The Ovens River upper sites were at the lower end of this range, with concentrations around 0.5 mg l⁻¹. Increasing DOC concentrations with distance downstream for the Logan and Ovens rivers may be a result of a build-up of refractory DOC originating from upstream sources and processes as described in the RCC (Vannote *et al.*, 1980). Increases in DOC may also result from local site specific inputs (i.e., direct riparian inputs (increased riparian cover, Table 1) or agricultural, industrial and urban inputs and instream production) as suggested by the riverine productivity model (RPM) of Thorp and DeLong (1994). This latter explanation is further supported by evidence of reasonably fast utilisation (in days) of DOC by planktonic-bacteria reported in inland rivers in Australia (Robertson *et al.*, 1999) which indicates that local reach inputs represent the main sources of bioavailable carbon detected at downstream sites in this study. Furthermore, the rapid use of some, but not all, of the added leaf leachate DOC and the fact that there was no detectable decreases in DOC in treatments that did not receive leachate in the bioassays (see below), suggest that much of the ambient DOC measured at the study sites at the time of this study was not labile.

The Gwydir River showed a different pattern of DOC concentrations, with the two sites immediately downstream of Copeton Dam being lower in DOC than GR1 and GR4. This suggests that processes in the reservoir may reduce DOC delivery to downstream sites. Similar impoundment and flow regulation findings have been reported elsewhere for DOC (Davis and Koop, 2006).

Water column productivity, as indicated by chlorophyll-*a* concentrations, was high in sites from the Logan and Gwydir Rivers and considerably lower in sites from the Ovens River. However, a downstream trend of increasing chlorophyll-*a* concentrations was observed for all three rivers. This finding is consistent with predictions of water column productivity in riverine systems, with water column productivity and biomass highest in the lower sections of rivers than in the more upstream reaches (Thorp and DeLong, 1994; Gawne *et al.*, 2007).

Enzymes

Although the activities of extracellular enzymes varied across the three rivers, enzyme activities also showed a reasonable capacity to demonstrate differences between sites within a river. For example, although aminopeptidase activities have previously been reported to increase with distance downstream (Admiraal and Tubbing, 1991; Ainsworth and Goulder, 1998; Ainsworth and Goulder, 2000), we also found that site-specific factors appeared to be more important than site location (e.g. slope or plain) in determining the overall differences in microbial activity levels at the time of our study.

When considering overall trends in microbial extracellular enzyme activities, the Logan and Ovens rivers were not significantly different from each other, but the Gwydir river did have responses that were different from the other two rivers. These differences were likely due to the different forms and availability of carbon in these different systems. For example, the activities of enzymes decomposing carbohydrates and those involved in obtaining phosphate were very low in the Gwydir River. In contrast, these groups of enzymes were highly active in the Logan and Ovens Rivers. These data suggest that the Logan and Ovens Rivers probably receive more external carbon inputs and have more concomitant leaf processing than does the Gwydir River. Whilst it is difficult to separate the natural and impoundment-related mechanisms behind these differences, the greater distances between sites on the Gwydir River (relative to those in the Logan and Ovens Rivers), the presence of a large reservoir and generally higher nitrogen and DOC loads may have contributed to these observed differences in microbial responses. Indeed, the significant influence of the releases from Copeton Dam are evident not only in the data for extracellular enzyme activities, but also the nutrient and DOC concentrations and the bioassay responses at sites GR2 and GR3.

Bioassays - DOC and inorganic nutrient addition

All sites in all rivers showed evidence of DOC limited respiration of the heterotrophic bacterial community, as evidenced by both the DO and DOC usage data at the

conclusion of the bioassay experiments, despite the variation in background DOC levels (Figure 4). Indeed, the greatest DOC limitation was in the Logan and Gwydir River sites, whose ambient DOC concentrations were much higher than those in the Ovens River. The only river to show any significant oxygen consumption from the initial population, without addition of DOC, was the Logan River at LR2, LR3 and LR4. Whilst this trend was not revealed in the DOC utilisation data, it is likely that some DOC from these sites was still available to the heterotrophic community at the beginning of the bioassay.

For treatments where inorganic nutrients (N and P) were added in combination with the leaf leachate DOC, a significant increase in DO usage and DOC utilisation (relative to the treatment where DOC was added only) was generally measured. To this end, inorganic nutrients co-limit heterotrophic productivity when DOC is supplied. This result was most pronounced for the Ovens River, and this may be related to the low ambient inorganic nutrient concentrations in this system. Further support for the critical limiting role of DOC lies with the fact that the addition of inorganic nutrients alone did not generally increase activity of the heterotrophic community, although in some sites of the Logan and Gwydir Rivers, a small but significant ($p < 0.05$) reduction in DO occurred.

A similar degree of DOC limitation of heterotrophic production has also been observed for the Namoi River and Hunter Estuary (Mitrovic and Westhorpe, unpublished data) in New South Wales. It is likely that many Australian rivers are DOC limited, with the heterotrophic community particularly limited by DOC at times when flows are low and DOC supply is reduced (Robertson *et al.*, 1999). DOC delivery during floods and freshes is therefore likely to be important in providing DOC to the heterotrophic community, especially in the lower parts of rivers. DOC concentration is likely to influence the bottom of the food chain, such as the heterotrophic plankton community, including bacteria, flagellates, ciliates which can utilise both inorganic and organic nutrients (Kaplan and Newbold, 1993). At the time of this study, the data suggests that the metazoan food webs of the study rivers were largely driven by autochthonous carbon sources. Our bioassay results suggest that autochthonous production may also be important to the microbial community, since it

appears that the ambient supply of DOC at the time of initiation of the experiment was not adequate to support substantial heterotrophic growth over the short duration (48 h) of the bioassay experiments.

Food Webs

The strong reliance of consumers on algal sources of carbon in all but one of the 12 sites sampled in this study supports our prediction of the dominance of algal carbon at the base of the food web in Australian rivers, but runs counter to the predictions of the River Continuum Concept (RCC) of Vannote *et al.*, (1980). Whilst the RCC suggests that riparian sources of carbon should be particularly important drivers of processes in heavily shaded headwater streams, our results suggest that even in these well shaded sites, benthic algae is the dominant source of organic matter fuelling the metazoan food web.

Our benthic chlorophyll-*a* and stable isotope analyses suggest that the food webs at the Logan, Gwydir and Ovens sites tend to behave in accordance with predictions from the RPM of Thorp and DeLong (1994). While the RPM was principally developed to conceptualise food web functioning in the mid- and lower- reaches of large rivers, our findings suggest that in-stream producers also tend to support consumers in headwater streams in many lotic ecosystems. Indeed, similar findings have been reported for heavily shaded rainforest streams in subtropical Brazil (Brito *et al.*, 2006) and sub-tropical and tropical Australia (Douglas *et al.*, 2005; Spears *et al.*, submitted). As Williams (1988) suggested, some of the conceptual models of riverine function developed in cool temperate northern hemisphere stream ecosystems may not be directly applicable to Australian aquatic environments.

As noted earlier, Robertson *et al.*, (1999) indicated that catchment land use and flow modifications have led to an ‘unnatural’ dominance of algae in inland waterways in Australia. In our study, although algal contributions to consumers reflected their dominance it should be noted that the Ovens River is largely unmodified in terms of flow and that it represents the least-impacted sub-basin in the Murray-Darling Basin. Similarly, recent studies of autochthonous carbon contributions to food webs in sub-

tropical and tropical rivers and streams has revealed that even in relatively pristine systems, benthic algal sources of carbon represent the dominant contribution of carbon to all consumer organisms (Brito *et al.*, 2006; Douglas *et al.*, 2005; Spears *et al.*, submitted). To this end, we suggest that the importance of algal carbon to metazoan food webs does not simply relate to the impaired nature of sites and is instead a generalisable feature of Australian river systems.

General trends in river functioning

The global ordination analyses suggested that for the sites examined in this study, and under the low flow conditions experienced in all three drought-affected catchments, carbon processes are more similar among sites within a river, and among rivers, than are ambient nutrient and DOC concentrations. In other words, although nutrient concentrations were highly variable, largely due to the impacts of the dam on the Gwydir River and an unidentified ^{15}N -enriched nutrient source in the lower Logan River, nutrient and DOC concentrations do not appear to alter the way that carbon is processed in these three river systems. This suggestion is also supported by the bioassay data, in which heterotrophic respiration was found to be limited by organic carbon at all 10 sites, irrespective of the ambient nutrient and DOC concentrations. Furthermore, despite differences in the biomass of algae (especially benthic algae) across the 12 sites, the results of the food web analyses suggest that benthic algae is the dominant source of carbon fuelling riverine food webs at the study sites, even those with low nutrient concentrations and low light conditions, whose food webs might have otherwise been predicted to be driven more strongly by allochthonous sources of carbon (*sensu* Vannote *et al.*, 1980). Together, these findings suggest that at the time of this study and across these three different river systems, pathways and mechanisms of carbon processing were apparently not influenced by changes in the forms and bioavailability of nutrients and DOC.

The implications of the findings of this study for river and catchment managers revolve around the fact that although both local and catchment-wide processes can influence the source, type and quantities of nutrients and carbon reaching riverine environments, the way Australian rivers process these elements may result in a high

level of consistency in the functioning of heterotrophic microbial communities and metazoan food webs. However, because this study was conducted under low flow conditions and eastern Australia has been in a severe period of drought for the last few years (Bond *et al.*, 2008), more work will be required during periods of non-drought to determine the generalisability of the findings presented here. Specifically, studies during periods of flow should aim to assess the consistency of the carbon processing trends observed in this study and to determine whether rivers as diverse as the Logan, Gwydir and Ovens follow different temporal trajectories given that flows tend to occur in different seasons in southern (winter and spring flows) and northern (summer flows) Australia.

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References

- Admiraal W, Tubbing GMJ. 1991. Extracellular enzyme activity associated with suspended matter in the River Rhine. *Freshwater Biology* **26**: 507-517.
- Ainsworth AM, Goulder R. 1989. Microbial organic-nitrogen transformations along the Swale-Ouse river system, Northern England. *The Science of the Total Environment* **210/211**: 329-355.
- Ainsworth AM, Goulder R. 2000. Downstream change in leucine amino peptidase activity and leucine assimilation by epilithic microbiota along the River Swale, northern England. *The Science of the Total Environment* **251/252**: 191-204.
- American Public Health Association (1998) *Standard methods for the examination of water and waste water*. American Public Health Association (APHA), Washington DC.

- Biggs TW, Dunne T, Martinelli LA. 2004. Natural controls and human impacts on stream nutrient concentrations in a deforested region of the Brazilian Amazon basin. *Biogeochemistry* **68**: 227-257.
- Bond NR, Lake PS, Arthington AH. 2008. The impacts of drought on freshwater ecosystems: an Australian perspective. *Hydrobiologia* **600**: 3-16.
- Boon PI, Bunn SE. 1994. Variations in the stable isotope composition of aquatic plants and their implications for food web analysis. *Aquatic Botany* **48**: 99-108.
- Brito EF, Moulton TP, De Souza ML, Bunn SE. 2006. Stable isotope analysis indicates microalgae as the predominant food source of fauna in a coastal forest stream, south-east Brazil. *Austral Ecology* **31**: 623-633.
- Bunn SE, Boon PI. 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. *Oecologia* **96**: 85-94.
- Bunn SE, Longeran NR, Kempster MA. 1995. Effects of acid washing on stable isotope ratios of C and N in penaeid shrimp and seagrass: Implications for food-web studies using multiple stable isotopes. *Limnology and Oceanography* **40**: 622-625.
- Bunn SE, Arthington AH. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management* **30**: 492-507.
- Bunn SE, Davies PM, Winning MA. 2003. Sources of organic carbon supporting the food web of an arid zone floodplain river. *Freshwater Biology* **48**: 619-635.
- Clark MJ, Cresser MS, Smart R, Chapman PJ, Edwards AC. 2004. The influence of catchment characteristics on the seasonality of carbon and nitrogen species concentrations in upland rivers of Northern Scotland. *Biogeochemistry* **68**: 1-19.
- Clarke KR, Warwick RM. 2001. *Change in marine communities: an approach to statistical analysis and interpretation*. UK, Primer-E Ltd.
- Connolly RM, Hindell JS, Gorman D. 2005. Seagrass and epiphytic algae support nutrition of a fisheries species, *Sillago schomburgkii*, in adjacent intertidal habitats. *Marine Ecology Progress Series* **286**: 69-76.
- Davis JR, Koop K. 2006. Eutrophication in Australian rivers, reservoirs and estuaries - a southern hemisphere perspective on the science and its implications. *Hydrobiologia* **559**: 23-76.

- Department of Natural Resources and Environment (1991) Protecting rivers and streams of high conservation value. <http://www.dse.vic.gov.au> Website accessed 1 July 2008.
- De Rose R, Barrett D, Marks A, Caitcheon G, Chen Y, Simon D, Lymburner L, Douglas G, Palmer M. 2005. *Linking sediment dynamics, riparian vegetation, erosion and sediment transport in the Ovens River Basin*. Canberra, Australia, CSIRO Land and Water Client Report.
- Dodds WK, Oakes RM. 2006. Controls on Nutrients Across a Prairie Stream Watershed: Land Use and Riparian Cover Effects. *Environmental Management* **37**: 634-646.
- Douglas MM, Bunn SE, Davies PM. 2005. River and wetland food webs in Australia's wet-dry tropics: general principles and implications for management. *Marine and Freshwater Research* **56**: 329-342.
- Drewry JJ, Newham LTH, Greene RSB, Jakeman AJ, Croke BFW. 2006. A review of nitrogen and phosphorous export to waterways: context for catchment modelling. *Marine and Freshwater Research* **57**: 757-774.
- Findlay S, Sinsabaugh RL, Fischer DT, Franchini P. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. *Ecosystems* **1**: 227-239
- France RL. 1995. Stable isotopic survey of the role of macrophytes in the carbon flow of aquatic foodwebs. *Vegetatio* **124**: 67-72.
- Gawne B, Merrick C, Williams DG, Rees G, Oliver R, Bowen PM, 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.
- Grey J, Jones RI, Sleep D. 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnology and Oceanography*, **46(3)**: 505-513.
- Hadwen WL, Bunn SE. 2004. Tourists increase the contribution of autochthonous carbon to littoral zone food webs in oligotrophic dune lakes. *Marine and Freshwater Research* **55**: 701-708.

- Hadwen WL, Bunn SE. 2005. Food web responses to low-level nutrient and ^{15}N -tracer additions in the littoral zone of an oligotrophic dune lake. *Limnology and Oceanography* **50**: 1096-1105.
- Hadwen WL, Arthington AH. 2007. Food webs of two intermittently open estuaries receiving ^{15}N -enriched sewage effluent. *Estuarine, Coastal and Shelf Science* **71**: 347-358.
- Hadwen WL, Russell GL, Arthington AH. 2007. Gut content- and stable isotope-derived diets of four commercially and recreationally important fish species in two intermittently open estuaries. *Marine and Freshwater Research* **58**:363-375.
- Hamilton SK, Lewis WMJ, Sippel SJ. 1992. Energy sources for aquatic animals in the Orinoco River floodplain: evidence from stable isotopes. *Oecologia* **89**:324-330.
- Harris GP. 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.
- ISO 1994. *Water Quality – Measurement of biochemical parameters – Spectrophotometric determination of the chlorophyll-a concentration*. International Organisation for Standardization, Geneva, Switzerland.
- Jarvie HP, Haygarth PM, Neal C, Butler P, Smith B, Naden PS, Joynes A, Neal M, Wickham H, Armstrong L, Harman S, Palmer-Felgate EJ. 2008. Stream water chemistry and quality along an upland–lowland rural land-use continuum, south west England. *Journal of Hydrology* **350**: 215-231.
- Junk WJ, Bayley PB, Sparks RE. 1989 The flood pulse concept in river-floodplain systems. In *Proceedings of the International Large River Symposium*, vol. 106 (ed. D. P. Dodge), pp. 110-127: Canadian Special Publication in Fisheries and Aquatic Sciences.
- Kaplan LA, Newbold JD. 1993. Biogeochemistry of dissolved organic carbon entering streams. In: *Aquatic microbiology an ecological approach*. (ed. T.E. Ford). Pp. 139-165. Blackwell Scientific Publications, Oxford.
- Kelly VJ. 2001. Influence of reservoirs on solute transport: a regional-scale approach. *Hydrological Processes* **15**: 1227-1249.
- McCutchan JHJ, Lewis WMJ, Kendall C, McGrath CC. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. *Oikos* **102**: 378-390.

- Mulholland PJ. 2003. Large-scale patterns in dissolved organic carbon concentration, flux, and sources. In: *Aquatic ecosystems: interactivity of dissolved organic matter*. (eds. S.E.G. Findlay and R.L. Sinsabaugh) Pp 139-159. Academic Press, San Diego, California.
- Neal C, Jarvie HP, Love A, Neal M, Wickham H, Harman S. 2008. Water quality along a river continuum subject to point and diffuse sources. *Journal of Hydrology* **350**: 154-165.
- Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* **18**: 293-320.
- Phillips DL, Gregg JW. 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* **136**: 261-269.
- Rees GN, Bowen PM, Watson GO. 2005. Variability in benthic respiration in three southeastern Australian lowland rivers. *River Research and Applications* **21**: 1147-1156.
- Robertson AI, Bunn SE, Boon PI, Walker KF. 1999. Sources, sinks and transformations of organic carbon in Australian floodplain rivers. *Marine and Freshwater Research* **50**: 813-829.
- Smith SV, Swaney DP, Buddemeier RW, Scarsbrook MR, Weatherhead MA, Humborg C, Eriksson H, Hannerz F. 2005. River nutrient loads and catchment size. *Biogeochemistry* **75**: 83-107.
- Spears MD, Hadwen WL, Kennard M. (under review) Implications of variable algal $\delta^{13}\text{C}$ for interpretation of food web structure and function in streams of southeast Queensland. Submitted to *Marine and Freshwater Research*.
- Thorp JH, DeLong MD. 1994. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. *Oikos*. **70(2)**: 305-308.
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 130-137.

- Ward AK, Johnson MD. 1996. Heterotrophic microorganisms. In: *Methods in stream ecology*. (eds. F.R. Hauer & G.A. Lamberti) Pp 233-268. Academic Press, San Diego, California.
- Wetzel RG, Likens GE. 2000. Organic Matter. In: *Limnological analysis*. 3rd Edn (R.G. Wetzel & G.E. Likens). Pp137-146. Springer-Verlag New York.
- Williams MR, Melack JM. 1997. Solute export from forested and partially deforested catchments in the central Amazon. *Biogeochemistry* **38**: 67-102.
- Williams WD. 1988. Limnological imbalances: an antipodean viewpoint. *Freshwater Biology* **20**: 407-420.

Table 1. Site characteristics for the Logan, Gwydir and Ovens rivers.

Catchment & site		Latitude & longitude	Elevation (m)	Landform type	Riparian canopy (approx %) & dominant species	Upstream & adjacent land use	Dominant bed types	Max. wetted width (m)	Average depth (m)	Annual flows (m ³ s ⁻¹)		
										Min	Max	Mean
Logan (Qld)	LR1 Mt Barney Creek	152°44'33"E 28°14'14"S	178	Slope	90% <i>Casuarina & Callistemons</i>	Cattle / reserve (upstream)	Cobble	9	0.36	0.08	2.38	0.95
	LR2 Panitz property	152°49'33"E 28°12'57"S	104	Slope/plain	60% <i>Casuarina & Callistemons</i>	Cattle	Cobble / sandy	20.7	1.05	0.54	3.44	1.59
	LR3 Cedar Grove	152°57'35"E 27°50'47"S	25	Plain	25% <i>Callistemons</i>	Cattle	Sandy	13	0.2	0.67	14.5	4.72
	LR4 Maclean Bridge	153°00'41"E 27°47'07"S	21	Plain	80% <i>Callistemons</i>	Rural residential / recreational	Sandy	12.8	0.1	-	-	-
	GR1 Yarrowyck	151° 22' E, 30° 28' S	740	Tableland	45% River she oak (<i>Casuarina cunninghamii</i>)	Cattle / recreational	Bedrock; pebble, granules / sandy	15	0.5	0.06	4.97	1.39
Gwydir (NSW)	GR2 Pinegrove	150° 38' E, 29° 54' S	300	Slope	75% River redgum (<i>Eucalyptus camuldensis</i>)	Cattle / recreational	Cobble, pebbles / granules	65	0.9	4.38	26.79	13.75
	GR3 Gravesend	150° 22' E 29° 35' S	255	Slope	40% River redgums & willows (<i>Salix babylonica</i>)	Grazing cattle	Granules & fines	40	1.5	8.35	48.08	21.9
	GR4 Mehi@ Bronte	148° 54' E 29° 29' S	135	Plain	70% River redgums & wattles (<i>Acacia stenophylla</i>)	Irrigated & dryland farming / livestock	Fines / silt	10	1.8	0.49	6.58	2.08
	OR1 Trout farm	36° 51' S 147° 4' E	477	Slope	80%	Grazing and native forest	Boulder/ Cobble	8	0.3	1.07	4.06	2.33
Ovens (Vic)	OR2 Cherry walk	36° 43' S 146° 58' E	323	Slope	80%	Forestry/ grazing	Cobble	15	1.0	5.4	1.93	5.42
	OR3 Bowmans	36° 30' S 146° 36' E	208	Plain	30%	Agriculture	Cobble and pebble	30	0.5	7.52	7.52	27.63
	OR4 Peechelba	36° 9' S 146° 14' E	140	Plain	90% river redgum (<i>Eucalyptus camuldensis</i>)	State forest native vegetation	Clay silt	30	2.0	2.14	72.56	32.78

Riparian canopy % & composition based on average canopy cover and composition from the left and right banks and bars within the defined 100-m reach.

*Annual flow rates calculated from nearest gauging stations from sites

Table 2. Physicochemical characteristics of study sites in the Logan, Gwydir and Ovens Rivers. Values are mean (\pm SE where 3 replicate measurements were taken) . ND = no data available.

Catchment	Site	pH	EC (ms cm^{-1})	Turbidity (NTU)	Temperature ($^{\circ}\text{C}$)	Discharge (L s^{-1})	DO (mg L^{-1})
Logan River (Qld)	LR1	7.04 (0.01)	0.09 (0.00)	2.27	21.6 (0.06)	155	8.58 (0.03)
	LR2	6.80 (0.01)	0.19 (0.00)	7.51	24.6 (0.00)	No flow	3.51 (0.09)
	LR3	7.70 (0.00)	0.60 (0.00)	29.2	25.8 (0.03)	106	8.59 (0.01)
	LR4	7.73 (0.01)	0.66 (0.00)	17.8	21.3 (0.08)	104	8.29 (0.05)
Gwydir River (NSW)	GR1	8.35 (0.00)	0.34 (0.00)	3 (0.03)	23.3 (0.2)	0.22	8.80 (0.20)
	GR2	7.86 (0.01)	0.14 (0.00)	11	16.5 (0.02)	43171	8.85 (0.00)
	GR3	8.77 (0.00)	0.16 (0.00)	24	23.3 (0.00)	25000	9.18 (0.00)
	GR4	8.60 (0.00)	0.24 (0.00)	475.4 (0.5)	27.0 (0.00)	86.3	6.65 (0.00)
Ovens River (Vic)	OR1	7.4	0.028	ND	14.0	ND	ND
	OR2	7.3	0.036	ND	20.0	ND	ND
	OR3	7.6	0.04	ND	22.0	ND	ND
	OR4	7.2	0.146	ND	24.2	ND	ND

Table 3. Mean \pm SE percent contribution of autochthonous and allochthonous sources of carbon to metazoan consumers in food webs from Logan (LR), Gwydir (GR) and Ovens (OR) Rivers, as determined using the IsoSource stable isotope mixing model software of Phillips and Gregg (2003).

	Logan River		Gwydir River		Ovens River	
	LR1 Mean	LR1 SE	GR1 Mean	GR1 SE	OR1 Mean	OR1 SE
% autochthonous	0.97	0.00	0.81	0.16	0.85	0.03
% allochthonous	0.03	0.00	0.19	0.16	0.15	0.03
	LR2 Mean	LR2 SE	GR2 Mean	GR2 SE	OR2 Mean	OR2 SE
% autochthonous	0.78	0.07	0.88	0.04	0.81	0.04
% allochthonous	0.22	0.07	0.12	0.04	0.19	0.04
	LR3 Mean	LR3 SE	GR3* Mean	GR3* SE	OR3 Mean	OR3 SE
% autochthonous	0.98	0.00	0.45	0.00	0.60	0.11
% allochthonous	0.02	0.00	0.55	0.00	0.40	0.11
	LR4 Mean	LR4 SE	GR4 Mean	GR4 SE	OR4 Mean	OR4 SE
% autochthonous	0.92	0.02	0.86	0.05	0.83	0.08
% allochthonous	0.08	0.02	0.14	0.05	0.18	0.08

* GR3 calculations conducted using the two-source mixing model presented in Bunn and Boon (1993).

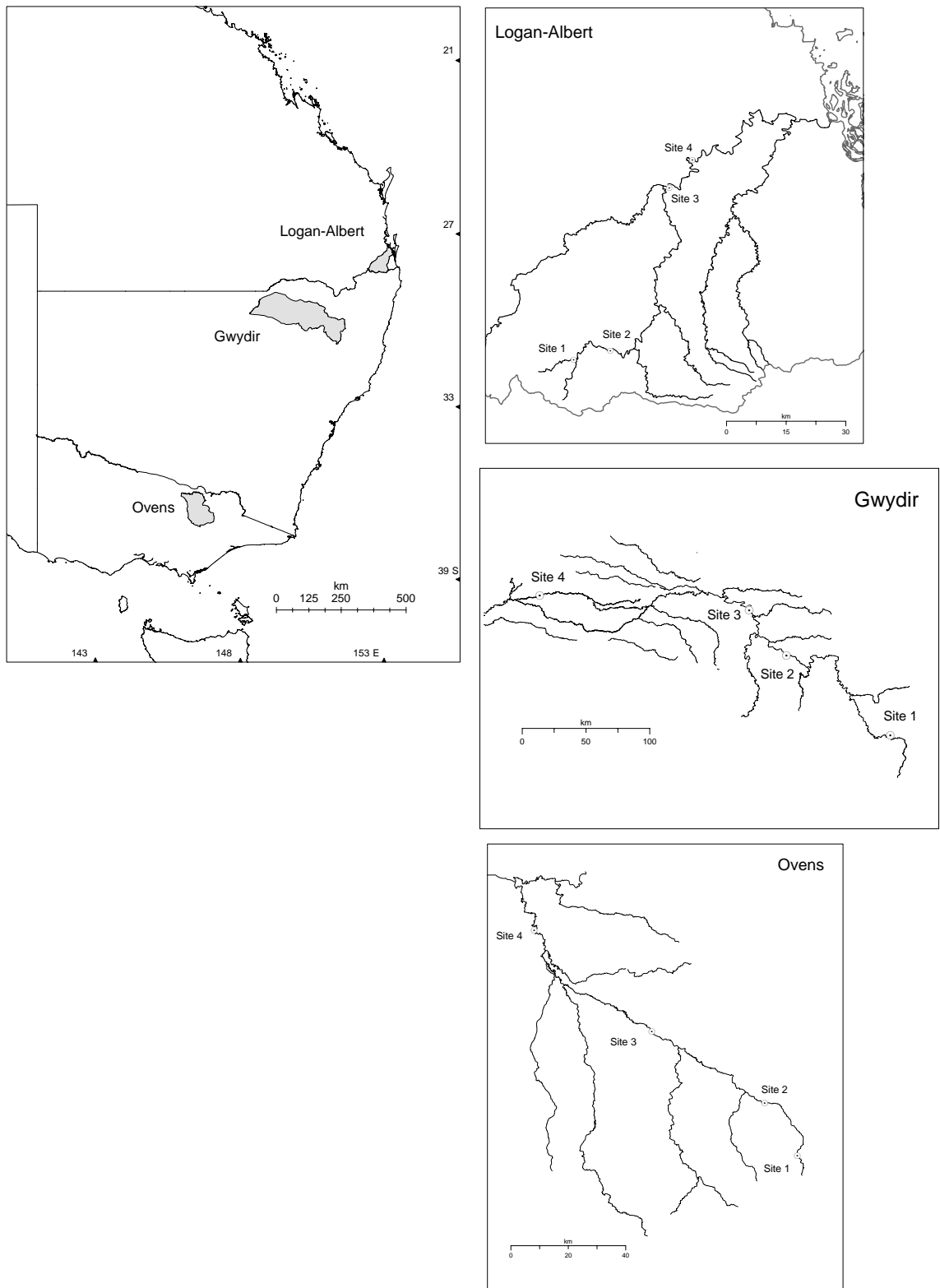


Figure 1. Map of eastern Australia and study area catchments. Insets show the location of the four sites sampled within each catchment.

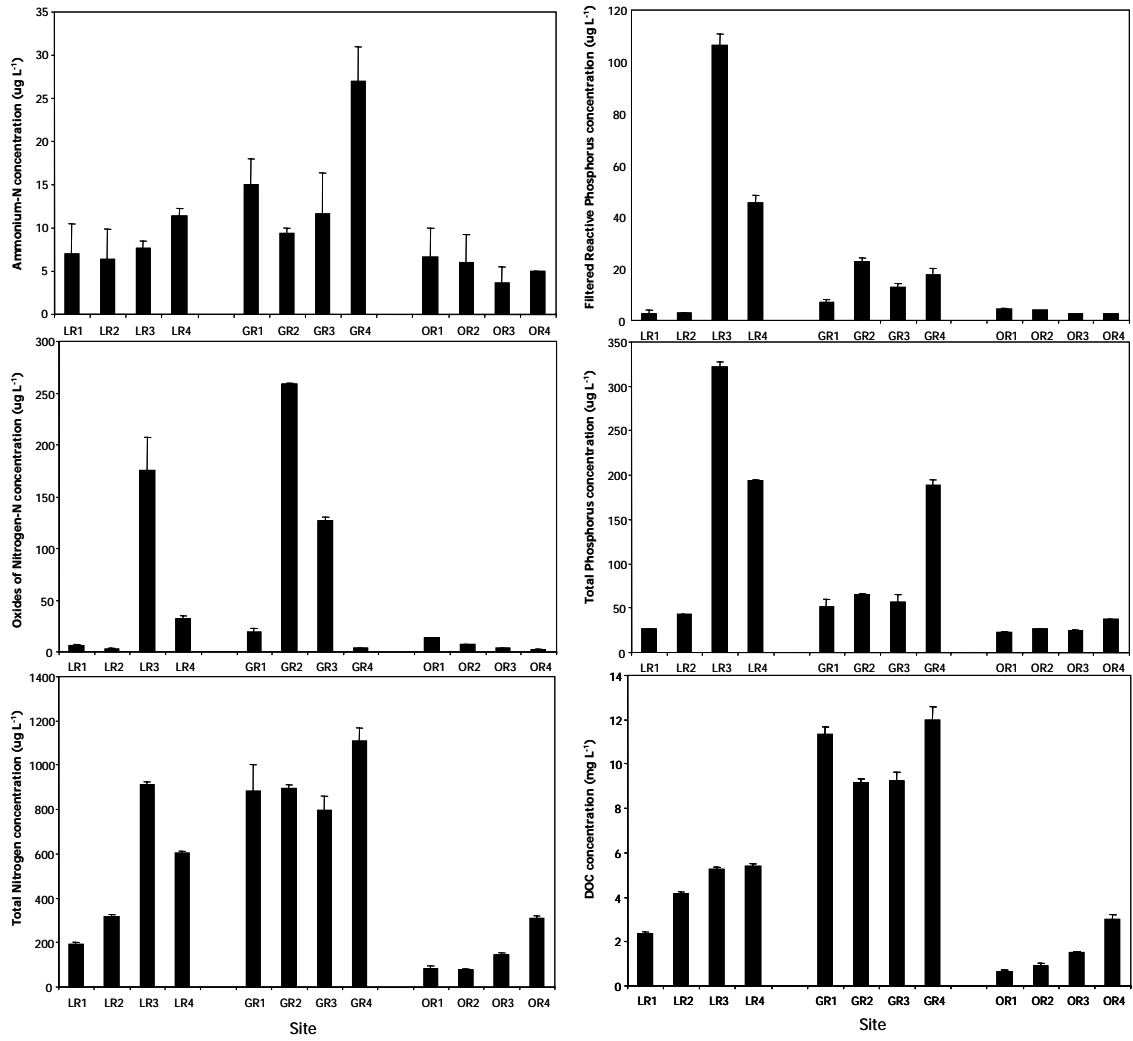


Figure 2. Mean (\pm SE) ambient nutrient and DOC concentrations from four sites in each of the Logan, Gwydir and Ovens Rivers between December 4 and December 8, 2006.

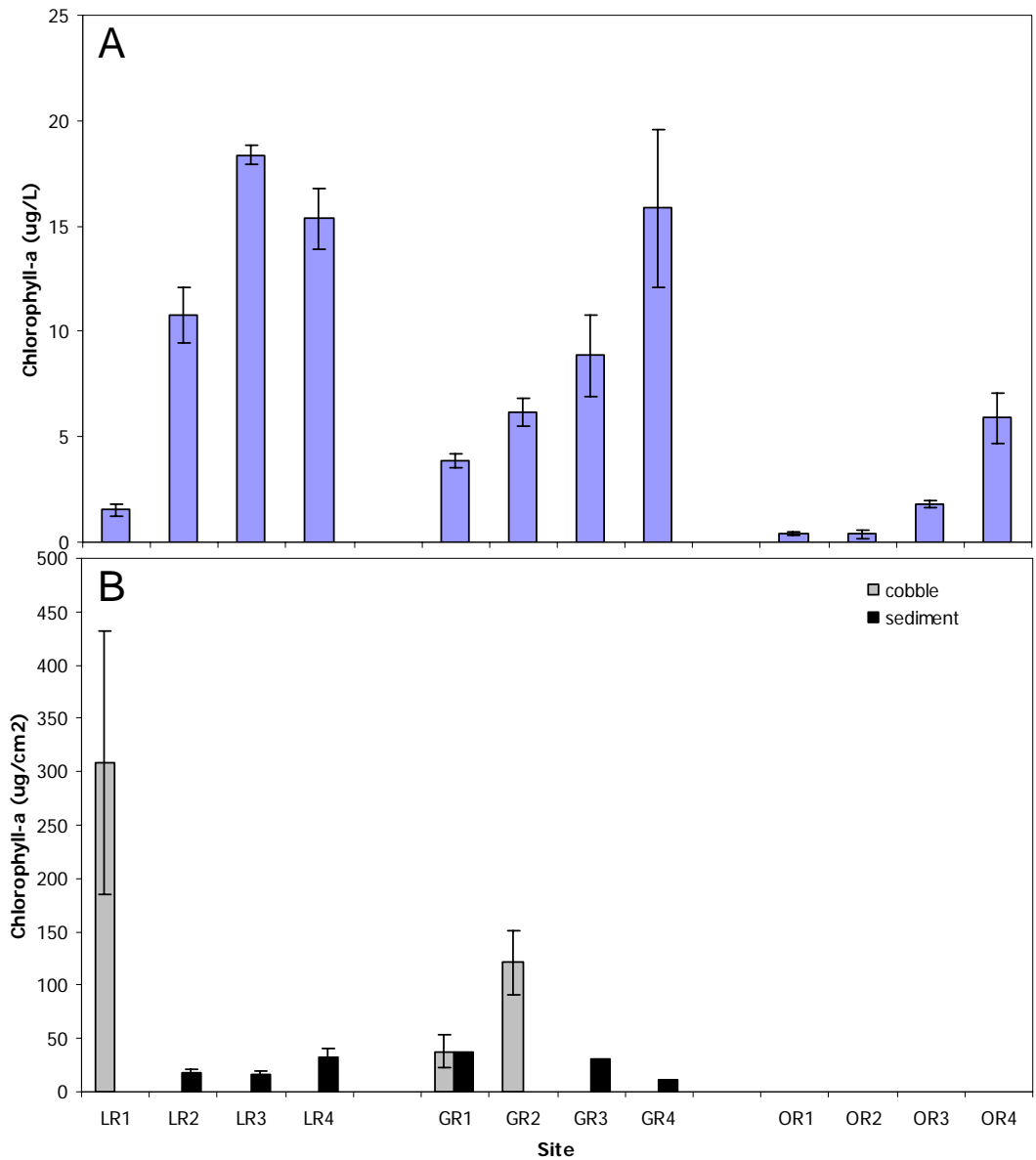


Figure 3. A) Water column, and B) cobble and sediment chlorophyll-*a* concentrations from four sites on the main stems of the Logan, Gwydir and Ovens Rivers between December 4 and December 8, 2006. NB. No data were available for cobble and sediment chlorophyll-*a* concentrations in the Ovens River.

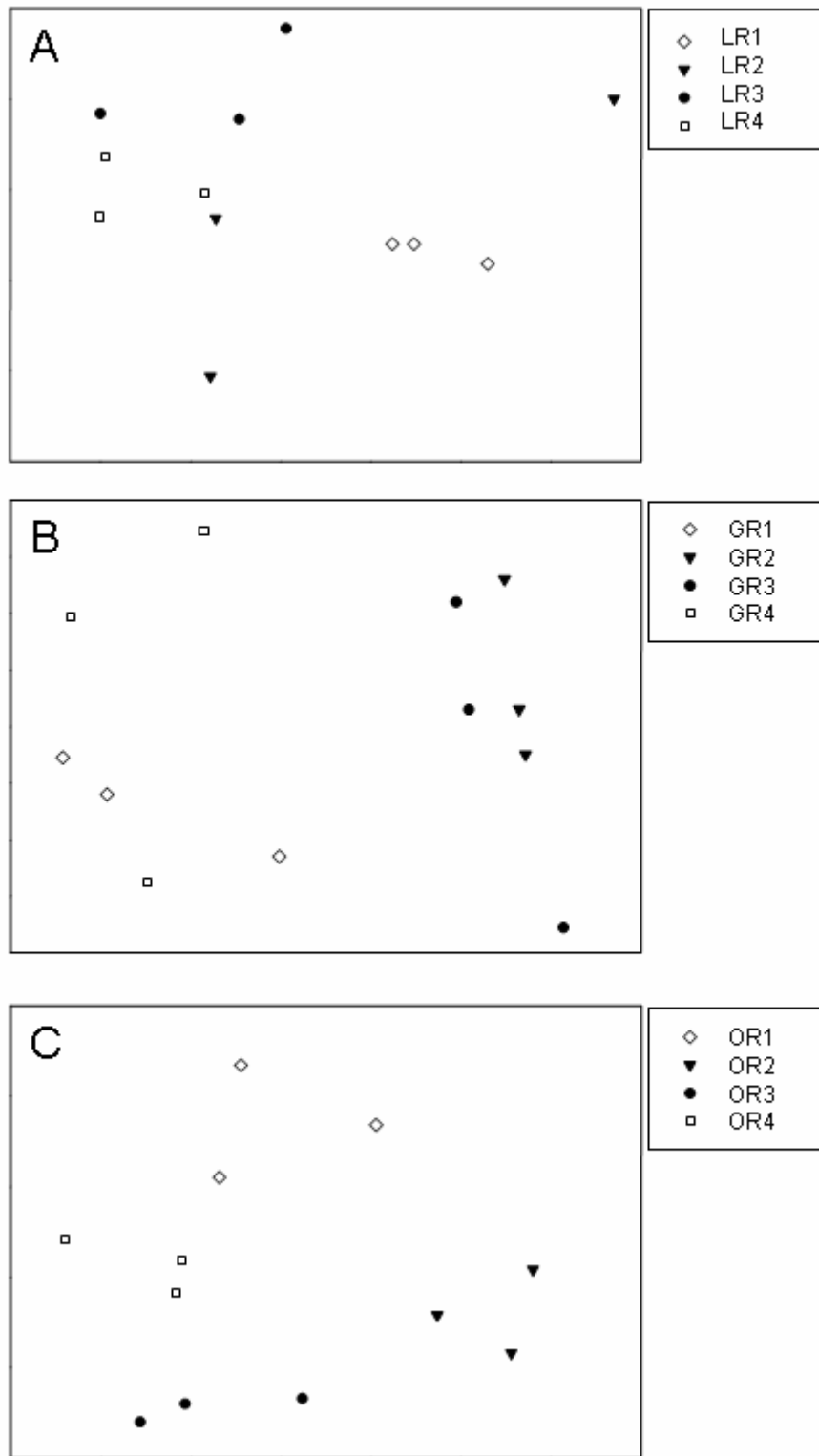


Figure 4. Ordination of extracellular enzyme activities at four sites along the main stems of the Logan (A), Gwydir (B) and Ovens (C) Rivers.

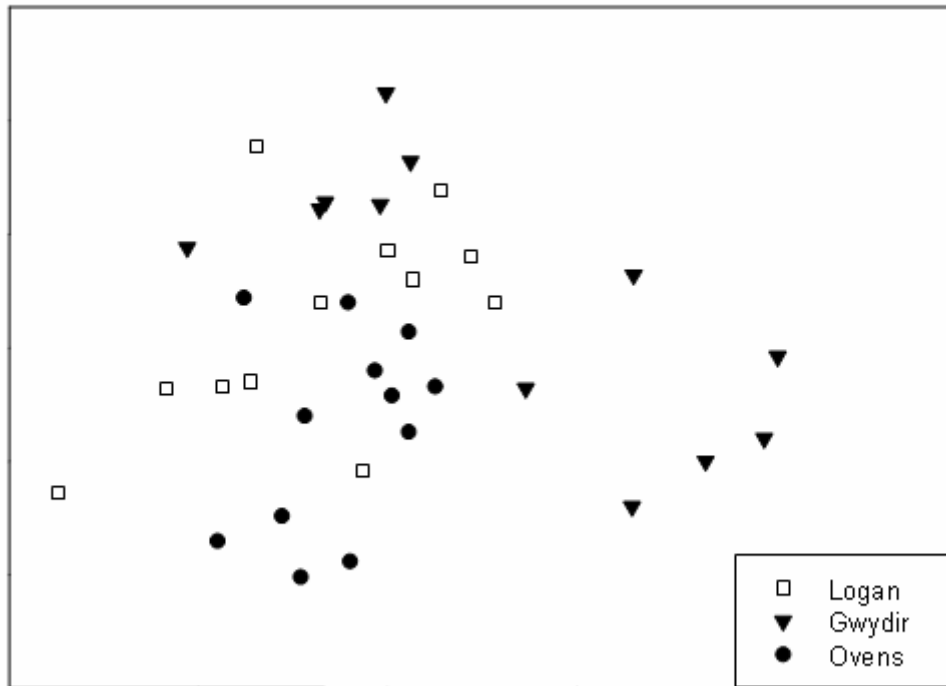


Figure 5. Ordination of extracellular enzyme activities at four sites along the main stems of the Logan, Gwydir and Ovens Rivers.

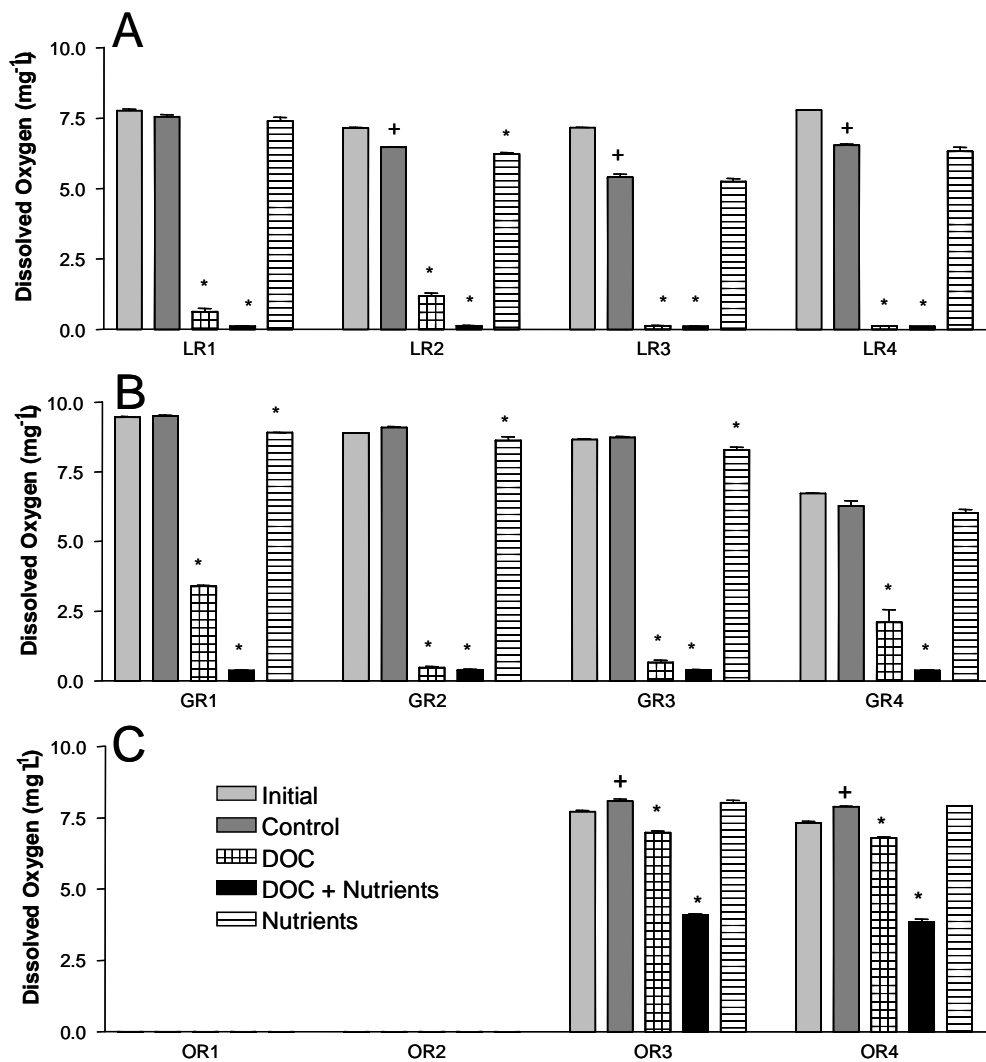


Figure 6. Dissolved oxygen concentrations from the DOC and nutrient addition bioassays conducted at four sites on the main stems of the A) Logan River, B) Gwydir River, and C) Ovens River. Initial concentrations are at the start of the incubation and other values are 48 h later. + indicates that the control is significantly different ($p < 0.05$) to the initial dissolved oxygen reading. * indicates that treatments were significantly different ($p < 0.05$) to the control.

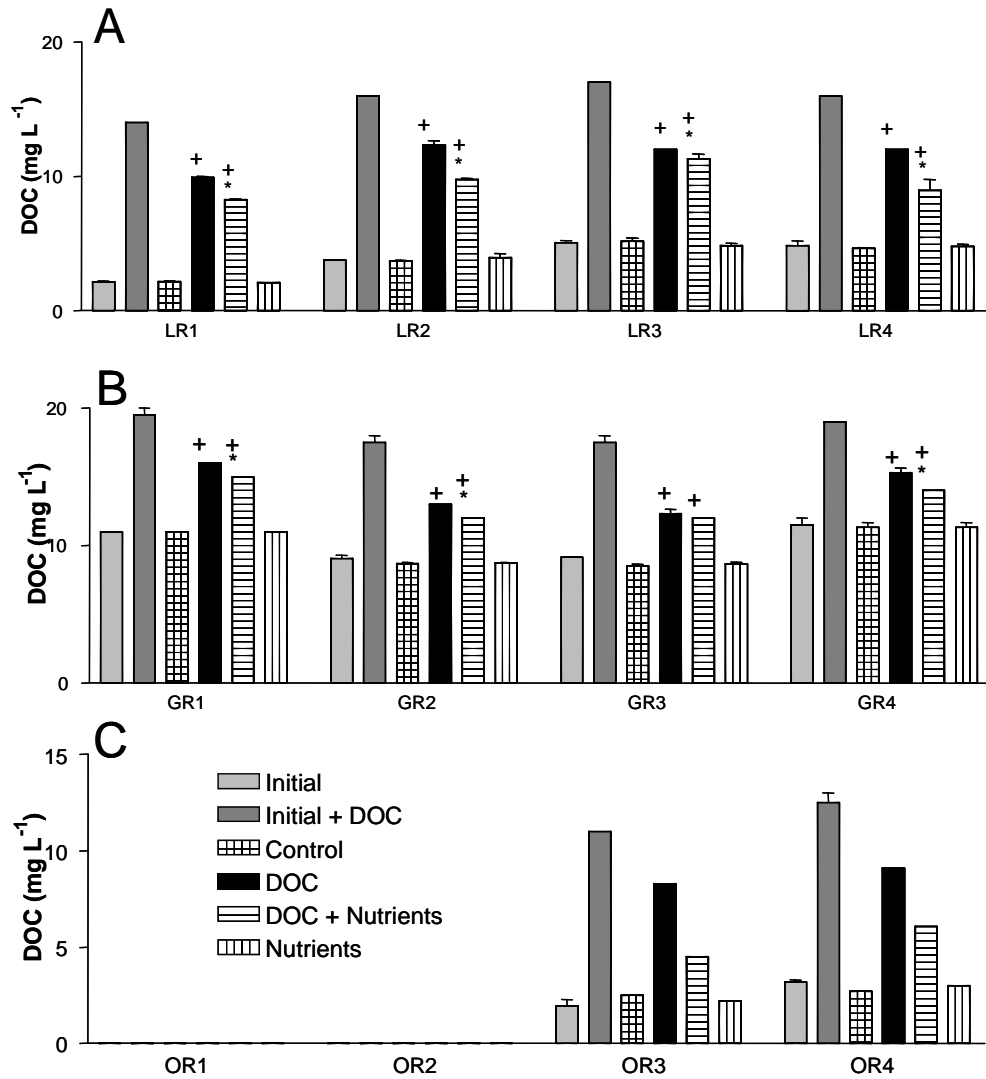


Figure 7. DOC results from the DOC and nutrient addition bioassays conducted at four sites on the main stems of the A) Logan River, B) Gwydir River, and C) Ovens River. Initial and Initial + DOC concentrations are at the start of the incubation and all other values relate to concentrations 48 h later. Initial, control and nutrients were statistically analysed separately from initial + DOC, DOC and DOC + nutrients. + indicates significant differences ($P < 0.05$) between the initial and control / nutrients or between initial + DOC and the DOC / DOC + nutrients. * indicates significant difference ($P < 0.05$) between the control and nutrients treatment or between DOC and DOC + nutrients treatment.

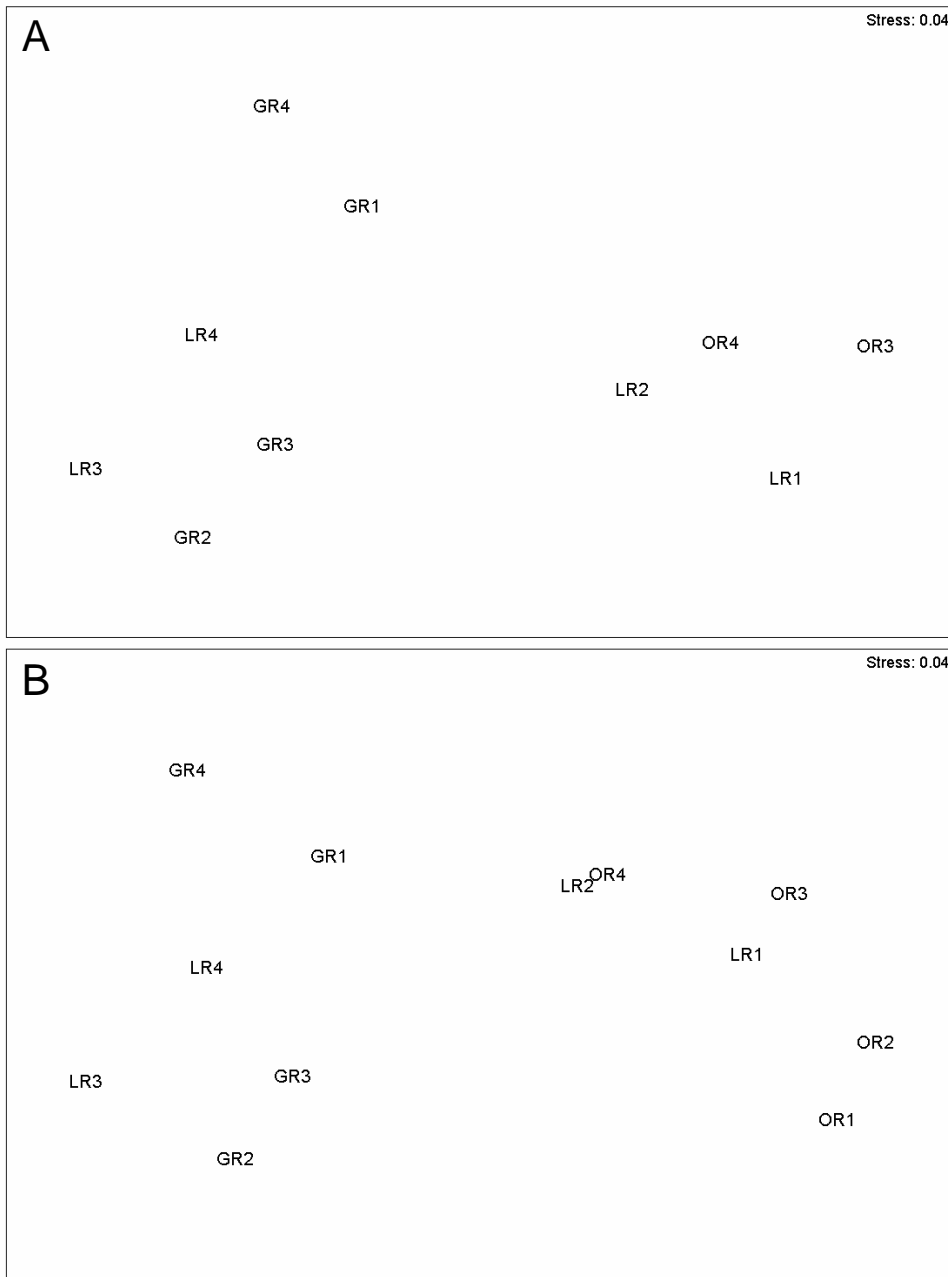


Figure 8. Plot of MDS ordination using A) all measures (minus bioassays) across all 12 sites in the Logan, Gwydir and Ovens Rivers, and B) all measures (minus sites OR1 and OR2) across the remaining 10 sites in the Logan, Gwydir and Ovens Rivers.