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Ecosystem metabolism in a dryland river waterhole

with 3 Tables and 3 Figures

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Abstract

Little is known about ecosystem processes in dryland rivers, despite the global distribution of these systems. Those in Australia are characterised by long periods of no flow in which they persist for many months as series of isolated, often turbid, waterholes. We assessed benthic and pelagic primary production, respiration, and bacterial production in one of these waterholes to determine the metabolic balance of the waterhole and resolve the relative importance of autochthonous and allochthonous sources of organic carbon. Despite a photic zone depth of only 0.25 m, three lines of evidence suggested that autochthonous sources of organic carbon were important for fuelling bacterial production under no flow conditions: the metabolic balance of the waterhole was not indicative of large allochthonous inputs; rates of gross primary production were great enough to meet a substantial fraction of estimated bacterial carbon demand; and pathways for allochthonous carbon to enter the waterhole were limited. These results suggest that models of lake metabolism based on temperate ecosystems can be expanded to include dryland river waterholes, which group with eutrophic lakes due to their high inorganic nutrients, low allochthonous inputs, and autotrophic metabolic balance.

Extra keywords: benthic, pelagic, gross primary production, respiration, autochthonous, allochthonous, heterotrophic bacteria

Introduction

The balance between gross primary production (GPP) and respiration (R) is a fundamental descriptor of carbon cycling in aquatic ecosystems and can provide insights into the sources of organic carbon available to heterotrophic organisms (Rosenfeld and Mackay 1987; Meyer 1989). GPP exceeds R in autotrophic ecosystems, resulting in excess organic carbon that is exported or stored. In contrast, R exceeds GPP in heterotrophic ecosystems, resulting in net consumption of organic carbon and a requirement for allochthonous organic carbon inputs to maintain R. Most streams and lakes studied to date appear to be heterotrophic (del Giorgio and Peters 1994; Cole 1999; Mulholland *et al.* 2001; Hanson *et al.* 2003). However, autotrophy has been reported for some desert streams (Minshall 1978; Fisher *et al.* 1982; Mulholland *et al.* 2001) and eutrophic lakes (del Giorgio *et al.* 1999; Hanson *et al.* 2003).

Across the entire gradient of aquatic ecosystems from autotrophic to heterotrophic, bacteria are important contributors to ecosystem respiration and organic carbon consumption (Cole 1999). Bacteria use both allochthonous and autochthonous sources of organic carbon for R and production (incorporation into biomass), with the availability of the two sources in the ecosystem expected to influence their relative importance to bacteria (Kritzberg *et al.* 2004; Kritzberg *et al.* 2005). The dominant source of organic carbon used by bacteria should therefore indirectly relate to the metabolic balance of the system, with autochthonous carbon being important in autotrophic systems and allochthonous carbon in heterotrophic systems. Relationships between chlorophyll *a* and bacterial production have been found in cross-system comparisons of pelagic zones (Cole *et al.* 1988; White 1991), suggesting the importance of autochthonous sources of carbon. These relationships

87 have been observed even in heterotrophic systems, suggesting that bacteria may
88 preferentially use autochthonous carbon (Kritzberg *et al.* 2005). However, in other
89 heterotrophic systems, no relationship is found between bacterial and algal
90 production, consistent with bacterial production from a non-algal source of carbon
91 (Findlay *et al.* 1991; Howarth *et al.* 1996). To date, studies of bacterial carbon use
92 have focused predominately on pelagic environments (del Giorgio *et al.* 1997; Cole
93 1999; Vadeboncoeur *et al.* 2002).

94 While a whole-ecosystem perspective requires measurements of multiple
95 aspects of ecosystem functioning, most studies of aquatic ecosystems investigate a
96 single process within a single habitat. In some cases, multiple aspects have been
97 measured, though usually a single process in both benthic and pelagic zones (e.g.
98 Fischer and Pusch 2001; Liboriussen and Jeppesen 2003) or both primary and
99 bacterial production in the pelagic zone (e.g. Chrzanowski and Hubbard 1988; Cole *et*
100 *al.* 1988; Findlay *et al.* 1991; Coveney and Wetzel 1995). Rarely have both zones and
101 both types of production been measured in a single system (but see Edwards and
102 Meyer 1987; Edwards *et al.* 1990; Meyer and Edwards 1990), in part because the size
103 or complexity of a given system makes the task very large, or because of a priori
104 expectations that either zone (or either process) would not contribute significantly at
105 the scale of the whole system. The benthic zone has been the main focus of studies of
106 small streams because its metabolism typically greatly exceeds that of the water
107 column (Keithan and Lowe 1985; Davies 1994). In contrast, lake studies have
108 predominately focused on the pelagic zone, given the generally greater water depths.
109 Both zones may be important to consider as rivers become larger (Minshall *et al.*
110 1992; Reynolds and Descy 1996) and lakes become shallower (Vadeboncoeur *et al.*
111 2002).

Although arid and semi-arid regions comprise greater than 60% of the earth's surface (Thomas 1989; Davies *et al.* 1994), little research has been done on dryland rivers compared to their counterparts in wetter regions (Bunn *et al.* 2006a). Dryland rivers are particularly well represented in Australia, with 75% of the continent classified as semi-arid or arid (Davies *et al.* 1994). Similar to many dryland rivers worldwide, large inland rivers in Australia exhibit extremely variable flow (Puckridge *et al.* 1998). Episodic floods inundate the extensive floodplains and networks of channels that characterise these river systems. Extended periods of no or low flow are typical between floods, therefore surface water is often limited to a series of disconnected channels and waterholes. Even under lentic conditions, mineral turbidity is high in these river systems due to fine clays in suspension (Bailey 2001; Sheldon *et al.* 2002; Bunn *et al.* 2003).

Recent work has begun to provide an understanding of benthic algal production and food webs in one turbid dryland river, Cooper Creek, Australia (Bunn and Davies 1999; Bunn *et al.* 2003). The combination of a large terrestrial-aquatic interface created by long, narrow channels and low light availability associated with high turbidity would be expected to create conditions in which allochthonous carbon from riparian vegetation would be more important than autochthonous production in waterholes. Because of the high turbidity, there are no aquatic macrophytes, and benthic algal production is restricted to shallow littoral margins where light levels are sufficient. Despite this limited spatial extent, stable isotope analysis suggested that benthic algae were the major source of energy supporting waterhole food webs during a period of no flow (Bunn *et al.* 2003). Rates of littoral benthic algal production were high enough under these conditions that the benthic zones of most waterholes were

net producers of carbon (i.e. autotrophic, GPP exceeded R), even though less than 10% of the benthic area was in the photic zone.

Our understanding of ecosystem functioning in dryland river waterholes is currently limited by the lack of ecosystem process measurements for these systems. It is likely that the general patterns observed in temperate, clear-water systems may not hold when applied to the high mineral turbidity and associated low light availability of dryland systems in Australia and elsewhere. While some studies have reported on components of primary production (Pieterse and Roos 1987; Bunn *et al.* 2003) and bacterial production (Boon 1991; Lind *et al.* 1997; Rees *et al.* 2005a) in turbid dryland aquatic systems, no study has examined the ecosystem as a whole. The purpose of this study was to quantify pelagic and benthic primary production, respiration and heterotrophic bacterial production to obtain an understanding of whole-ecosystem carbon cycling in a turbid, dryland waterhole. Specific aims were to: 1) assess rates of waterhole metabolism during no flow conditions and 2) determine the relative importance of autochthonous and allochthonous sources of carbon for fuelling bacterial metabolism. We used methods measuring changes in dissolved oxygen as well as those employing radioisotope tracers to provide information on different aspects of carbon cycling, including variation in GPP within the narrow photic zone, and to provide independent constraints on components of the waterhole carbon budget.

Materials and Methods

Site Description

The study was conducted at Glen Murken waterhole (S 25°26.9' E 142°40.7'), an in-channel waterhole in Cooper Creek near Windorah, Queensland, Australia.

With a total area of approximately 296,000 km², Cooper Creek is one of the major sub-catchments of the Lake Eyre Basin. The Cooper Creek catchment is in a relatively undisturbed state with no intensive agriculture, low cattle stocking densities (1.2 animal equivalents km⁻², Fisher *et al.* 2004) and a small human population (approximately 12,000 in the catchment; <http://www.lakeeyrebasin.org.au/archive/media/cooper.pdf>). Cooper Creek has an extremely low gradient and is characterised by multiple, anastomosing channels, particularly in the Channel Country near Windorah (See Figure 1 in Bunn *et al.* 2003). The catchment has an arid to semi-arid climate with mean annual rainfall varying from 400 to 500 mm in the northern part of the catchment, 292 mm at Windorah, and to less than 100 mm at its entry to Lake Eyre. Maximum daily air temperatures at Windorah range from 21.4 to 38.1 °C, and minima range from 24 to 7 °C. Stream flow is generated by seasonal monsoons as well as periodic local rainfall, resulting in high inter-annual variability in flow (Puckridge *et al.* 1998; Puckridge *et al.* 2000). With high rates of evaporation (2.9 m yr⁻¹ pan evaporation at Windorah) and high flow variability, long periods of no flow are typical, with surface water confined to isolated waterholes. Analysis of the flow data at the Currareeva gauge near Windorah showed an average of 3 dry spells (no surface flow) per year during the period 1939 – 1989, with more than half lasting longer than 25 days and a quarter lasting greater than 164 days (Bunn *et al.* 2006b). Further description of waterholes in the region can be found in Arthington *et al.* (2005) and Marshall *et al.* (2006), with respect to fauna; McGregor *et al.* (2006), with respect to algae; and Hamilton *et al.* (2005), with respect to hydrology and the physical persistence of waterholes.

The study was conducted approximately 9 months after the last flow, on 28 and 29 September 2001. A large flood event took place in March-April 2000 (see

Bunn *et al.* 2006a), and was followed by a small in-channel flow in December 2000-January 2001. There were no other flow events between then and when the study was conducted. Dimensions of the waterhole were estimated using a combination of field surveys of waterhole cross sections and a long section and remotely sensed imagery (Davis *et al.* 2002). Glen Murken is small to moderate in size compared to other waterholes in the Windorah area (Bunn *et al.* 2003; Hamilton *et al.* 2005). At the time of sampling, the dimensions of Glen Murken were as follows: surface area 13,971 m², perimeter 1,556 m, width 17.5 m, length 771 m, and mean depth 1.03 m.

Community metabolism measurements using changes in dissolved oxygen

Benthic metabolism was determined by measuring changes in DO concentrations in two types of enclosed transparent chambers under both light and dark conditions (Bott *et al.* 1985; Bunn *et al.* 2003; Fellows *et al.* 2006). Four dome-shaped perspex chambers (diameter = 0.295 m, surface area of substrate enclosed = 0.068 m², total height = 0.25 m; 'large chambers'), similar to those used by Bunn *et al.* (2003), were run for 24 h in the photic zone. Smaller cylindrical chambers (diameter = 0.095 m, surface area of substrate enclosed = 0.007 m², total height = 0.010 m; 'small chambers') were also used to make shorter-term measurements at three different depths within the shallow photic zone. The small chambers were deployed in pairs and run for 2 h around mid-day (10:40 – 12:40), with one of each pair covered with metal foil to create dark conditions (total of 6 chambers).

Both large and small chambers had dissolved oxygen (DO) sensors (YSI 5739) located in the top and a pump recirculated water through the chamber to ensure flow saturation across the membrane of the oxygen probe. Each probe was attached to a data-logger (TPS 601), which recorded DO and temperature at 10-min intervals. The

211 chambers were pushed into the sediment to depths of approximately 0.10 m for the
212 large chambers and 0.05 m for the small chambers, with the actual depths of insertion
213 measured and used to calculate water volume for each chamber. The thick clay
214 substrate provided a reliable seal at these depths of insertion. A light meter (Testo
215 545, Testo GmbH & Co., Lenzkirch, Germany) was deployed on the bank near the
216 benthic chambers to record photosynthetically active radiation (PAR) every 10 min
217 over the duration of metabolism measurements. Measurements in units of lux were
218 converted to $\mu\text{mol m}^{-2} \text{ s}^{-1}$ (Wetzel and Likens 1991). Benthic chamber water was
219 partially exchanged with surface water if gas bubbles formed in the chambers due to
220 DO concentrations exceeding 100% saturation.

221 Different components of benthic metabolism were calculated using the rate of
222 DO change in the chambers under daylight and dark conditions. For large chambers,
223 the mean rate of change for 6 to 8 h after dark was taken as the rate of R, and daily
224 respiration was calculated by assuming the rate was constant over 24 h. GPP was
225 calculated as the sum of the DO produced during daylight hours plus the DO
226 consumed by respiration during that period of time based on the night-time respiration
227 rate. Hourly average rates of GPP were also calculated for comparison with the small
228 chambers and the ^{14}C incubations, which lasted for 2 h. Changes in DO
229 concentrations over time ($\text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$) were multiplied by chamber volume and
230 divided by substrate surface area to obtain values in units of $\text{mg O}_2 \text{ m}^{-2} \text{ h}^{-1}$. These
231 rates were converted to units of carbon assuming that one mole of carbon is
232 equivalent of one mole of O_2 for both respiration and photosynthesis (i.e. $1 \text{ mg O}_2 =$
233 0.375 mg C , Lambert 1984; Bender *et al.* 1987; Davies 1994). Calculations of R and
234 GPP for the small chambers were similar to those for the large chambers, except the
235 dark chamber in each pair was used to obtain the rate of respiration. The photic zone

was divided into four sections based on the positions of the three pairs of small chambers. A weighted mean for the whole photic zone was obtained using the proportion of the photic zone represented by each section and either the mean of two rates (at the ends of the section) or single rates (for the shallowest and deepest sections). The rates obtained from the large chambers were applied over the entire photic zone and are presented as the mean of 4 replicates.

Primary production using ^{14}C incorporation

Rates of water column and benthic sediment primary production were determined by incorporation of ^{14}C during 2 h incubations around mid-day (Wetzel and Likens 1991). Water was collected from two depths: 0.03 m (within photic zone) and 0.65 m (below photic zone). 100 ml water samples were placed in 250 mL polycarbonate bottles with 20 μCi of [^{14}C]- NaHCO_3 per bottle (20 $\mu\text{Ci mL}^{-1}$ solution; ICN Biomedicals, Sydney, Australia). Samples were incubated between 11:00 and 13:00 at seven different light intensities created using shade cloth bags: 0 (dark control), 2, 4, 8, 35, 70, and 100% of incident light, with one sample per depth per light level. At the end of the incubations, the bottles were put on ice in the dark, and two 25-mL sub-samples were filtered from each bottle onto 0.45 μm pore size cellulose acetate filters. Both filters were placed into a scintillation vial, and 300 μL of 5 M HCl was added to release inorganic carbon as $^{14}\text{CO}_2$.

Benthic algal production was quantified using in-situ incubations in polystyrene micro-chambers (diameter = 0.025 m, volume approximately 20 mL) from 12:40 – 14:40. The chambers were transparent screw-cap vials modified to have an open bottom and fitted with a rubber septum in the cap. The chambers were deployed at five depths ranging from 0.02 – 0.19 m (with a 6th chamber as a dark

control at the middle depth) by inserting the open bottoms into the clay substrate by at least 0.05 m. The lids were then screwed on and 20 μCi of $[^{14}\text{C}]\text{-NaHCO}_3$ was injected into each chamber using a 1 mL insulin syringe. At the end of the 2 h incubation, the chambers were retrieved by carefully digging below the chamber and inserting a rubber stopper at the base of the chamber. The top 3 mm of benthic sediment was collected by extruding the core contained within the chamber and slicing the sediment into a vial. 1 mL of 5 M HCl was added to each vial immediately following sediment collection.

All samples were kept refrigerated at 4 °C until processed in the laboratory within 1 week of collection. The water column filters were left to dry for 19 h and then 200 μL of distilled water and 7 mL of scintillant were added. Benthic samples were also left for 19 h and then sonicated for 10 sec at 15W (Branson Sonifier 450) to homogenise the samples. Three replicates of 100 μL of benthic sediment were dispensed into scintillation vials, dried at 30 °C for 1.5 h, and 7 mL of scintillant added. Radioactivity was measured using a liquid scintillation counter (Packard 2000 Beta Scintillation Counter Tri-Carb 2000CA Series System). The benthic sample values reported are the mean values of the replicate sub-samples.

Primary production was calculated as described in Wetzel and Likens (1991). Total inorganic carbon concentrations were calculated from measured alkalinity, temperature and pH. Rates from different light intensities (water column) and different depths (benthic) were used to scale rates to the entire photic zone using a weighted mean (as described above for small DO chambers). For water column rates, a representative depth was calculated for each light intensity using the measured extinction coefficient.

286 *Bacterial production*

287 Bacterial carbon production was determined by ^3H -thymidine incorporation
288 into DNA (Moriarty and Pollard 1982; Azam *et al.* 1983). To measure
289 bacterioplankton production, several litres of water from the photic zone (0.03 m
290 deep) were collected and 15 mL samples were incubated with 50 μL of [^3H -methyl]
291 thymidine (2 Ci mmol^{-1} ; ICN Biomedicals, Sydney, Australia) starting at 16:00. A
292 time course was conducted from 0 – 30 min in 5 min intervals to assess the linearity
293 of incorporation (total of 7 samples). At each time point, the incubation was stopped
294 with 1 mL of 100 mmol unlabelled thymidine solution followed by 300 μL of 37%
295 formaldehyde (buffered to pH 7). Samples were kept at 4 $^{\circ}\text{C}$ until processed in the
296 laboratory, where 5-mL sub-samples were taken from each 15-ml sample and filtered
297 onto 0.2 μm pore size polycarbonate filters (pre-soaked in 1 mmol unlabelled
298 thymidine). The filter was then washed with 5% cold trichloroacetic acid followed
299 by 80% cold ethanol, and 1 mmol unlabelled thymidine. Filters were placed in
300 scintillation vials with 200 μL 5 M HCl, heated at 100 $^{\circ}\text{C}$ for 30 min, and then 250 μL
301 of distilled water and 5.5 mL of scintillant added. The filters were radioassayed using
302 a liquid scintillation counter within 1 week of collection. Each 15 ml sample yielded
303 3 sub-samples that were filtered and counted separately, and their resulting values
304 averaged to represent the time point. The rate of thymidine incorporation reported is
305 the mean of the six rates determined from time points 5 – 30 min. Rates of thymidine
306 incorporation were converted to rates of bacterial production in units of carbon as
307 described by Pollard and Greenfield (1997). A value of carbon content per cell is
308 required for this conversion, and values vary substantially across environments
309 (Nagata 1986). Two values were considered to provide high and low bounds to the
310 rates of bacterial carbon production. The lower value was set at 6 fg cell^{-1} given by

Rees et al. (2005a) for bacterioplankton in another Australian dryland river, the lower Murray River. The high value was 34 fg cell^{-1} based on average cell size of $34 \mu\text{m}^{-3}$ obtained from measurements of water column and benthic bacteria from a waterhole near Glen Murken (M. Burford and A.Cook, unpublished data) and a carbon concentration of $100 \text{ fg } \mu\text{m}^{-3}$ taken as typical from a review of multiple pelagic systems (Nagata 1986).

To determine bacterial abundance, three 15 mL samples of the water were also taken and preserved with formaldehyde for bacterial counts. In the laboratory, three sub-samples were taken from each sample, diluted to a ratio of 1:10 with distilled water, sonicated, and filtered onto black polycarbonate $0.2 \mu\text{m}$ pore size filters. Bacteria were counted using epifluorescence at magnification of 1000x following staining with SYBR Green II (Molecular Probes). A minimum of 65 cells were counted per subsample. Cell densities of the three sub-samples were averaged to represent each sample, and the value of water column bacterial abundance reported is the mean of the three samples.

Benthic bacterial production was measured similarly to that of bacterioplankton, with the addition of two more concentrations of ^3H -thymidine due to the uncertainties associated with using the method in sediments with a high clay content. A composite sample of benthic sediment was collected with a flat trowel by scraping the top 3 mm of sediment from a delineated area in the photic zone (0.06 m^2). The sediment was homogenised and $200 \mu\text{L}$ of this slurry used for each sample. A time course starting at 17:00 was conducted for each of 3 different concentrations (50, 100, $150 \mu\text{L}$) of [^3H -methyl] thymidine (2 Ci mmol^{-1}) to assess linearity of incorporation and determine the appropriate concentration. The incubations were conducted as described for bacterioplankton above, and kept at 4°C until processed in

the laboratory. The samples underwent serial dilutions using 18 MΩ deionised water, and were sonicated prior to removal of sub-samples that were filtered onto 0.2 µm pore size polycarbonate filters. Three sub-samples were filtered, each containing 1/175 of the original sediment sample. The filters were then processed and rates of bacterial production calculated as described above for the bacterioplankton.

Three 200 µL samples of slurry were also taken at the time of sampling and preserved with formaldehyde for bacterial counts. The procedure followed was similar to that for the bacterioplankton counts, with greater dilution and fewer subsamples. Two sub-samples were taken from each sample, and serial dilutions with sonification and vortexing were carried out. The final amount of sediment on each filter was 1/2500 of the original sample. Cell densities of the two sub-samples were averaged to represent each sample, and the value of benthic bacterial abundance reported is the mean of the three samples.

Production and respiration at the waterhole scale

Waterhole dimensions and measured rates of GPP and R were used to calculate rates of carbon consumption and production for the entire waterhole. For consistency of comparison between GPP and R, values were taken from 24 h large chamber incubations, from which both measurements were obtained. Because of the shallow bathymetry, the waterhole benthic zone was assumed to have the same surface area as that of the waterhole. The benthic photic zone area was calculated as the width of the zone parallel to the bank slope, multiplied by the perimeter. The mean rate of benthic GPP was applied to the photic zone area. The portion of the benthic zone not in the photic zone was assumed to make no contribution to primary production (see Bunn et al. 2003). For benthic respiration, the rates measured in the

photic zone were treated the same way as GPP. The rest of the benthic zone was assumed to have a rate of R of $0.05 \text{ g C m}^{-2} \text{ d}^{-1}$, the mean value of channel bottom R measured using similar benthic chambers in Murken waterhole, a waterhole in close proximity to Glen Murken (2 locations within the waterhole measured September 1996 and May 1997; range 0.047 to $0.054 \text{ g C m}^{-2} \text{ d}^{-1}$; mean water depth of chambers = 0.52 m ; Sites ID49 and ID50, Table 2 in Bunn *et al.* 2003).

The volume of water in the photic zone was calculated by multiplying the photic zone depth by the surface area of the waterhole. The remainder of the waterhole volume was calculated as the average waterhole depth minus the photic zone depth times the surface area. Water column GPP and R were obtained in a nearby Murken waterhole in October 2004. Three of the large chambers used for benthic measurements were fitted with lids (water volume 9 L) and were suspended in the water column in the photic zone ($0 - 0.25 \text{ m}$) and two chambers below the photic zone (approx. $0.70 - 1 \text{ m}$). Measurements were made over 24 h , and calculations were made as described for the large benthic chamber incubations. Conditions were comparable with those at the time of sampling Glen Murken, including greater than 6 months since flow, the same photic depth of 0.25 m and similar mean water temperature (21°C). Murken had slightly lower water column chlorophyll a (5 mg m^{-3} compared to 11 mg m^{-3}) but higher nutrient concentrations ($\text{TN} = 1.5 \text{ mg L}^{-1}$, nitrate- $\text{N} = 0.49 \text{ mg L}^{-1}$, total phosphorus (TP) = 0.48 mg L^{-1} ; compare to Table 1 values for Glen Murken).

The values from Murken water column metabolism were applied directly to Glen Murken as opposed to using an approach of estimating GPP based on TP or chlorophyll a (sensu del Giorgio and Peters 1994) because 1) the rankings of TP and chlorophyll a concentrations were in opposite directions for the two waterholes; and

2) the waterhole photic depths were identical, and it is assumed that light limitation is the dominant control on GPP in these extremely turbid systems. This assumption is supported by the strong relationship between benthic GPP and turbidity reported by Davis et al. (2002) from a study of 15 Cooper Creek waterholes that included Murken and Glen Murken. Rates of GPP and R from water column chambers in photic zone were applied to the volume of the photic zone, and the deeper chamber values (which had no measurable GPP) were used to calculate R for the rest of the waterhole volume. Net ecosystem production (NEP) was also calculated for the waterhole components by subtracting R from GPP.

Comparisons of bacterial metabolism with rates of GPP and R

Because bacterial production measurements were restricted to the photic zone, they were principally compared to primary production and respiration rates on the basis of a unit area of the benthic photic zone and a unit volume of the water column photic zone. Rates of bacterial production were used to calculate bacterial respiration and carbon demand over a range of bacterial growth efficiency (BGE) values. BGE was defined as bacterial production divided by bacterial carbon demand (the sum of bacterial production and respiration, Cole *et al.* 1988). Plots of calculated bacterial respiration ($\text{mg C m}^{-2} \text{ h}^{-1}$ or $\text{mg C m}^{-3} \text{ h}^{-1}$) versus BGE were compared to values of community R measured using DO chambers (both large and small for benthic R). Similarly, calculated total bacterial carbon demand (bacterial production plus respiration; $\text{mg C m}^{-2} \text{ h}^{-1}$ or $\text{mg C m}^{-3} \text{ h}^{-1}$) was compared to values of GPP measured using DO chambers (both large and small for benthic GPP). These comparisons are a simplification of the actual carbon dynamics of the system, as community R includes the R of all autotrophs and heterotrophs in the system, not just bacteria, and similarly,

not all of GPP is available as carbon for consumption by bacteria. However, these comparisons provide a perspective on the relative magnitudes of the processes. For comparison at the entire waterhole scale, bacterial rates were assumed to be constant over 24 h, benthic rates were multiplied by the total area of the waterhole, and bacterioplankton rates were applied to the entire volume of the waterhole.

Water chemistry and other supporting data

Sediment and water column samples were collected for chlorophyll *a* analysis. Sediment samples consisted of the top 5 mm of sediment pooled from three cores taken at each of the five depths where ¹⁴C benthic incubations took place. Samples were kept frozen and in the dark until extraction with acetone and spectrophometric analysis for chlorophyll *a* (with acidification correction for phaeophytin) was conducted in the laboratory (Lorenzen 1967). Benthic chlorophyll was expressed as mg chlorophyll *a* m⁻² using the total surface area of the cores. Water column samples were collected at 4 depths, including the two depths at which water was collected for ¹⁴C water column incubations. Each sample consisted of 4 pooled subsamples, each being a 0.45 µM cellulose membrane filter which had been used to filter 30 mL of water. Samples were extracted in acetone and absorbance measured on a spectrophotometer as described above.

The water column was characterised with a combination of in situ measurements and samples collected and preserved for later analysis. Irradiance was measured at incremental depths using a Licor quantum sensor (LI-192, LI-COR, Lincoln, USA). The resulting light profile was used to calculate the extinction coefficient and the depth of photic zone was defined as 1% of incident light (Wetzel and Likens 1991). Ambient water temperature and DO at 0.2 m and 0.65 m deep

were recorded at 10 min intervals for 24 h using DO probes fitted with stirring devices and data-loggers (TPS 601). Turbidity and pH were measured on a depth-integrated 10 L sample of the water column. An additional integrated sample of the water column was collected and two subsamples stored for water chemistry analysis in the laboratory. One sample was frozen and analysed for total phosphorus, total nitrogen, and nitrate. The other sample was kept at room temperature and analysed for alkalinity. Standard analysis methods were used and more details can be found in Marshall et al. (2006).

Results

Water quality

As is typical for Cooper Creek waterholes, the water column of Glen Murken was very turbid, with a value of over 250 nephelometer turbidity units (NTU) and a photic zone depth of 0.25 m (Table 1). The bank slope in the area in which benthic metabolism measurements were made was 23 degrees, and coupled with the photic zone depth, resulted in a calculated width of 0.64 m for the benthic photic zone (parallel to the bank slope). Nutrient concentrations were relatively high, at 1.2 mg L⁻¹ total nitrogen and 0.27 mg L⁻¹ total phosphorus. Deep and shallow dissolved oxygen and temperature values over 24 h indicated that the water column was stratified. Within the photic zone, diel water temperature ranged from 21 to 28°C, but the temperature was consistently lower deeper in the water column (mean 20°C) with a temperature range of only 1 degree (Table 1). Dissolved oxygen concentrations were also consistently lower in the deeper water, with a mean of 3.7 mg L⁻¹ compared to 8.4 mg L⁻¹ at the shallow depth. The shallow depth was at or above saturation for

about half the time, ranging from 80 to 130% saturation, while the deeper depth was below saturation the entire time, ranging from 30 to 70%.

Community metabolism using changes in dissolved oxygen concentrations

Large chambers covered the benthic zone in water 0.09 – 0.19 m deep, approximately half of the benthic photic zone. The average water depth at the centre of the chambers was 0.14 m. Gross primary production had a mean value of 0.55 g C m⁻² d⁻¹ (SE ± 0.02), while mean R was slightly lower at 0.49 g C m⁻² d⁻¹ (SE ± 0.03), resulting in mean GPP/R of just over 1. Rates of GPP generally increased with increasing irradiance until 11 am, when GPP decreased despite high irradiance (Figure 1). Maximum rates of GPP occurred between 9:00 and 11:00, with lower hourly averages during the periods of ¹⁴C benthic incubations (Figure 1; Table 2). DO concentrations in the chambers exceeded 100% saturation for 4 to 6 h starting from approximately midday. Removal of gas bubbles and partial exchange of chamber water did not alter the saturation status noticeably because surface water was greater than 100% saturation during the same time. Saturated conditions may have resulted in an underestimate of benthic GPP. The decrease in GPP after 11:00 also may have been a result of photoinhibition.

The mean rate of GPP measured in small chambers was very similar to that of the large chambers for the same time period (Table 2). Changes in GPP over time in the small chambers tracked the changes in large chambers, with values decreasing after 11:30 (Figure 1). Respiration in the dark chambers caused DO concentrations to drop rapidly, and therefore only the first 30 min of data for both GPP and R were used in calculating rates for comparison with other methods. Rates of GPP were similar across the three different depths at which chambers were deployed, with a slight

485 decrease with depth and a mean of $96 \text{ mg C m}^{-2} \text{ h}^{-1}$ ($\text{SE} \pm 5$). The rate of R in the
486 shallow depth was somewhat higher than the other two depths, and the mean across
487 the three depths was $72 \text{ mg C m}^{-2} \text{ h}^{-1}$ ($\text{SE} \pm 7$).

488 Rates of water column GPP and R obtained from photic zone chamber
489 incubations in Murken waterhole in October 2004 were $2.43 \text{ g C m}^{-3} \text{ d}^{-1}$ ($\text{SE} \pm 0.18$)
490 and $0.82 \text{ g C m}^{-3} \text{ d}^{-1}$ ($\text{SE} \pm 0.09$), respectively. A GPP/R ratio greater than one is
491 consistent with the ambient DO measurements made in Glen Murken which showed
492 DO concentrations greater than 100% saturation for 13 hr, from 11 am to midnight.
493 Chambers outside the photic zone had no measurable GPP, and a rate of 0.31 g C m^{-3}
494 d^{-1} ($\text{SE} \pm 0.07$) for R. These values scaled to the photic zone and total depths of Glen
495 Murken were $0.61 \text{ g C m}^{-2} \text{ d}^{-1}$ for GPP and $0.44 \text{ g C m}^{-2} \text{ d}^{-1}$ for R.

496

497 *Rates of primary production using ^{14}C incorporation*

498 Values of benthic algal production measured using ^{14}C incorporation were
499 lower than those measured using changes in DO, ranging from $7 \text{ mg C m}^{-2} \text{ h}^{-1}$ at 0.14
500 m deep to $32 \text{ mg C m}^{-2} \text{ h}^{-1}$ at 0.07 m deep, with a mean of $15 \text{ mg C m}^{-2} \text{ h}^{-1}$ ($\text{SE} \pm 4$)
501 (Table 2). The area weighted rate for the whole photic zone (using the rates from all 5
502 depths) was $10 \text{ mg C m}^{-2} \text{ h}^{-1}$. Chlorophyll *a* values ranged 3.8 to 14.7 mg m^{-2} , and
503 showed no consistent pattern with depth.

504 Rates of water column algal production using ^{14}C ranged from $10 \text{ mg C m}^{-3} \text{ h}^{-1}$
505 for deep water with 2% light availability to $65 \text{ mg C m}^{-3} \text{ h}^{-1}$ for shallow water with
506 35% light. The highest production values were at 70 and 35% light transmission for
507 both depths of water incubated. Photic zone water had greater rates of production
508 than that from below the photic zone for all light levels except for 70%. Across the 6
509 light intensities, the mean rates of production for shallow and deep water were 34 mg

C m⁻³ h⁻¹ (SE ± 9) and 24 mg C m⁻³ h⁻¹ (SE ± 10), respectively. These values were similar to the average rate during daylight hours of 22 mg C m⁻³ h⁻¹ obtained using changes in DO in chambers suspended in the photic zone. However, when the rates of production using ¹⁴C were volume-weighted over the depth of the photic zone (using values from the shallow water incubation), the value was lower (9 mg C m⁻² h⁻¹). Chlorophyll *a* values at the two depths from which water was sampled for ¹⁴C incubations were very similar, at 15 and 13 mg m⁻³. The mean of the four depths from which chlorophyll samples were taken was 11 mg m⁻³ (SE ± 2).

Bacterial production

The rate of thymidine incorporation by bacterioplankton was linear ($r^2 = 0.98$, $p = 0.00002$), and the mean rate of bacterial production across the 6 time points was 0.067 g C m⁻³ h⁻¹ (SE ± 0.004) using the conversion of 6 fg C cell⁻¹ and 0.373 g C m⁻³ h⁻¹ (SE ± 0.02) using the conversion of 34 fg C cell⁻¹. Assuming the rate was consistent throughout the water column and over 24 h, the rates of bacterial production were 1.63 g C m⁻² d⁻¹ (SE ± 0.10) and 9.22 g C m⁻² d⁻¹ (SE ± 0.50), respectively. Mean bacterioplankton abundance was 5.48 x 10¹³ cells m⁻³ (SE ± 8.26 x 10¹²).

The rate of thymidine incorporation by benthic bacteria was linear for all three thymidine concentrations ($r^2 > 0.7$; $p < 0.01$ for all 3). The two higher concentrations (100 and 150 µL) yielded similar calculated rates, and were higher than the rate calculated for 50 µL. The rate from the intermediate concentration was used based on the higher blank value (time zero) for the highest concentration, and the assumption that the lower concentration was not saturating. The mean rate of benthic bacterial production across the 6 time points was 0.073 g C m⁻² h⁻¹ (SE ± 0.013) using a

conversion factor of 6 fg C cell⁻¹ and 0.441 g C m⁻² h⁻¹ (SE ± 0.071) using 34 fg C cell⁻¹. These values yield daily rates of 1.75 g C m⁻² d⁻¹ (SE ± 0.30) and 9.93 g C m⁻² d⁻¹ (SE ± 0.17), respectively. Mean benthic bacteria abundance was 5.39 x 10¹² cells m⁻² (SE ± 1.55 x 10¹²).

Production and respiration at the waterhole scale using large metabolism chambers

A very small portion of the waterhole benthic zone was in the photic zone (just under 8%). In contrast, because of the shallow mean depth of the waterhole, 24% of the water volume was in the photic zone. The benthic photic zone alone was marginally autotrophic with values of 1.1 for P/R and 59 g C d⁻¹ for NEP (Table 3). However, with the inclusion of the deeper benthic habitat, the overall benthic zone was heterotrophic with a P/R value of 0.5 and NEP of -589 g C d⁻¹. The water column photic zone alone was highly autotrophic, with a P/R value of 3.0 (NEP = 5624 g C d⁻¹), and even with the addition of the deeper water, the water column was still autotrophic (P/R = 1.4; NEP = 2273 g C d⁻¹). The benthic zone contributed only 5.7% of waterhole GPP and 15.5% of waterhole R, and due to this relatively small influence, the overall whole waterhole was autotrophic with a P/R of 1.2 and NEP of 1683 g C d⁻¹ (Table 3).

Comparisons of bacterial metabolism with rates of GPP and R

Calculations of bacterial R using the higher value of 34 fg cell⁻¹ for carbon content resulted in values that exceeded measured total R for all values of BGE for both the benthic and pelagic zones (BGE = 0.2 – 0.8; Figure 2). In contrast, for values of 6 fg cell⁻¹, calculated benthic bacterial R was equal to measured benthic R at a value of BGE of 0.51 for the maximum rate of measured respiration (small DO

chambers) and a value of 0.78 for the average (large DO chambers, night time) rate. Calculated bacterioplankton R (using 6 fg cell⁻¹) and measured water column R were equal at a value of BGE of 0.66. Similar to comparisons of calculated bacterial R and measured R, bacterial C demand greatly exceeded GPP for all values of BGE for calculations using 34 fg cell⁻¹. For the benthic zone, calculated bacterial C demand also exceeded the maximum value of measured GPP for the entire range of BGE values when 6 fg cell⁻¹ was used, but values of demand and GPP were similar at high BGE values. In contrast, water column GPP exceeded bacterial carbon demand for values of BGE greater than 0.3 when 6 fg cell⁻¹ was used.

Water column and benthic bacterial production were very similar per m² of waterhole. Benthic bacterial production extrapolated to the whole waterhole was 24,400 g C d⁻¹ (lower cell carbon content) or 139,000 g C d⁻¹ (higher cell carbon content). Bacterioplankton production was 22,700 g C d⁻¹ or 129,000 g C d⁻¹, respectively. Either the benthic or pelagic zones taken separately (for either C conversion factor) were considerably more than the 9,000 g C d⁻¹ GPP of the entire waterhole.

Discussion

Waterhole metabolism

At the scale of the entire waterhole, Glen Murken was autotrophic for this snapshot in time based on results from the large metabolism chambers. With an overall P/R value of 1.2, the waterhole was only slightly autotrophic, with GPP in excess of R by 1,700 g C d⁻¹. A significant import or storage/export term for organic carbon was not required to balance the carbon budget of the waterhole given the nearly equal values of GPP and R.

Cross-site comparisons have suggested a relationship between trophic status and water column metabolic balance, with metabolic balance shifting from heterotrophic to autotrophic as trophic status becomes more enriched (del Giorgio *et al.* 1997; Cole *et al.* 2000; Hanson *et al.* 2003). Following this relationship, Glen Murken would be grouped with eutrophic lakes based on its slightly autotrophic status (this does not imply cultural eutrophication of the waterhole; both land use intensity and human population density are very low in the catchment). Current models based on northern hemisphere temperate lakes suggest that not just trophic status, but specifically both total phosphorus (TP) and dissolved organic carbon (DOC), are important controls on the metabolic balance of epilimnia, with high TP and low DOC resulting in autotrophy and the opposite resulting in heterotrophy (Hanson *et al.* 2003). To expand this model to a wider range of waterbodies, we propose that these two factors could be more generally expressed as inorganic nutrient concentrations and allochthonous C inputs. Glen Murken falls on the high inorganic nutrient and low allochthonous (see below) end of the spectrum, and this is expected to be the case for the entirety of the no flow period. Glen Murken metabolism rates for the water column separately and for the entire waterhole are consistent with lakes in this group being autotrophic. This general model seems to apply to Glen Murken despite its development focusing on epilimnion metabolism and not explicitly including turbidity as a factor, but the potential influence of light limitation will need to be explored by testing the model in a wider range of turbid systems.

Because Glen Murken and most other waterholes in the catchment are very shallow, the benthic zone would be expected to be important to overall waterhole metabolism based on general trends observed for shallow lakes (Vadeboncoeur *et al.* 2002). However, because of the high turbidity, very little of the benthic area was in

the photic zone and the contribution of benthic GPP to total waterhole GPP was small (6% based on large DO chamber measurements). Because of the limited areal extent of the photic zone, the contribution of the benthic GPP would still be minor even if rates of benthic GPP were underestimated due to oxygen saturation. Although light penetration in Glen Murken is limited by inorganic turbidity, the reduced role of benthic GPP is similar to that reported for many eutrophic lakes in which high phytoplankton densities shade the benthic zone (Vadeboncoeur *et al.* 2002; Liboriussen and Jeppesen 2003). In contrast, respiration is not constrained by light, and so the overall benthic contribution might be expected to be somewhat greater than for GPP. While the benthic zone did make a larger contribution to total R than to total GPP, the contribution was still only 16% because rates of R were relatively low outside the photic zone. Low rates of R outside the photic zone suggest a tight link between algal production and R, with the autotrophs themselves contributing to R as well as heterotrophs respiring autochthonous carbon. Evidence of this link is also provided by the highly significant linear relationship between benthic GPP and R across 12 sites reported by Bunn *et al.* (2003).

The Glen Murken benthic zone was heterotrophic, in contrast to the findings of Bunn *et al.* (2003) across 12 waterholes in the same catchment. Similar to this study, they found that the photic zone was typically a small proportion of the waterhole benthic zone (4 – 8%), but at the time of their measurements, benthic GPP was so high that the overall benthic zone was autotrophic ($GPP = 2.02 \pm 0.25$; $R = 1.36 \pm 0.18$; (mean $g\ C\ m^{-2}\ d^{-1} \pm SE$)). Their study was conducted in September 1996 and May 1997, and while there were small flow events in 1994 and between their sampling times, overall there was limited flow since the last major floods in 1990 and 1991. In comparison, we sampled Glen Murken approximately 20 months after a

major flood in 2000. While drawing general conclusions about trophic status would require temporal assessment of metabolism, the differences in time since flood and benthic GPP between these two studies suggest that longer durations of low flow or no flow may lead to a greater degree of autotrophy, at least for the benthic zone. This supports the proposal of Bunn et al. (2006b) that long periods of flow stability may lead to the formation of productive littoral zone 'bath-tub' rings of algae. While Bunn et al. (2003) did not specifically measure water column metabolism in their study, maximum values of ambient dissolved oxygen in all waterholes exceeded 100% saturation during the day, suggesting that the epilimnia were also autotrophic. Water column metabolism dominated the waterhole totals in the current study, pointing to the need for further research to understand relationships between flow regime and both water column and benthic production (Bunn *et al.* 2006b).

Bacterial production and sources of carbon

Rates of bacterial production varied by more than 5x based on using low and high values of cell carbon content (6 and 34 fg C cell⁻¹), but some insight can be gained into where the actual values might lie based on measured rates of community R. If values of community R obtained using dissolved oxygen changes in metabolism chambers are used to set an upper bound for bacterial R, then the value of cell carbon content is closer to 6 fg C cell⁻¹. Community R measurements may have underestimated total R because changes in dissolved oxygen concentration do not account for anaerobic respiration. It is unlikely that anaerobic processes are significant in the water column, but they could be in the sediment. Some evidence of benthic methane production was found in similar waterholes by Bunn et al. (2003) based on the ¹³C-depleted carbon stable isotope signatures of chironomid larvae

collected from leaf packs. Depending on the fraction of R that is anaerobic, actual benthic R could be higher than what was measured in the present study, suggesting higher bacterial cell carbon contents/lower BGE, a more heterotrophic benthic zone, and a greater reliance on allochthonous carbon.

The potential importance of autochthonous carbon for fuelling bacterial carbon demand is suggested both by the autotrophic metabolic balance of Glen Murken waterhole and the lack of available sources of allochthonous carbon. The whole-waterhole P/R value of 1.2 (based on scaling up large DO chamber measurements) suggests that there are not large amounts of allochthonous organic carbon currently being respired. In fact, few pathways for allochthonous inputs to the waterhole exist under conditions of no flow. Allochthonous carbon enters other types of aquatic ecosystems through groundwater inputs, transport from upstream or inlet streams, and from riparian and catchment vegetation (Fisher and Likens 1973; Aitkenhead *et al.* 2003). Across a wide range of waterholes in this area of the Cooper Creek catchment, there are no groundwater inputs (Hamilton *et al.* 2005). During no flow conditions, there are limited opportunities for surface hydrologic transport, and whereas riparian vegetation could fall directly in or be blown in, both riparian and terrestrial vegetation are sparse in this environment (Capon 2003). Additionally, there does not appear to be large amounts of particulate organic matter ($>250\ \mu\text{m}$) stored in the littoral sediments, with a mean of $55 \pm 18\ \text{g C m}^{-2}$ (mean $\pm$ SE, $n = 14$) in nearby Murken waterhole (S. Bunn, unpublished data). Allochthonous inputs may be of increasing importance with in-channel flows and overbank floods, but algal production is still a major source of carbon during floods and benthic GPP exceeds R on the inundated floodplain within a few days of wetting (Bunn *et al.* 2006a).

The magnitude of GPP relative to calculated bacterial carbon demand also suggests the relative importance of autochthonous carbon to bacteria. Bacterial production measurements were conducted only during the day and in the photic zone, but at least for this time point, it appears that bacterial organic carbon demand in the photic zone could be met by autochthonous production (assuming the lower value of cell carbon content). Benthic bacterial carbon demand exceeded benthic GPP values (from both small and large DO chambers), but water column GPP (from large DO chambers) substantially exceeded water column bacterial demand. There are difficulties in scaling measurements to longer time frames and larger spatial areas than those at which the measurements were made, but if rates are assumed to be constant over time and throughout the water column/benthic zone, bacterial demand exceeds autochthonous production in the waterhole by approximately 5 times. However, if bacterial rates track GPP, then they would likely be lower at night and outside the photic zone, and demand might be entirely met from within-waterhole production. While multiple lines of evidence point to an important role for autochthonous carbon in bacterial production, a more quantitative assessment requires greater certainty of bacterial carbon content and BGE, and more information on spatial and diel variation in bacterial production.

Comparing methods of measuring primary production

Daily values of benthic and water column GPP were required for constructing a waterhole carbon budget, but at the same time, spatial variation within the photic zone was also of interest. Within the photic zone, rates of production obtained using ¹⁴C incubations showed more variation than those obtained using small DO chambers. From the range of 0.02 – 0.19 m depth over which the ¹⁴C chambers were deployed,

709 rates of production were lower both at shallow and deep depths. This suggests that
710 conditions may be harsher at the water's edge, possibly due to wave action, changes
711 in water level over time, and/or photoinhibition. It is also possible that heterogeneity
712 in the benthic community at the scale of the 0.025 m diameter chambers, and not a
713 systematic difference in algal production, could have resulted in the variation
714 observed. A lower rate was not observed in the shallowest of the small DO chambers,
715 with a decrease in GPP observed across the three chambers from 0.02 to 0.15 m
716 depth. However, the shallowest chamber protruded from the water by 0.01 m,
717 effectively increasing the overlying water depth, and possibly lowering the light
718 enough to prevent photoinhibition. Within the water column ^{14}C incubations,
719 photoinhibition was suggested at 100% light transmission, with higher rates at 70%
720 and 35% light; below 35%, rates decreased with decreasing light levels.

721 Rates of GPP in large and small benthic DO chambers were very similar for
722 the period of time in which the incubations overlapped, while rates from ^{14}C
723 incubations were lower. Water column ^{14}C rates were also lower than rates from DO
724 chambers, but this comparison is complicated by the fact that the values are from
725 different waterholes at different times. Radioisotopes and DO methods measure
726 different aspects of metabolism, and differences in rates have been reported by several
727 comparative studies (e.g. Bender *et al.* 1987; Bott *et al.* 1997; Ostrom *et al.* 2005).
728 Values obtained from ^{14}C incubations are generally thought to lie somewhere between
729 GPP and net primary production (Bender *et al.* 1987), and are therefore expected to be
730 lower than GPP measured by DO changes. In the case of these incubations, the static
731 nature of the small ^{14}C incubation chambers compared to the water circulation in the
732 DO chambers also may have influenced the rates obtained. While the two types of
733 methods yielded different rates of primary production, they showed consistent trends

in comparing water column and benthic primary production. Per m² of the photic zone, ¹⁴C values for benthic and water column production were very similar to each other, at 10 and 9 mg C m⁻² h⁻¹, as were values obtained from the large DO chambers at 0.55 and 0.61 g C m⁻² d⁻¹.

Dryland waterholes compared to other aquatic ecosystems

Rates of benthic GPP and R for the photic zone of Glen Murken were at the low end of the range of values reported for desert streams and rivers in a review by Bunn et al. (2006a). Glen Murken rates were much lower than most rivers in the USA, as well as rivers in Egypt and Spain, but were similar to or somewhat higher than the Warrego, Ord, and Robe rivers in Australia. Values of benthic R measured across multiple seasons in three Australian lowland rivers were all lower than that of Glen Murken, with the exception of two high summer values (Rees *et al.* 2005b). Rates of water column GPP for Glen Murken were within the range of values reported for phytoplankton production in the Vaal River, South Africa, and were slightly greater than annual average values for a shallow turbid lake in Mexico (Lind *et al.* 1997).

Water column and benthic bacterial production in Glen Murken were generally greater than that reported for a range of rivers and shallow lakes. The rate of water column bacterial production in Glen Murken was 5 – 7 times greater than those in the lower Murray, and was comparable to the highest value measured during an elevated flow event that was hypothesised to bring in high amounts of allochthonous carbon (Rees *et al.* 2005a). Water column production at Glen Murken was also greater than at wetlands and the main channel of the Murray River measured by Boon (1991); a shallow turbid lake in Mexico (Lind *et al.* 1997); shallow

759 macrophyte-rich lakes in Denmark (Theil-Nielsen and Sondergaard 1999); a sand-bed
760 river in Germany (Fischer and Pusch 2001); and a blackwater river (Edwards *et al.*
761 1990) and a wetland (Stanley *et al.* 2003) in the southern United States. Three of the
762 studies also measured benthic bacterial production, and production in Glen Murken
763 was greater than that reported by Edwards *et al.* (1990) and Stanley *et al.* (2003), but
764 fell within the range of values reported by Fischer and Pusch (2001). Fischer and
765 Pusch (2001) suggest that the high autochthonous production associated with
766 extensive submerged macrophytes as well as the rapid exchange of pore water in the
767 sand bed of the River Spree may have led to the observed greater abundance and
768 productivity of sediment bacteria compared to most other systems that have been
769 studied (which have been dominated by allochthonous carbon inputs). While the two
770 systems are quite different in most respects, it also may be that autochthonous
771 production in Glen Murken explains the relatively high bacterial production in the
772 waterhole compared to most other studies. Studies with concurrent measurements of
773 primary production, respiration, and bacterial production are needed both for the
774 different information that these methods yield, but also for complementary nature of
775 the methods in terms of providing constraints on some of the assumptions made.

776 Despite high inorganic turbidity and an associated narrow photic zone, Glen
777 Murken waterhole was slightly autotrophic under the no flow conditions of the study.
778 With primary production presumably limited by light in this turbid, high nutrient
779 environment, the waterhole might be expected to be strongly heterotrophic. However,
780 allochthonous inputs appeared to be low. High turbidity also resulted in a limited
781 contribution of the benthic zone to overall waterhole GPP, in contrast to observations
782 of clear shallow lakes, but similar to those for shallow eutrophic lakes with biogenic
783 turbidity. While pelagic zone metabolism dominated overall waterhole metabolism,

previous studies using stable isotopes have emphasised the importance of benthic algal carbon to waterhole food webs in this region (Bunn and Davies 1999; Bunn *et al.* 2003). Our findings suggest that autochthonous carbon also may be quantitatively important for fuelling bacterial production and to overall ecosystem carbon budgets of these turbid, dryland river waterholes.

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1003 **Table 1. Physical and chemical characteristics of the water column at Glen**
 1004 **Murken waterhole.**
 1005 Water temperature and dissolved oxygen (DO) were logged in situ for 24 h. See text
 1006 for additional details of measurements.

Parameter	
Light extinction coefficient (m^{-1})	18.6
Photic zone depth (m)	0.25
Turbidity (NTU)	266
Alkalinity (meq L^{-1})	1.52
Total nitrogen (mg L^{-1})	1.2
Nitrate-nitrogen (mg L^{-1})	0.50
Total phosphorus (mg L^{-1})	0.27
pH	7.5
Mean water temperature 0.20 m deep (min and max) ($^{\circ}\text{C}$)	23.7 (21.2 – 28.2)
Mean water temperature 0.65 m deep (min and max) ($^{\circ}\text{C}$)	19.9 (19.6 – 20.5)
Mean DO concentration 0.20 m deep (min and max) % of saturation	100 (79 – 134)
Mean DO concentration 0.65 m deep (min and max) % of saturation	41 (26 – 70)

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1009 **Table 2. Comparison of benthic primary production rates obtained using**
1010 **changes in dissolved oxygen and $^{14}\text{C-HCO}_3$ incubations in sealed, in-situ**
1011 **chambers.**
1012 Individual values and means ($\pm$ SE) are shown, with the exception of the large
1013 dissolved oxygen chambers for which only the mean ($\pm$ SE) of 3 replicates is given.

Method	Mean depth (m)	Min. and Max depth (m)	Primary production ($\text{mg C m}^{-2} \text{ h}^{-1}$)	Respiration ($\text{mg C m}^{-2} \text{ h}^{-1}$)
Dissolved oxygen, large chambers (0.30 m diameter)				
10:40 – 11:10	0.14	0.09-0.19	82 ± 4	21 ± 1
12:40 – 14:40	0.14	0.09-0.19	51 ± 3	21 ± 1
Dissolved oxygen, small chambers (0.10 m diameter), 10:40 – 11:10				
	0.03	0.02-0.04	104	85
	0.09	0.08-0.10	97	69
	0.14	0.13-0.15	86	61
mean			96 ± 5	72 ± 7
area weighted mean			93	69
$^{14}\text{C-HCO}_3$, microchambers (0.025 m diameter), 12:40 – 14:40				
	0.02	--	12	--
	0.05	--	15	--
	0.07	--	32	--
	0.14	--	7	--
	0.19	--	10	--
mean			15 ± 4	
area weighted mean			10	--

1014

Table 3. Whole-waterhole production and respiration.

Rates of gross primary production (GPP) and respiration (R) obtained using changes in DO in large metabolism chambers incubated for 24 h in the benthic zone and water column were scaled to the entire waterhole using the relative area or volume of the habitat type.

	Benthic Zone					Water Column					Waterhole Totals			
	area (m ²)	GPP (g C d ⁻¹)	R (g C d ⁻¹)	NEP (g C d ⁻¹)	P/R	volume (m ³)	GPP (g C d ⁻¹)	R (g C d ⁻¹)	NEP (g C d ⁻¹)	P/R	GPP (g C d ⁻¹)	R (g C d ⁻¹)	NEP (g C d ⁻¹)	P/R
Photic zone	996	550	491	59	1.1	3493	8488	2864	5624	3.0	9038	3355	5683	2.7
Aphotic zone	12975	0	649	-649	0	10897	0	3351	-3351	0	0	4000	-4000	0
Total	13971	550	1139	-589	0.5	14390	8488	6215	2273	1.4	9038	7355	1683	1.2

Figure legends

Figure 1. Benthic primary production measured with three methods. Changes in dissolved oxygen (DO) concentrations in large (open circles) and small (closed circles) metabolism chambers. Gross primary production values from a single chamber of each type are shown. Benthic algal production using ^{14}C incorporation is represented as dashed lines for the 2 h incubation period, with mean (bold line), minimum, and maximum values shown. Photosynthetically active radiation (PAR) is also shown (solid line).

Figure 2. Calculated bacterial respiration and carbon demand at differing values of bacterial growth efficiency (BGE) compared to measured total respiration and gross primary production. The curved solid lines represent calculated values of bacterial respiration or carbon demand for different values of BGE using two values of cell carbon content – upper lines = 34 fg cell^{-1} , lower lines = 6 fg cell^{-1} . Panels A and C show benthic values and panels B and D show water column values. Horizontal lines represent values of respiration (R) or gross primary production (GPP) measured using changes in dissolved oxygen in benthic metabolism chambers (A and C) or in large water column chambers (B and D). For benthic metabolism, a maximum value (from mid day incubation in small chambers; Table 2) is given as well as a daily average (from large chambers). Only daily averages are given for water column metabolism. Calculated bacterial R is compared to measured R (A and B), while calculated bacterial carbon demand is compared to measured GPP (C and D).

Figure 1

Figure 2