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Flow variability and longitudinal characteristics of organic carbon in the Lachlan River, Australia

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Abstract. Heterotrophic organic-carbon cycling is a major source of energy to aquatic food webs, yet there are few studies into patterns of heterotrophic productivity in large lowland rivers. The Lachlan River experienced a period of extreme flow variability from September 2010 to February 2011; for example, daily discharge (ML day⁻¹) at one site reached >22 times its 10-year average. Heterotrophic cycling of dissolved organic carbon (DOC) and particulate organic carbon (POC) were assessed over this period at six sites on the Lachlan River. Concentrations of total organic carbon (TOC) ranged from 7 to 30 mg L⁻¹, of which the majority was in dissolved form. Concentration of DOC was positively correlated with daily discharge. Biochemical oxygen demand of TOC over 5 days (BOD₅) showed significant variability, ranging from 0.6 to 6.6 mg O₂ L⁻¹. BOD₅ did not appear related to discharge, but instead to a range of other factors, including regulation via weirs, lateral and longitudinal factors. Partitioning of DOC and POC showed that POC had an influence on BOD₅ comparable to DOC. This is relevant to environmental-flow management in the Lachlan River, the Murray–Darling Basin and rivers generally, by showing that flow variability influences a fundamental ecosystem characteristic, namely organic carbon.

Additional keywords: biogeochemistry, catchment management, flow regulation, Murray-Darling system, organic matter.

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Introduction

Organic carbon as an energy source is a key component of riverine ecosystems whether this is via longitudinal and riparian inputs (Vannote *et al.* 1980), lateral and floodplain connectivity (Junk *et al.* 1989), vertical connectivity (Boulton *et al.* 2010) or in-stream autotrophic productivity (Thorp and Delong 1994). Studies conducted globally have reported that carbon dynamics and hydrological variability are closely linked because of interactions between flow rate and organic-matter exchanges across floodplains, riparian zones and sediments (e.g. South American, Petry *et al.* 2003; Australian, Westhorpe and Mitrovic 2012; North American, Dalzell *et al.* 2007). Therefore, to characterise patterns of organic carbon, their sources and variability should be analysed across a range of hydrological conditions (Tank *et al.* 2010).

Australian lowland rivers generally have high flow variability relative to river systems globally (Puckridge *et al.* 1998) and are intensely regulated (Thoms and Sheldon 2000). Given the hydrological variability, it has been hypothesised that floodplain-derived carbon is an important source of energy for aquatic food webs; however, regulation has limited the contribution of floodplain-derived carbon (Gawne *et al.* 2007; Robertson et al. 1999). Recent studies have found that autochthonous carbon plays a more significant role than predicted in riverine primary productivity (Oliver and Merrick 2006). For example, in three eastern Australian rivers, Hadwen et al. (2010) found that the heterotrophic community was DOC limited and the ambient DOC concentration was low and generally recalcitrant. In the regionally significant Murrumbidgee River, Vink et al. (2005) found that the ecosystem was predominantly heterotrophic during a period of in-channel flow but there was a trend of increasing autotrophic activity downstream. Additionally, Gawne et al. (2007) found that both heterotrophic and autotrophic productivity were significant contributors to the productivity of the Murray River but that this varied seasonally. These studies have largely taken place under extended low-flow conditions (Bond, Lake et al. 2008); however, recent floods in New South Wales, Australia, provided the opportunity to assess carbon dynamics under both low and high flows.

The influence of total organic carbon (TOC) is not only related to flow but also to its concentration, composition and bioavailability (Cleveland *et al.* 2004; Wallace *et al.* 2008). The dissolved fraction (DOC) is commonly the larger fraction by an ~ 10 : 1 ratio, so is considered a more important source of energy

to heterotrophic bacteria than the particulate organic fraction (POC) (Volk et al. 1997; Wetzel 2001). Within the DOC fraction, bioavailability is related to the composition and the proportions of functional groups present, such as humic substances and hydrophilic acids, which are influenced by a broad range of factors (Qualls 2005). This includes the type and age of leaf litter, the rate of leaching DOC from litter (Baldwin 1999) and its bioavailability, the amount of litter and debris that has accumulated before flooding, whether or not the litter has undergone previous flooding and the extent and timing of rainfall or flooding (Whitworth et al. 2012). Interactive effects between organic matter and floodplain soil systems are also factors influencing DOC composition (Cleveland et al. 2004). Standardised bioassays can be used to assess the biochemical oxygen demand (BOD) and the biodegradability of DOC, POC and TOC generated by heterotrophic metabolism. The addition of nutrients and or labile carbon can help diagnose whether or not carbon metabolism is limited by nutrient availability and/or the presence of active heterotrophic bacteria. This technique was used to address the hypothesis that although DOC concentration will be related to flow, bioavailability will depend on the source of the carbon.

Recently, reinstating a natural (i.e. more variable) hydrograph through environmental flows has become a priority to achieve favourable ecosystem outcomes, such as supporting sustainable fisheries and ecologically significant wetlands (Walker *et al.* 1995; Kingsford 2011). This was recommended in the Murray–Darling Basin and specifically in the Lachlan River where a 44–67 GL year⁻¹ increase in environmental allocations has been proposed (Murray–Darling Basin Authority 2010). However, environmental flows may also have a downside if the organic carbon is readily bioavailable, which may lead to hypoxia, fish deaths and other undesirable water-quality characteristics (Howitt *et al.* 2007; Hladyz *et al.* 2011).

Materials and methods

Catchment and site description

The Lachlan River catchment (85532 km^2) flows west from the Great Dividing Range in New South Wales (NSW), generally terminating in the Great Cumbung Swamp. The climate is temperate, with rainfall averaging 370 and 780 mm per year in the western and eastern reaches, respectively (CSIRO 2008). The rainfall occurs predominantly in winter (June–August), leading to highly variable seasonal flow (Kemp 2010). Flows are highly regulated with 14 weirs plus the Wyangala Dam (~301 GL), which is located in the upper catchment. The influence of Wyangala Dam often delays peak flows in the western lowlands to July–October (McMahon and Finlayson 2003).

Study sites (S1–S6) were distributed longitudinally across a \sim 225-km (point-to-point) stretch of the Lachlan River, from Condobolin (upstream) to Booligal (downstream) (Fig. 1). This western lowland region is characterised by a shallow gradient and majority agricultural land use (Kemp 2010). There is a trend of decreasing channel size and average flow from upstream to downstream sites; for example, bankfull width is 48 m at Condobolin (S1) and 27 m at Booligal (S6) (Kemp 2010). This is largely due to the natural diversion of water to distributaries that do not return to the main channel. At all sites, the riparian

vegetation consists of sclerophyll forest dominated by river red gums (*Eucalyptus camaldulensis*) and river-adjacent land use is overwhelmingly agricultural.

Locations for sampling are natural pools that maintain a 3–5-m depth in low-flow (\sim 150 ML day⁻¹, \sim 1.74 m³ s⁻¹) conditions, with shallow (\sim 1.0–1.5 m) upstream and downstream runs. This ensured that sites did not completely dry up during the study period. Sites are evenly distributed between 'weir pool' sites immediately upstream of weir pools (S1, S3, S6) and free-flowing sites located outside of the direct influence of weirs (S2, S4, S5). The distribution of sample sites was designed to account for possible longitudinal variability in heterotrophic metabolism and the characteristics of the organic carbon pool (Kelly 2001; Stanford and Ward 2001).

Field sampling

Sampling occurred during 21–23 September 2010, 18–21 January 2011 and 21–23 February 2011. Water samples were collected with a Schindler trap (2 L) from a boat anchored at three locations per site (approximately midstream, 50–100 m apart), giving three replicate samples. At each location, three trap samples were mixed to give a depth-integrated composite sample, one each from the top (0.3 m below the air-water interface), middle (approximated from river depth) and bottom (0.3 m above the sediment–water interface) of the water column.

Samples (~1.2 L) in opaque jars were immediately transferred to an insulated cool box (esky) and stored in the dark until transferred (typically within 30 min of sample collection) to a portable refrigerator at ~4°C. Samples for filtered treatments were pre-filtered through pre-washed Whatman GF/C (Whatman International, Maidstone, Kent, UK) filters at the end of each sampling day. All sample jars were subsequently placed in salted ice-slurry for 3–4-day transport to the laboratory. At the laboratory, samples were subsequently filtered through pre-washed Millipore PVDF 0.45 µm membrane filters (Merck Millipore, Billerica, Massachusetts, USA). This excluded most particulate material but retained dissolved carbon and nutrients within the filtrate. The pore size, 0.45 µm, was chosen so as to maintain consistency with previous studies and datasets from regionally relevant river systems.

Discharge data (ML day⁻¹, 1 ML day⁻¹ = $0.01157 \text{ m}^3 \text{ s}^{-1}$) were taken from the nearest gauging station to each site from the New South Wales State Government (State Water, http:// waterinfo.nsw.gov.au, accessed 30th April 2011; Fig. 2). Under low flow conditions, the weirs between S1/S2 and S3/S4 would provide a significant hydrological disparity in water-level variability and flow rates between these sites, but their influence on discharge data was considered negligible, given the generally high flow and relatively strong (given the structural barrier) longitudinal connectivity during sampling. The ability to make direct comparisons between sites on the basis of discharge is limited because the sampling occurred at different positions on their respective hydrographs and because several sites share a gauging station.

The S6 gauging station was offline between 15 September 2010 and 1 October 2010. Discharge on the 23 September 2010 sampling period was approximated by averaging the daily discharge from the date immediately before (15 September 2010) and after (1 October 2010) the disruption.



Fig. 1. The Lachlan River catchment map, including the locations of sites and gauging stations. Adapted from NSW Government: http://www.environment.nsw.gov.au/ieo/Lachlan/map.html (accessed 30 April 2011).



Fig. 2. Hydrographs at gauging stations: S1 and S2 = Condobolin Bridge (Station #412006); S3 and S4 = Hillston Weir (Station #412039); S5 = Whealbah (Station #412078) and S6 = Booligal (Station #412005; note missing data 16–30 September 2010). S1 and S2 as well as S3 and S4 share gauging stations because of geographical proximity. Nonetheless, because sampling was carried out over several days (\uparrow) (21–23 September 2010; 18–20 January 2011; 21–23 February 2011), flow among sites varied.

Laboratory bioassays - nutrient, carbon addition

Standardised bioassays based on a modification of the American Public Health Association (APHA) Standard Method (5210B) 5-day test (BOD₅; Eaton and Franson 2005) were used to assess the biodegradability of the DOC and TOC and the BOD generated by heterotrophic metabolism. A nitrification inhibitor, 2-chloro-6-(trichloro methyl) pyridine ($10 \ \mu g L^{-1}$), was used to ensure that BOD was not influenced by nitrogenous oxygen demand from the biological oxidation of reduced forms of nitrogen (ammonia, organic nitrogen). Bioassays undertaken with this nitrification inhibitor are typically referred to as carbonaceous biochemical oxygen demand (CBOD). The influence of temperature and autotrophic metabolism were minimised by conducting bioassays at 20°C in the dark (standard conditions). To ensure the presence of a heterotrophic community, all samples were seeded with 20 mL L⁻¹ of unfiltered water collected from the Lachlan River (S6) that had been stored in a jar in salted ice slurry for 2–3 days.

September 2010 samples were analysed using independent incubation bottles (310 mL Wheaton BOD bottles; Wheaton Industries, Millville, NJ, USA) for Day-0 and Day-5 time steps to minimise issues associated with pseudo-replication and repeated measures. Samples were diluted using reverse-osmosis (RO) water acclimatised for 24 h at 20°C to 60%, i.e. a sample to RO (including additions) ratio of 60:40. Samples were diluted to avoid reaching anoxia within the 5-day period and O₂ (cf. organic carbon) becoming the limiting factor. The treatments included the following:

- (1) filtered with no nutrient or carbon addition,
- (2) filtered with addition of NaNO₃ and K_2 HPO₄ to 500 µg N L^{-1} and 50 µg P L^{-1} ,

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- (3) filtered with addition of β -Glucose to 1000 µg C L⁻¹, and
- (4) controls: seed/dilution water, dilution water.

The focus of the September bioassays was to test whether the availability of labile carbon or nutrient concentrations were limiting heterotrophic metabolism of DOC.

Laboratory bioassays – DOC/POC partitioning and leachate addition

Unlike the nutrient-addition bioassays, January and February 2011 treatments were designed to assess how the heterotrophic community would respond to allochthonous carbon inputs and also how BOD and carbon concentration were partitioned between POC and DOC fractions. A leachate was prepared from floodplain litter collected from six litter bins suspended above the floodplain adjacent (<10m) to the main channel at S4 and S5. Litter collected from January 2011 accumulated between 28 October 2010 and 19 January 2011, and litter from February 2011 accumulated between 19 January 2011 and 22 February 2011 at S4 and 19 January 2011 and 23 February 2011 at S5. The collected material was air-dried and 40 g of intact litter (including bark, twigs, leaves randomly selected by hand) was leached in 20 L of RO water for 6 h at 20°C. Leachate was screened with 45-µm mesh before use. Although this does not reflect all possible allochthonous carbon sources, this does reflect one typical allochthonous input that would occur across the study sites.

January and February samples were not diluted before the bioassays, given the relatively low BOD results of the September samples. The same bottle was sampled on Days 0, 1, 2, 3, 5. Treatments included the following:

- (1) filtered with no addition,
- (2) unfiltered with no addition,
- (3) unfiltered, 5% (15.5 mL) leachate addition, and
- (4) controls: dilution water; seed with dilution water; leachate and seed with dilution water.

BOD results of the filtered and unfiltered samples were related to the BOD of dissolved carbon fractions (BOD_{DOC}) and total carbon (BOD_{TOC}), respectively, such that the metabolism of DOC and POC (BOD_{POC} = BOD_{TOC} – BOD_{DOC}) could be partitioned across sites and sampling periods. This is based on the assumption that the interactive effect between BOD_{DOC} and BOD_{POC} is negligible.

Organic carbon concentrations and nutrient analysis

Thirty millilitre samples were taken from each bioassay bottles at Day 0 and analysed for DOC, to establish initial carbon concentrations (DOC_i). Filtered and unfiltered samples were submitted to the Environmental Analysis Laboratory (EAL), Southern Cross University, NSW, Australia, for measurement of DOC and TOC. As the September samples underwent nutrientaddition treatment, the ambient nutrient concentrations were also obtained (total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), orthophosphate, nitrate, nitrite, ammonia).

Data analysis

The program GraphPad Prism (Version 5.01, GraphPad Software, San Diego, CA, USA) was used for regressions and

one-way and two-way ANOVA analyses. The specific ANOVA analyses conducted are outlined in the results below. A significance level of P = 0.05 was used for all analyses. Data transformations were not required.

Results

Discharge

Flow conditions included two catchment-derived flow pulses between September–October 2010 and December–January 2011 (Fig. 2). Flow conditions and relative positions on the hydrograph differed among sites because of longitudinal variations in the hydrograph and dates sampled. For example, in September 2010, S1 was sampled at the peak of a flow pulse, whereas S6 was sampled at the front end of that pulse. In addition, the magnitude of the flow pulses decreased among sites, such that the December–January flow pulse at S1 peaked on 22 December 2010 at 12 481 ML day⁻¹ (~144.46 m³ s⁻¹), whereas at S6, the pulse peaked on 28 January 2011 at 2988 ML day⁻¹ (~34.58 m³ s⁻¹). These pulses were exceptionally high flows, ~18 times the 10-year average at S1 and ~23 times that at S6. Nonetheless, flows did not exceed bankfull flows, which are ~15 000 ML day⁻¹ (~172 m³ s⁻¹) at S1 and 5000 ML day⁻¹ (~60 m³ s⁻¹) at S6 (Kemp 2010).

The relationship between discharge and DOC

The initial DOC concentrations ([DOC_i], Table 1) at each site were positively related with daily discharge at sampling (Fig. 3). Linear regression of relative discharge and [DOC_i] gave slopes for S1–S5 that were not significantly (P = 0.6343) different from each other. Pooled data from these sites showed a significantly positive relationship, with a slope of 610.1 mg C ML⁻¹ (ML day⁻¹)⁻¹. S6 varied from all other sites, with a significantly (P < 0.0001) higher slope. The slope at S6 was 6123 mg C ML⁻¹ (ML day⁻¹)⁻¹.

BOD₅ nutrient addition

Addition of nitrogen and phosphorus (above ambient concentrations, Table 2) to September 2010 samples (Fig. 4) did not significantly influence BOD₅ (two-way ANOVA, P > 0.05). In contrast, the addition of β -glucose led to a significant increase in BOD₅ (two-way ANOVA, P < 0.0001, average increase in BOD₅ = 2.4 mg O₂ L⁻¹). The non-significant interaction effect between site and addition treatment (P = 0.8993) indicated that the magnitude of the increase in BOD₅ was independent of site. Direct assessment of the BOD increase as a result of carbon addition (BOD₅ (+ Carbon) – BOD₅ (No addition)) indicated that the magnitude of BOD increase was constant across all sites (one-way ANOVA, P = 0.9394).

Unlike carbon concentration, BOD₅ did not show a relationship with discharge, nor did BOD₅ show a statistically significant relationship with [DOC_i] or site. Linear regression of BOD₅ and discharge gave slopes that were not significantly different from zero at all sites (P > 0.05, $r^2 = 0.0014$ (S2), 0.0001 (S3), 0.3761 (S4), 0.0521 (S5), 0.0620 (S6)). The exception was S1, which was significantly positive (slope = $2.675 \pm 0.400 \times 10^{-4}$, $r^2 = 0.8644$) and was significantly (P = 0.0003) different from zero.

Table 1. Initial concentrations of dissolved organic carbon (DOC) and
total organic carbon (TOC) from all sites and sampling periodsValues are means and standard deviations are given. All values are mg L⁻¹

Site	S 1	S2	S3	S4 S		S6				
DOC										
September 2010	12.19	11.85	9.07	11.14	10.25	10.34				
s.d.	1.78	3.47	1.05	1.46	0.30	2.50				
January 2011	11.89	11.58	13.33	13.70	14.83	25.59				
s.d.	0.34 0.40 1.01 0.38		0.38	0.44	1.02					
February 2011	2011 7.18 7.29 9.70 9.85		9.85	10.34	10.46					
s.d.	0.17	0.09	0.13	0.32	0.32	0.38				
TOC										
January 2011	11.47	11.26	14.40	12.25	14.31	28.46				
s.d.	0.35	2.28	0.58	0.21	0.30	0.27				
February 2011	8.08	8.08	10.95	11.17	11.26	12.49				
s.d.	0.13	0.12	0.43	0.57	0.38	0.88				
30 - 25 - 20 - 15 - 20 - 10 - 5 - 5 - 0 -	L & s -) **	6 9	S1-S5				
100			10 000							
Daily flow ML day ⁻¹										

Fig. 3. Daily discharge at sampling and [DOC_i] concentrations are positively related. The pooled data from S1–S5 has a positive slope of 0.0006101 ± 0.0001272 ($r^2 = 0.3486$), which is significantly (P < 0.0001) different from zero. S6 also gives a positive slope of 0.006123 ± 0.0007128 ($r^2 = 0.9134$), which is significantly (P < 0.0001) different from zero. Error bars represent ± 1 s.e.

Leachate-addition bioassays

The change in BOD because of leachate addition was analysed using the difference between the BOD of leachate addition and no addition bioassays on Days 1, 2, 3 and 5 (i.e. $\Delta BOD_x =$ BOD_x (unfiltered + leachate) - BOD_x (unfiltered)). Leachate control bioassays were used to remove the BOD attributable to consumption of carbon from the leachate itself; therefore, ΔBOD_x reflects the oxygen demand attributable to the influence of the leachate on the organic carbon of the samples. BOD_x can be modelled using a first-order rate reaction (O'Connell *et al.* 2000); however, in this case, a linear model was used because linear regressions gave r^2 -values of >0.95 in all cases. This linear response may result from short bioassay duration and a high organic carbon concentration, where depletion of carbon does not cause BOD_x to asymptote within the bioassay period.

A response to leachate addition was detected in both sampling periods using linear regressions of ΔBOD_x and time at each site. For January 2011 samples, ΔBOD_x produced slopes significantly different from zero over 5 days at all sites (S1–S5, P < 0.0001; S6, P = 0.0007) and the relationship did not differ significantly (P = 0.1439) among sites. In February 2011 samples, ΔBOD_x were significantly non-zero over 5 days at all

Table 2. Ambient nutrient concentrations in September 2010 bioassays

Values are means and standard deviations are given. All values are mg L^{-1}

Site	S 1	S2	S3	S4	S5	S6
Total dissolved nitrogen	1.035	0.905	0.850	0.899	0.990	0.641
s.d.	0.377	0.084	0.135	0.061	0.281	0.064
Nitrate	0.087	0.117	0.189	0.208	0.278	0.088
s.d.	0.031	0.004	0.037	0.005	0.216	0.035
Nitrite	0.004	0.004	0.003	0.005	0.008	0.003
s.d.	0.001	0.000	0.000	0.001	0.006	0.001
Ammonia	0.179	0.150	0.174	0.188	0.189	0.141
s.d.	0.045	0.012	0.011	0.014	0.022	0.027
Total dissolved phosphorus	0.052	0.043	0.037	0.039	0.058	0.036
s.d.	0.004	0.007	0.003	0.005	0.037	0.003
Orthophosphate	0.012	0.012	0.010	0.012	0.028	0.008
s.d.	0.003	0.001	0.001	0.002	0.033	0.004



Fig. 4. A two-way ANOVA was used with *site* and *treatment* as the factors. *Treatment* significantly (P < 0.0001) affected biochemical oxygen demand of total organic carbon (TOC) over 5 days (BOD₅). Bonferroni post-tests were used to find differences among treatment groups at each site. Nutrient addition treatment and No addition treatment were not significantly (P > 0.05) different at all sites. Carbon was significantly (P < 0.0001) higher than other treatments at all sites. The *interaction* among factors was insignificant (P = 0.8993), so the influence of treatment can be interpreted independent of *site*. Error bars represent ± 1 s.e.

sites excluding S3 (S1–6 P = 0.0230, 0.0207, 0.0583, < 0.0001, 0.0409, 0.0030) and the relationship did not differ among sites (P = 0.6380).

The magnitude of the response differed between sampling periods, with the mean increase in BOD₅ as a result of leachate in January 1.302 mg O₂ L⁻¹ (standard deviation = 0.230), whereas in February, this was 0.384 mg O₂ L⁻¹ (standard deviation = 0.161). It is relevant that the oxygen demand (BOD₅) exerted by the leachate used in the January bioassays was ~1 mg O₂ L⁻¹, compared with 0.2 mg O₂ L⁻¹ in the February bioassays. This may have influenced the different responses observed between the two sampling times.

POC/DOC concentrations and BOD

The DOC : POC ratio can be calculated from [TOC] and [DOC] concentrations (Table 1) as follows: [POC] = [TOC] – [DOC]). In January 2011, the initial DOC : POC ratios were >10 at all sites, excluding S6 which was slightly lower (8.9:1). In the February 2011 samples, ratios ranged from 7.4–11:1 at S1–S5,

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Fig. 5. The differences in biochemical oxygen demand of dissolved organic fraction (BOD_{DOC}) and particulate organic fraction (BOD_{POC}) were assessed with a two-way ANOVA, using *site* and *fraction* factors with Bonferroni post-tests (critical P = 0.05). Significant differences between BOD_{DOC} and BOD_{POC} at sites are represented by different letters. Letter sets for time period and site are independent. In addition, longitudinal variations in BOD₅ within each sampling period and fraction were assessed using four individual one-way ANOVAs. Across the sampling periods, S6 had significantly higher BOD_{DOC} and BOD_{POC} than did all other sites, excluding BOD_{DOC} in February (P < 0.05). Error bars represent ± 1 s.e.

and were 5.2:1 at S6. The results of the BOD₅ analysis demonstrated that for the majority of sites in January and February 2011, BOD_{POC} was equal to or greater that BOD_{DOC} (Fig. 5).

Longitudinal patterns in BOD

BOD₅ from September 2011 showed no overall longitudinal trend but did differ between non-weir and weir pool sites. Sites could be divided into two groups with weir pool sites (S1, S3, S6) significantly higher than non-weir sites S2 and S4, with S5 being intermediate (Fig. 6). There was no significant difference between weir and non-weir sites at any other sampling period.

In January and February 2011, significant variations in BOD_{DOC} and BOD_{POC} showed that S6 had the highest BOD_5 , except for BOD_{DOC} in February 2011 (Fig. 5).

BOD per unit carbon (i.e. BOD : TOC ratios) may be used as a measure of the relative bioavailability of the carbon pool (Wallace 2006). An assessment of BOD : TOC among sites and sampling periods demonstrated a longitudinal trend of BOD : TOC decreasing downstream for sites S1–S5 in January and February. However, site S6 displays a BOD : TOC similar to that observed at S1 (Fig. 7).

Discussion

Carbon limitation of heterotrophic metabolism

Leachate addition to samples stimulated BOD over a short period, i.e. 6-h leachate preparation and 5-day incubation,



Fig. 6. Analysis of biochemical oxygen demand of total organic carbon (TOC) over 5 days (BOD₅) from September 2011 used one-way ANOVA with Bonferroni post-tests (critical P = 0.05). BOD₅ varied significantly (P = 0.0012) with site. The same letters represent sites that are not significantly different. All *B* sites are non-weir sites. Error bars represent ± 1 s.e.



Fig. 7. Biochemical oxygen demand of total organic carbon (TOC) over 5 days (BOD₅) per unit TOC (mg O₂ (mg C)⁻¹) appears to decrease longitudinally from S1 to S5, whereas at S6 it is significantly higher than at S3–S5 in both periods. Analysis used one-way ANOVAs for each of the sampling period. Different letters represent a significant difference at P = 0.05. Letter sets from January 2011 and February 2011 are independent. Error bars represent ± 1 s.e.

supporting the proposition that fresh input of leaf leachate will stimulate a rapid biological response (Baldwin 1999). Relative DOC : N and DOC : P ratios suggest that inorganic nutrients will limit BOD (Geider and La Roche 2002). Nonetheless, nutrient additions had no influence, whereas additions of bioavailable carbon (i.e. β -glucose) significantly increased BOD₅. One way to account for this is if a significant proportion of the organic carbon pool is recalcitrant, causing heterotrophic metabolism to be limited by labile carbon subsidies. This is consistent with estimates of the recalcitrant nature of global riverine carbon (Søndergaard and Middelboe 1995).

Carbon utilisation rates vary among litter types (e.g. leaf, bark, twigs; O'Connell *et al.* 2000) and between aged and fresh leaf litter (Baldwin 1999). Watkins *et al.* (2010) showed that the timing of inundation was critical to Australian river floodplains as litter fall peaks over summer and aging of river red gum leaves increased their ability to be decomposed. The significantly lower response to leachate addition in February 2011 is likely to have been influenced by the carbon composition of leachate derived from litter fall collected at different times, which vary with age.

The uniform BOD response to leachate addition across sites suggested that there was no longitudinal variation in the microbial community's ability to respond to organic carbon addition. Factors such as the nature and composition of the natural organic matter (NOM) inputs can cause variations in the abundance and composition of the microbial community and heterotrophic functional groups present (Foreman and Covert 2003; Gulis and Suberkropp 2003). It is unknown whether the heterotrophic communities did respond to leachate addition uniformly. Differences in BOD among sites were therefore assumed to be the result of shifts in the composition and bioavailability of the organic carbon pool and not of variations in the microbial community (Volk *et al.* 1997).

The relationship between relative discharge and carbon

Across the Lachlan River, there was a positive relationship between discharge and DOC concentration at each site during the study period. Allochthonous carbon inputs are predicted to increase with catchment flows as a result of increased longitudinal inputs plus increased riparian and floodplain connectivity (Tockner *et al.* 2000). This occurs where flows exceed bankfull and interact directly with the floodplain, i.e. the 'flood pulse' concept (Junk *et al.* 1989), but also when increased flow is below bankfull, i.e. the 'flow pulse' (Puckridge *et al.* 1998).

The flow pulses during the present study were catchment derived as opposed to regulated dam-release flows. Impoundment of water because of weirs/dams is able to influence the composition of organic matter and reduce its bioavailability to downstream sites (Stanford and Ward 2001). As such, flow pulses that are sourced largely from storage releases are less likely to input carbon longitudinally but can release NOM from riparian sources, floodplain and sediment interactions (Walker *et al.* 1995; Robertson *et al.* 1999). Nonetheless, storage flows would not necessarily produce identical results as the flows observed in the study period, and the difference between these, would benefit from further study.

POC/DOC partitioning

In the Lachlan River, DOC : POC ratios largely reflected the 10:1 ratio suggested by Wetzel (2001), with some variation, such as S6 having a DOC : POC ratio of <10 at both periods. Contrasting this, the BOD_{POC} was generally equal to or higher than BOD_{DOC}. Independent data from the Lachlan River (January 2011; data not shown) did not detect chlorophyll concentrations at high levels such that phytoplankton respiration would account for the high BOD_{POC}. Because BOD_{POC} was measured in the presence of DOC, interactive effects between BOD_{POC} and BOD_{DOC}, such as increased substrate area, may have contributed to the observed elevated BOD_{POC}.

Elevated BOD_{POC} can contribute to water-column anoxia related to a high concentration of organic carbon. Anoxia is common in blackwater events and can cause widespread fish kills (Howitt *et al.* 2007). Although the results from the Lachlan River did not directly show that POC was more labile than DOC because BOD does not directly relate to the amount

of carbon consumed (Wallace *et al.* 2008), they did show that POC and DOC can have a comparable influence on ecosystem characteristics. Furthermore, Cole *et al.* (2006) showed that POC can be an important source of carbon to high trophic-level species. As such, studies focusing on the DOC component of organic carbon may be excluding a critical part of the energy subsidy in a river system.

Longitudinal patterns in carbon cycling

Variations in BOD could not be clearly associated with any one factor, e.g. site, flow or [DOC_i], but this is not unexpected, considering the BOD variation may result from multiple complex factors. Longitudinal position, flow conditions and seasonal factors all appeared to exert some influence on BOD.

Differences in BOD between weir and non-weir sites in September 2010 suggested that regulation and changes to retention time can cause variation in the carbon pool. On a catchment scale, regulatory structures fragment hydrological and ecological processes while limiting the lateral connectivity (Nilsson et al. 2005). Particularly, in dryland rivers, there is a significant difference in the hydrology of weir pools versus midand upper reaches (Davies et al. 1995). This may be seen as analogous with the serial discontinuity concept, which argues that major regulatory mechanisms, e.g. dams, influence longitudinal patterns in physical parameters, nutrient conditions and ultimately community composition in rivers (Ward and Stanford 1983). Because hydrology and aquatic metabolism appear to be linked (Bernot et al. 2010), the observed BOD differences between weir and non-weir sites may have been the result of regulation. As a weir effect was observed only once, making further conclusions is speculation and longer-term studies focusing on the influence of regulation on carbon composition and carbon cycling are needed.

The peak of an anoxic flow pulse coincided with January 2011 at S5 and S6, which corresponded to significantly elevated DOC and TOC concentrations and BOD_{TOC}. Blackwater events, where high flows cause increased carbon inputs, BOD stimulation, DO depletion and anoxia, make it difficult for managers who wish to institute patterns of floodplain inundation while avoiding fish kills. Howitt et al. (2007) modelled the occurrence of blackwater from two flooding events in the Barmah-Millewa river red gum forests. Temperature was one key factor, wherein summer high-flow periods were significantly more likely to lead to an anoxic water column because of the influence of temperature on microbial activity. Results from the Lachlan River supported this because the January 2011 flow pulse corresponded with anoxic conditions (DO = $3.00 \text{ mg} \text{ O}_2 \text{ L}^{-1}$ (S5), 0.49 mg $O_2 L^{-1}$ (S6)) and heightened water temperatures of 27.06°C (S5) and 25.37°C (S6). September 2010 sampling also coincided with a flow pulse but DO at S1-S6 ranged between 7.55 and 8.45 mg $O_2 L^{-1}$ and temperature was 14.98–16.27°C. Litter-fall rates of Australian sclerophyll vegetation are characteristically highest during summer months (Pressland 1982). This may also have contributed because litter fall was observed significantly higher in the November-January period than in September-October (data not shown). Nonetheless, there are a range of contributing factors to any given blackwater event and multi-factor models, as in Howitt et al. (2007), are the most useful predictive tool.

BOD_{TOC}/[TOC] may give an indication of longitudinal factors influencing observed variations in BOD. BOD_{TOC}/ [TOC] can relate to carbon bioavailability because high BOD per unit carbon suggests that a higher proportion of carbon is being consumed, although it has been observed that BOD does not directly relate to the amount of carbon consumed (Wallace *et al.* 2008). As such, BOD_{TOC}/[TOC] relates to the relative influence of a particular pool of carbon on the BOD of the water column and can be only loosely related to bioavailability.

In the Lachlan River, the observed BOD_{TOC}/[TOC] decreased longitudinally between S1 and S5. This is consistent with one of the mechanisms underlying the River Continuum concept, i.e. that upstream communities consume the most bioavailable organic carbon fraction and predominantly recalcitrant carbon will be transported downstream (Vannote et al. 1980). This phenomenon may influence the characteristics of the allochthonous carbon pool along a river, resulting in a trend of increasing concentration downstream but decreasing bioavailability. This would be exacerbated by the concentrating effect of water losses via evaporation/transpiration. This trend was observed by Hadwen et al. (2010) in the Ovens and Logan Rivers. The accumulation of recalcitrant carbon as a package of water progresses downstream may influence organic carbon by increasing the concentration of organic carbon ([DOC]) but decreasing the influence on the water column (BOD), which may be due to lower bioavailability.

S6 defied the BOD_{TOC}/[TOC] trend. A hypothesis to explain this and the significant increases in BOD and carbon concentration from S5 to S6 incorporates differences in river-adjacent land use that can cause significant variations in organic carbon and disrupt longitudinal patterns in carbon (Jarvie *et al.* 2008; Neal *et al.* 2008). As the Lachlan River flows towards its terminus, it contracts, branches and anabranches (Kemp 2010). Unlike upstream where land use is overwhelmingly agricultural, the dominant landscape feature between S5 and S6 is a river red gum forest, the Moon Moon State Forest.

Unlike carbon concentrations, the observed BOD suggests that carbon composition and bioavailability are unlikely to be characterised by a single factor. It is clear that flow variability has an influence on organic carbon characteristics of the Lachlan River, but directly relating this to heterotrophic carbon cycling is more complex, let alone relating it to aquatic biota. Both the advantages of environmental flow pulses, such as carbon availability for the food web, and potential deleterious effects, such as blackwater events, need to be considered when managing flow regimes in regulated river systems.

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