Benthic metabolism as an indicator of stream ecosystem health

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SUMMARY

1. We tested direct and indirect measures of benthic metabolism as indicators of stream ecosystem health across a known agricultural landuse disturbance gradient in southeast Queensland, Australia. Gross primary production (GPP) and respiration (R_{24}) in benthic chambers in cobble and sediment habitats, algal biomass (as chlorophyll *a*) from cobbles and sediment cores, algal biomass accrual on artificial substrates and stable carbon isotope ratios of aquatic plants and benthic sediments were measured at 53 stream sites, ranging from undisturbed subtropical rainforest to catchments where improved pasture and intensive cropping are major landuses.
2. Rates of benthic GPP and R_{24} varied by more than two orders of magnitude across the study gradient. Generalised linear regression modelling explained 80% or more of the variation in these two indicators when sediment and cobble substrate dominated sites were considered separately. Both catchment and reach scale descriptors of the disturbance gradient were important in explaining variation in GPP and R_{24}. Model fits were poor for net daily benthic metabolism (NDM) and production to respiration ratio (P/R).
3. Algal biomass accrual on artificial substrate and stable carbon isotope ratios of aquatic plants and benthic sediment were the best of the indirect indicators, with regression model R^2 values of 50 % or greater. Model fits were poor for algal biomass on natural substrates for cobble sites and all sites. None of these indirect measures of benthic metabolism was a good surrogate for measured GPP.
4. Direct measures of benthic metabolism, GPP and R_{24}, and several indirect measures were good indicators of stream ecosystem health and are

recommended in assessing process-related responses to catchment land use change and the success of ecosystem rehabilitation actions.

Introduction

Stream and river health assessment has been traditionally dominated by the measurement of the distribution and abundance of plant and animal species (Marchant, Mitchell & Norris, 1984; Bunn, 1995; Harris, 1995; Reid *et al.*, 1995; Whitton & Kelly, 1995; Wright, 1995). However, there is growing concern that measures of ecosystem health should include not only aspects of their organization (e.g. biodiversity, species composition, food web structure), but also their vigour (e.g. rates of production, nutrient cycling) and resilience (e.g. ability to recover from disturbance) (Rapport, Costanza & McMichael, 1998; Bunn, Norris & Storey, this issue). Furthermore, many goals of river management relate to the maintenance of natural ecological processes and ecosystem function yet measurement of these processes is often neglected in assessment programs (Bunn & Davies, 2000).

Bunn, Davies & Mosisch (1999) and Bunn & Davies (2000) have previously argued that direct measures of ecosystem processes, such as benthic community metabolism, are important considerations in aquatic ecosystem health monitoring. Benthic community metabolism is likely to be an important indicator because the component processes of metabolism, respiration and primary production, both respond to environmental variables that are commonly influenced by catchment disturbance, such as light and temperature regimes and nutrient loads (Bunn *et al.*, 1999). Gross primary production (GPP) in forested streams should be low and light-limited due to shading by riparian vegetation at minimally disturbed or “reference” sites but should

increase across a gradient of catchment disturbance due to increased light and nutrient availability. Respiration (R_{24}) may also be expected to increase with increasing disturbance, not only due to higher in-stream GPP but also because of inputs of organic carbon and sediment from the catchment (Bunn *et al.*, 1999).

Methods used to assess benthic metabolism vary in the level of information obtained as well as in the cost and technical expertise required. Three common methods are: 1) direct measures of benthic community metabolism using dissolved gas fluxes in enclosed chambers (Bott *et al.*, 1985); 2) measures of static biomass of primary producers (Morin, Lamoureux & Busnarda, 1999); and 3) growth rate of primary producers measured by biomass accrual on bare substrate (Kevern & Ball, 1965). Direct measurements of rates of benthic community metabolism have been used as an important tool in stream ecosystem ecology for nearly four decades (McIntire *et al.*, 1964; Bott *et al.*, 1978; Dodds *et al.*, 1996; Craft, Stanford & Pusch, 2002), but have not been widely adopted in monitoring ecosystem health (but see Hill *et al.*, 2000), perhaps due in part to the perception that the measurements are technically difficult.

Many studies and monitoring programs measure static algal biomass on natural substrate as a surrogate of primary production (e.g. Morin *et al.*, 1999), which is less expensive and less time consuming than making rate measurements. It is important to realize that algal biomass may not necessarily relate directly to the rate of metabolism because it represents the result of interactions between net primary production, activity of grazing invertebrates, and the physical disturbance regime. Additionally, the relationship between primary production and biomass has been shown to be density dependent (Pfeifer & McDiffet, 1975; Morin *et al.*, 1999). Nonetheless,

production and biomass are often found to be positively correlated in many different types of systems (Enríquez *et al.*, 1996; Morin *et al.*, 1999). Direct measures of algal biomass may be a useful indicator of stream health independent of its relationship with production because high algal biomass is often viewed as a symptom of unhealthy streams. Measuring algal biomass on artificial substrates placed in streams for a set duration can provide an estimate of algal growth and may help to standardize inter-site comparisons by controlling substrate type and biofilm age (e.g. Mosisch, Bunn & Davies, 2001).

An additional and perhaps novel way of estimating benthic primary production in streams may be derived from the measurement of stable carbon isotope signatures of algae and other aquatic plants. Stable isotopes have been used extensively to determine the energy base of stream and river food webs (Peterson & Fry, 1987; Finlay, 2001). Difficulties in interpretation often arise because many factors other than the mode of photosynthesis influence the carbon isotope signatures of aquatic plants, including light intensity, water velocity, and CO_2 concentration (O'Leary, Madhavan & Paneth, 1992; France, 1995; France & Holmquist, 1997; MacLeod & Barton, 1998; Finlay, 2001). In recent work on a range of biomes in Australia, Bunn *et al*. (1999, and unpublished data) have found that a significant proportion of the observed variation in $\delta^{13}C$ values of algae is explained by variation in benthic GPP. Measures of the carbon isotope signatures of plant tissues are likely to reflect the rate of primary production, especially if other key factors such as water velocity do not vary greatly across sites. Depending on the source of organic carbon in sediments, $\delta^{13}C$ values of sediment might also reflect benthic GPP. Although the measurement of $\delta^{13}C$ requires the use of a technically precise analytical instrument (isotope ratio

mass spectrometer), it is relatively easy to collect the samples and the analyses are routinely undertaken in many research laboratories at low cost.

The aim of this study was to compare the performance of these four measures of benthic metabolism across a diffuse landuse gradient, as part of a larger study investigating potential indicators of ecosystem health for streams and rivers in southeast Queensland, Australia (Bunn *et al.*, this issue). A particular focus was on the relative response of the four ecosystem process measures to reach scale *versus* catchment scale descriptors of disturbance.

Methods

The Southeast Queensland Study

This study forms part of the scientific work undertaken for the Southeast Queensland Regional Water Quality Strategy (referred to herein as the Strategy). The study area covers six catchments and 15 major rivers of the Moreton region of Queensland in southeastern Australia (22,353 km^2) and incorporates 19 local government regions. The region lies in a transitional zone between tropical and temperate climates, though much of the rainfall (55%) occurs during the summer wet season (December to March). Stream flow varies greatly with season and many streams, particularly in the headwaters, are ephemeral and flow only during the wet season. Upland endemic riparian vegetation includes notophyll vine rainforests, dry eucalypt-dominated forests, and fern thicket/hoop pine (*Araucaria cunninghamii*) scrub. Endemic riparian vegetation of lowland areas is dominated by semi-evergreen vine thickets/hoop pine scrub and dry notophyll vine forest, river sheoak (*Casuarina spp.*), red bottlebrush (*Calistemon spp.*) and lilly-pilly (*Syzygium spp.*). Riparian zones along southeast

Queensland's streams have been heavily disturbed since European settlement and less than 60% of endemic vegetation remains in many subcatchments (Catterall & Kingston, 1993). Further details of the study region can be found in Storey *et al*. (this issue).

The project on Design and Implementation of a Baseline Monitoring program for streams and rivers in the region (DIBM) formed a key component of the Strategy (1999-2001). The aim was to develop a cost-effective, coordinated ecosystem health monitoring program for freshwaters of the region that can be used to measure and report on current status and future changes in ecological health. To do this, the DIBM study adopted an approach similar to that previously used to detect anthropogenic impacts in marine systems (Bayne *et al.*, 1988; Addison & Clarke, 1990; Stebbing & Dethlefsen, 1992). These Group of Experts on Environmental Pollution (GEEP) studies evaluated a broad range of indicators against a known disturbance gradient and identified those that best responded. Further details of the Strategy and the approach can be found in Bunn *et al*. (this issue).

The major land uses in southeast Queensland are grazing and cropping, and these were chosen as the primary disturbance gradient against which indicators were evaluated. Data on the percentage of catchment cleared was derived from GIS, while other attributes or *descriptors* of the disturbance gradient were measured in the field (see Storey *et al.*, this issue). The disturbance gradient descriptors were assigned to one of six broad categories (Table 1) to simplify reporting and allow direct comparison of different indicators. A detailed description of the disturbance gradient can be found in Storey *et al*. (this issue). A suite of potential indicators of stream

health was measured at 53 sites varying in the degree of land use disturbance (from undisturbed rainforest to cleared catchments) in September and October 2000. These indicators fell into five groups: macroinvertebrates, fish, water chemistry, nutrients and nutrient cycling, and benthic metabolism. The response of these indicators to descriptors of reach and catchment scale disturbance was investigated using generalised linear regression modelling (see below).

Direct measurement of benthic metabolism

In many streams and rivers, the benthic zone is the major region of organic matter processing, and negligible rates of metabolism occur in the water column (Keithan & Lowe, 1985; Davies, 1994). This is especially the case in small streams, which were the particular focus of work undertaken within the DIBM project (see Storey *et al*., this issue).

Benthic metabolism was determined by measuring the net change in dissolved oxygen within a dome-shaped perspex chamber (diameter = 29.5 cm, total height = 25 cm, total volume = 10 L) over a 24 hr period at each site. A dissolved oxygen (DO) sensor (YSI 5739, USA) was located in the top of each chamber and a pump recirculated water to reduce boundary layer effects at the sediment-water interface and ensure flow saturation across the membrane of the oxygen probe. Each probe was attached to a data-logger (TPS 601), which recorded DO and water temperature at 10 minute intervals. Where the streambed consisted predominantly of large cobbles, one or more cobbles were placed inside the chamber with a plastic base to provide a watertight seal. In streams with a substrate of sediment (sand or mud), the chambers were pushed into the sediment to a depth of approximately 10 cm, with an enclosed

surface area of substrate of 0.068m^2. The volume of water in the chamber was measured by subtracting the volume of the cobble or sediments from the total volume. Cobble surface area was measured by wrapping the cobbles in aluminium foil, weighing the foil used to cover the rock, and using a weight-area relationship for the foil to calculate area (after McCreadie & Colbo, 1991). The metabolically "active" surface area of each cobble was assumed to be half the total cobble area (Naiman, 1983; Davies, 1994).

Different components of benthic metabolism were calculated by comparing the rate of change of DO concentration in the chambers at different times of the day. The mean rate of change at night was taken as the rate of respiration, and daily respiration (R_{24}) was calculated by assuming the rate was constant and multiplying by 24 hrs. Gross primary production (GPP) was calculated as the sum of the DO production during daylight hours plus the DO consumed by respiration during that period of time based on the night-time respiration rate. Net daily metabolism (NDM) was calculated as the difference between GPP and R_{24} and P/R ratio was calculated as GPP divided by R_{24}. Changes in DO concentrations over time (mg O_2 L^{-1} hr^{-1}) were multiplied by chamber volume and divided by substrate surface area to obtain values in units of mg O_2 m^{-2} hr^{-1}. These rates were converted to units of carbon assuming that one mole of C is equivalent of one mole of O_2 for both respiration and photosynthesis (i.e. 1 mg O2 = 0.375 mg C, Lambert, 1984; Bender *et al.*, 1987).

Benthic metabolism measurements were made using duplicate domes at 51 of the 53 sites. Of these 51 sites, the benthic substrate was dominated by cobbles at 26 sites

and finer sediment at 25 sites. Poor equipment performance at two of the sediment substrate sites meant that usable data were collected from 49 of the sites.

Algal biomass on natural substrates

Different approaches to measuring algal biomass can be grouped into three broad categories: ash-free dry mass, pigment analysis, and biovolume of algal cells (Steinman & Lamberti, 1996). Pigment analysis using chlorophyll *a* content of benthic biofilms was used to measure benthic algal biomass in this study because it is a relatively simple technique and it removes the influence of other potential organic components of the biofilm (Steinman & Lamberti, 1996). Measurement of chlorophyll *a* on natural substrates on a particular day represents a static measure of algal biomass.

Samples were collected from the stream bed for algal biomass determination using different techniques for the two types of bed substrate. For cobbles, algae were collected from the top surface using a Perspex cylinder that isolated 0.0015 m^2 with a gasket and contained a circular brush. The biofilm was scrubbed loose as ambient stream water was pumped through the cylinder and into a collection vessel. The slurry was filtered onto 0.7 μM glass fibre filters using a hand vacuum pump, and filters were frozen until analysis. A sample was taken from each cobble used in benthic chamber measurements as well as one additional cobble, for a total of three samples from each of the 26 sites. A sample of the ambient stream water at each site was also filtered and analysed to correct the cobble values for any chlorophyll *a* in the stream water.

For sites without cobbles, small cores (surface area = 0.0006 m^2) were taken using modified 60 ml plastic syringes to collect algae on sediment substrates. The top 2 cm of each core was retained and frozen until analysis. Two cores were taken at each site near the location where benthic chambers were deployed. Removal of the chambers disturbed the sediment too much to allow sediment samples to be taken from the sediment that had been enclosed in the chambers. Samples were obtained from 21 of the 22 sediment sites.

Chlorophyll *a* analysis was performed according to the methods of Parsons, Maita & Lalli (1984). Following extraction in 90% acetone, the solution was centrifuged and the supernatant analysed for chlorophyll *a* concentration by spectrophotometer, using an acidification step to account for phaeophytin content. Chlorophyll *a* concentration was expressed as mg m^{-2} for all substrates.

Algal growth on artificial substrates

The control treatment of an algal bioassay experiment (see Udy *et al*., this issue) provided an artificial substrate for measuring growth of benthic algae. The biomass of algae at the end of deployment represents net algal accrual over the period and was considered a measure of net algal growth. Artificial substrates were made from plastic pots with lids containing a 6 cm diameter circle of 100 µm nylon mesh. Control pots used here did not have added nutrients (treatment pots contained slow-release fertiliser; Udy *et al*., this issue). Two sets of pots were deployed at each site the day after benthic metabolism measurements were made, and were left for approximately four weeks prior to collection. The mesh and any attached algae was removed and frozen until analysis, resulting in two replicate samples per site.

Chlorophyll *a* concentration was measured as described for natural substrates. Data from artificial substrates were not obtained for 19 of the 51 sites due to a variety of factors including exposure from falling stream levels, burial by sediment, and vandalism.

Stable carbon isotopes

Where present, aquatic plant samples (filamentous algae and submerged vascular macrophytes) were collected by hand for $\delta^{13}C$ analysis (23 sites). Sediment samples were collected using modified 60 ml plastic syringes, with the top 5 cm of sediment retained. Sediments were collected from all the sites dominated by sediment substrate as well as cobble sites where pockets of sediment could be found, for a total of 43 sites. These samples presumably included microalgae growing on the sediment surface as well as any particulate organic matter present. All samples were frozen during transportation to the laboratory and subsequently kept frozen until prepared for stable isotope analysis. Plant samples were cleaned and rinsed in distilled water and oven-dried at 60°C for 36-48 h. Sediment samples were dried at 60°C until completely dry (up to 6 days). Dried plant and sediment samples were ground to a powder-like consistency using mortar and pestle. Ground samples were oxidised at high temperature using an elemental analyser and the resultant CO_2 was analysed with a continuous-flow ratio mass spectrometer (IsoPrime, Micromass, UK). Ratios of $^{13}C/^{12}C$ were expressed in δ notation as the relative per mil (‰) difference between the sample and conventional standard (PeeDee Belemnite carbonate):

$\delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 1000$; where $R = {}^{13}C/{}^{12}C$.

Data analysis

In keeping with the GEEP-style approach (Bayne *et al.*, 1988), a protocol for data analysis was devised to simplify the process of comparing the eight indices of benthic metabolism (GPP, R_{24}, P/R, NDM, chlorophyll a on natural substrate, chlorophyll a on artificial substrate, $\delta^{13}C$ plants, and $\delta^{13}C$ sediment). This subsequently allowed direct comparison of all the results across the various ecological indicators (Harch et al.; Smith et al., this issue). Initially, distributional properties of the data were checked to identify outliers and any required transformations for subsequent statistical analyses. Preliminary investigation of relationships between descriptors of the disturbance gradient and indices were explored using scatter plots and Spearman rank correlation coefficients to ascertain whether any simple bivariate relationships existed. A Generalised Linear Modelling (GLM) framework was used to determine whether particular metabolism indices could be used to detect the underlying disturbance gradient. While a number of multivariate approaches could have been taken, stepwise regression modelling was employed because it not only accommodates for the different distributional forms of the indices (e.g. normal, poisson, binomial), but it also identifies which disturbance gradients account for the variability in each of the indices, and additionally quantifies the proportion of variation accounted for by each disturbance measure. Indicators were assessed in terms of the approximate amount of variation explained (approximate R^2 value) by the model and the proportion of this variation explained by individual descriptors of the disturbance gradient. Data were analyzed using the S-PLUS 2000 - Professional Release 3 (MathSoft Inc.) statistical software.

Over 80 disturbance descriptors of the catchment land use disturbance gradient were derived from measurements made at the sites as well as catchment data collated from various GIS sources (see Storey et al., this issue, for a complete description). The descriptors were grouped into 6 broad categories (Table 1). A limited number of the disturbance descriptors were included in the GLM to avoid over-parameterization of the regression models. In the case of the benthic metabolism indicators, 13 descriptors from 4 of the categories were chosen as the most appropriate (Table 1). For example, direct measurements of riparian canopy cover at the metabolism sites using fish eye lens photography (see Bunn *et al.*, 1999) were used in preference to other measurements (e.g. reach scale visual estimates of cover and densiometer measurements). These 4 categories were described as containing measures made at the catchment scale (*Landuse*), the reach scale (*Channel Conditions* and *Riparian Conditions*), or influenced by both scales (*Water and Sediment Chemistry*).

Due to the potential for large differences between streams of different substrate types, cobble and sediment streams were analysed both separately and combined, so that it was possible to identify trends that occurred in only one of the substrate types as well as overall trends. Mean site values were used in analyses for measures where there were two or more replicates per site. Two sites were downstream of sewage treatment plants and had total and dissolved nutrient concentrations that were two orders of magnitude greater than most other sites. These high values prevented the successful transformation of the nutrient data, so the two sites were removed from the dataset. No transformations of descriptors were required after these two sites were removed. The two sites at which benthic metabolism equipment failed were also dropped from the data set, and further analyses therefore involved 22 sediment and 25 cobble sites.

Simple linear regression analysis was performed between GPP and riparian cover for comparison with previous studies as well as between several of the different measures of benthic production to look for predictive relationships. The relationship between R_{24} and GPP was investigated to assess whether GPP explained a substantial proportion of the variation in R_{24}, suggesting that autocthonous carbon was important to respiration or whether the relationship was weak, suggesting allocthonous carbon was important.

Results

Direct measures of benthic metabolism

Rates of GPP and R_{24} varied by up to two orders of magnitude among the 47 sites included in analyses. GPP and R_{24} exhibited similar maximum, minimum, and mean values. Mean GPP was 610 mg C m^{-2} d^{-1} with a range from 0 to 2990, and mean R_{24} was 600 mg C $m^{-2}d^{-1}$, with a range of 10 to 2340. NDM ranged from -1140 to 1840 mg C $m^{-2}d^{-1}$ with a mean of -10, and P/R ranged from 0 to 8.6 with a mean of 1.3. Slightly over half of the sites had values of P/R greater than 1. Sediment sites had greater mean values of GPP and R_{24} compared to cobble sites, but mean NDM and P/R were greater at cobble sites. The mean value of GPP for the 25 cobble sites was 490 mg C m^{-2} d^{-1} and the mean value of R_{24} was 330 mg C m^{-2} d^{-1}. Mean P/R was 1.7 and mean NDM was 150 mg C m^{-2} d^{-1}. The 22 sediment sites had mean values of 750 mg C m^{-2} d^{-1} for GPP, 900 for R_{24}, -150 for NDM, and 0.8 for P/R.

Regression modelling showed that much of the observed variability among sites in GPP and R_{24} could be explained by disturbance gradient descriptors in the W*ater and*

sediment chemistry, *Riparian condition*, and *Landuse* categories (Table 2). For cobble-bed streams, 89% of the variation in GPP could be explained by the overall model (Figure 1a). Descriptors in the *Water and sediment chemistry* category contributed most to the high approximate R^2 value (Table 2), with Total N concentration alone contributing 59%. The ions gradient explained an additional 11%, and the relationships of both variables with GPP had positive slopes. The ability of the disturbance gradient descriptors to explain the variability in GPP for sites with sediment substrate was slightly weaker (R^2 = 79%, Figure 1b) and included factors related to *Riparian condition* and *Water and sediment chemistry* (Table 2). Riparian vegetation explained 44% of the variation and the relationship had a negative slope, while relationships with Turbidity and the Ions gradient had positive slopes and R^2 values of 11 and 10%, respectively. The model for all sites explained less of the variation in GPP than either substrate alone (Figure 1c) and also included *Riparian condition* and *Water and sediment chemistry* as major predictors (Table 2). Similar to the model for sediment sites, the relationship with Riparian vegetation explained the largest portion of the variation (32%) and exhibited a negative slope, while NH_4^+ concentration and the Ions gradient had R^2 values of 14 and 13%, respectively, and both had positive slopes with GPP.

Canopy cover alone explained 41% of the variation in GPP across all sites when analysed using simple linear regression analysis ($p < 0.001$; Figure 2). GPP decreased with increasing canopy cover, and the relationships were very similar when sites were partitioned by substrate, with R^2 values of 42% for cobbles and 37% for sediment. Note that in the case of the cobble stream sites, this relationship was not obvious in the GLM, as much of this variance is likely to have been removed in the stepwise

model by Total N concentration in the *Water and sediment chemistry* category (Table 2).

As with GPP, a large proportion of the variation in R_{24} was explained by *Water and sediment chemistry* (Table 2). For cobble sites, *Landuse* was also an important descriptor category, contributing over half of the total approximate model R^2 of 84% (Figure 1d). The two *Landuse* descriptors, % Crop cover and % Cleared, made similar contributions to the total R^2 at 24 and 21%, respectively. Both of these descriptors and Maximum temperature (partial R^2 = 38%) had relationships with R_{24} of positive slope. Descriptors in the *Riparian condition* category explained nearly half of the total 85% for sediment sites (Figure 1e), with values of 22% for Hemiphot cover and 17% for Riparian vegetation. Similar to GPP, values of R_{24} decreased with increasing values for both *Riparian condition* descriptors. The Ions gradient in the *Water and sediment chemistry* category explained another 23% and exhibited a positive slope. Only *Water and sediment chemistry* descriptors contributed to the model for all sites (total R^2 = 58%; Figure 1f). The Ions gradient and Total N contributed 35 and 23%, respectively, and both relationships with R_{24} had positive slopes.

With the exception of NDM at cobble sites, descriptors of the disturbance gradient did not explain as much of the variability in net NDM and P/R as they did for GPP or R_{24} (Table 2). For both of these variables, model values of R^2 were much lower for sediment sites than cobble sites, and the combined models had intermediate values. The model fit for P/R of sediment sites was very poor and little of the observed variation was explained by any disturbance parameters.

Algal biomass

Chlorophyll *a* concentrations on cobble substrates ranged from 1 to 23 mg Chl *a* m^{-2}, with a mean of 7, while those from sediment substrates were typically higher, ranging from 5 to 614 mg Chl *a* m^{-2}, with a mean of 105. Very little of the variation in chlorophyll *a* concentrations at cobble sites or all sites combined was explained by the disturbance gradient (Table 2, Figures 3a and c). The model for sediment sites explained much of the variation in ambient Chl *a* concentrations (Figure 3b), with about half of this contributed by *Water and sediment chemistry* (35% from the Ions gradient) and half from *Riparian condition* category (37% from Riparian vegetation) (Table 2). The relationship between Chl *a* and the Ions gradient had a positive slope, while the relationship with Riparian vegetation had a negative slope.

Algal growth on artificial substrate

Chlorophyll *a* concentrations on artificial substrates ranged from 1 to 64 mg Chl *a* m^{-2} (mean = 11). These equate to net biomass accrual rates of 0.03 to 2 mg Chl *a* m^{-2} d^{-1}. Regression modelling showed that about two thirds of the variability in the Chl *a* concentrations on the artificial substrates could be explained by disturbance gradient descriptors of *Water and sediment chemistry*, *Landuse*, and *Riparian conditions* (Table 2, Figure 4). The main descriptors contributing to the model were Maximum temperature (22%), PO_4 (15%), and % Cleared (14%). All three had positive relationships with Chl *a*.

Stable carbon isotopes

The $\delta^{13}C$ values of filamentous algae and vascular macrophytes ranged from -39‰ to ⁻15‰ for the 20 sites from which samples were available. Regression modelling showed that 60% of the variability in $\delta^{13}C$ values for aquatic vegetation could be explained by descriptors of *Water and sediment chemistry*, and *Landuse* (Figure 5a, Table 2). Within *Water and sediment chemistry*, the Ions gradient contributed 26% and had a relationship of positive slope, while $NO_2 + NO_3$ explained 14% but had a negative slope. An additional 15% was explained by % Cleared, with a positive slope. The range in the $\delta^{13}C$ values of the sediment (-29‰ to -14‰, n = 43 sites) was smaller than the range of values obtained for aquatic plants. Although the model fit for $\delta^{13}C$ values was weaker (49%), it was also dominated by descriptors of *Landuse* (% Cleared, 35% with a positive slope) and *Water and sediment chemistry* (Ions gradient, 11%, with a positive slope)(Figure 5b, Table 2).

Relationships among measures of benthic metabolism

No strong relationships were found between direct measures of benthic metabolism and potential indirect measures. There was a reasonable positive relationship between GPP and chlorophyll *a* on natural substrates at cobble sites ($R^2 = 44\%$, $p < 0.001$) but the relationship for sediment sites was not significant ($R^2 = 18\%$, $p = 0.054$; Figure 6). The trend was quite weak when all sites were considered ($R^2 = 12\%$, $p = 0.021$). There was no relationship between GPP and algal biomass accrual (as chlorophyll *a*) on artificial substrates ($R^2 < 1\%$, $p = 0.68$). Aquatic plants from sites with higher GPP generally had more enriched $\delta^{13}C$ values ($R^2 = 34\%$, $p = 0.007$; Figure 7a). A similar relationship was found between sediment $\delta^{13}C$ values and GPP, but GPP explained only 19% of the variation (Figure 7b). When sites of differing substrate

were considered separately, the relationship was improved for sediment sites (R^2 = 31%, p = 0.009) but was not significant for cobble sites (R^2 = 15%, p = 0.08; Figure 7b).

The relationships between gross primary production and R were all significant (p < 0.001). A substantial portion of the variation in R_{24} was explained by GPP, with R^2 values of 58, 61, and 70% for all sites, cobble, and sediment sites, respectively.

Discussion

Performance of ecosystem process indicators

The most important feature of a good indicator of ecosystem health is that it responds to the disturbance gradient of interest. For this study, the first criterion on which the indicators are judged is the R^2 values of the regression models developed using Generalised Linear Modelling. However, there are other features of indicators that should be taken into consideration. A good indicator should also have measured values spanning a relatively large range, to provide for the possibility of distinguishing intermediate levels of disturbance as well as reference *vs*. impacted sites. From a practical standpoint, obtaining measurements of the indicator must be feasible and yield usable results at the range of sites under consideration. For example, indicators needed to perform well for both cobble and sediment substrates in this study. Another important attribute is that there should be a clear conceptual understanding of how and why the indicator will change in response to disturbance. In the case of most of the measures of benthic metabolism, the observed response to changes in light and nutrient regimes associated with land-use change and riparian degradation were as predicted: GPP, R_{24}, Chl *a* on natural susbtrates, and $\delta^{13}C$ values

of plants generally increased with increasing percentage of total catchment area cleared and increasing nutrient and ion concentrations and decreased with increasing riparian vegetation cover.

Of the eight indicators of benthic metabolism evaluated in this study, GPP and R_{24} were the best overall indicators of ecosystem health. Both measures exhibited a range of values over three orders of magnitude, and a high proportion of their variation could be explained by descriptors of the disturbance gradient, especially when cobble and sediment substrate sites were considered separately. Both reach scale and catchment scale factors could be considered important for these two indicators since *Water and sediment chemistry* and *Riparian Conditions* were the categories of descriptors that explained most of the variation, and *Landuse* was important in the case of cobble R_{24}.

Algal growth on artificial substrates and stable isotopes of plants and sediment appeared to be moderately good indicators, with *Water and sediment chemistry* and *Landuse* explaining most of the variation in these indicators. Similar to GPP and R_{24}, both reach and catchment scale descriptors of the disturbance gradient were important for these three indicators. The model R^2's were lower for these indicators than for GPP and R_{24}, but the more important limitation was the low number of sites for which data were successfully obtained for two of the three indicators. Sampling of aquatic plants was limited by the fact that they were present at fewer than half the sites. As mentioned in the Methods section, a variety of factors led to a relatively poor retrieval rate of 60% for the artificial substrates. Sediment samples for stable isotope analyses

were collected for over 90% of the sites, but the $\delta^{13}C$ values did not show as large a range in values as the plant samples and had a lower model R_2 value.

With the exception of algal biomass for sediment sites and NDM for cobbles, NDM, P/R, and algal biomass on natural substrates were not adequate indicators. The poor performance of NDM and P/R compared to GPP and R_{24} may be due to the fact that NDM and P/R are composites of GPP and R_{24}, and these two processes may be affected by different aspects of the disturbance gradient. Because minimally impacted sites generally have intact riparian vegetation and substantial shading of the stream channel, these sites would be predicted to have very low GPP and low R_{24}, yielding P/R ratios much lower than 1, and small, negative values of NDM. Both GPP and R_{24} are expected to increase with increasing catchment disturbance, but not necessarily in a way that leads to directional changes in NDM or P/R for these sites. For example, an increase in plant growth leading to P/R ratios exceeding 1 could be an indication of an impacted site. However, since disturbance may also increase sediment and organic matter input, R_{24} could increase independently of the increase in GPP, leading to disturbed sites with P/R less than 1.

Comparison of benthic metabolism rates with other studies

The range of sites in this study included catchments with very little clearing as well as predominately agricultural catchments, and riparian vegetation canopy cover levels of zero to almost 100%, so it is not surprising that values of GPP measured in this study (0 to 2990 mg C m^{-2} d^{-1}) nearly span the range of values reported in the literature. The lower end of the values for GPP measured in the current study compare well to the range of 20 to 710 mg C m^{-2} d^{-1} measured for forested streams in North America,

while the maximum value is less than half the value of 7500 mg C m^{-2} d^{-1} reported for a desert stream (Mulholland *et al.*, 2001; values in units of oxygen converted to C using a factor of 0.375 as described in the Methods). In contrast, values of R_{24} from this study (10 to 2340 mg C m^{-2} d^{-1}) are nearly all lower than the range of 2360 to 12,120 mg C m^{-2} d^{-1} for the 7 forested, 1 desert, and 1 grassland streams studied in Mulholland *et al.* (2001). These differences in respiration are expected given that the Mulholland *et al.*(2001) study used an open-system dissolved oxygen technique to measure metabolism, and as pointed out by those authors, open system measurements of respiration are often higher than benthic chamber measurements due to differences in how much subsurface sediment respiration is included. However, the ranges of values for this study were very similar to those from a longitudinal study of the Taieri River, a grassland river in New Zealand, (GPP 110-3600; R_{24} 260-3680 mg C m^{-2} d^{-1}, also converted from units of oxygen), which also used open system measures (Young & Huryn, 1996). Values of GPP and R_{24} from benthic chamber measurements made by Bunn *et al.* (1999) in forested streams of the Johnstone River catchment in north Queensland, the Northern jarrah forest, Western Australia, and the Mary River catchment, just north of the study area, fall at the lower end of the range of values of this study, with GPP ranging from 90 to 200 mg C m^{-2} d^{-1}, and R_{24} ranging from 170 to 380 mg C m^{-2} d^{-1}. When sites in the Mary River with varying amounts of grazing landuse are considered in addition to the forested sites, the maximum GPP and R_{24} values increase to 2100 and 1550 mg C m^{-2} d^{-1}, respectively (Bunn *et al.*, 1999), but are still lower than the maximum values from this study. It is interesting to note that the slope, intercept, and R^2 for the relationship between GPP and riparian canopy cover from this study (Figure 2) are almost identical to that for reach level data from the 20 Mary River sites presented in Bunn *et al.* (1999).

Surrogate measures of GPP

None of the indirect measures of benthic metabolism proved to be adequate surrogates for GPP. Most of the relationships between individual indirect measures and GPP were not significant when explored using simple linear regression. Of those that were significant, the highest R^2 was 44%. In general, these indicators all would be expected to respond to similar factors such as light, nutrients, stream velocity, etc. The lack of relationship is probably influenced by different factors in each situation, but one possibility is the different time scales over which the indicators respond. GPP and R_{24} are measured over 24 hrs and are influenced by conditions on that day, as well as the condition of substrate biofilm. Chlorophyll *a* content of biofilm on natural substrate likely reflects influences over the weeks to months during which the biofilm develops (4 weeks in the case of the artificial substrates) and is influenced by grazing and physical disturbance regimes. Similarly, material collected for stable isotope analysis integrates days to weeks for filamentous algae and even longer for aquatic macrophytes. Since indirect measures may be responding to different factors, and over different time scales, they appear to be complementary to direct measures, as opposed to serving as surrogate measures.

The lack of relationship between GPP and chlorophyll *a* on sediment and low R^2 for cobbles is surprising considering that the substrates sampled for chlorophyll *a* were near where the chambers were deployed or actually in the chamber in the case of cobbles. Sites varied as to whether or not filamentous algae were present, and undoubtedly there were other less obvious differences in algal species across sites. Different types of algae exhibit different relationships between photosynthetic rate

and chlorophyll *a* content (Krause-Jensen & Sand-Jensen, 1998). For example, sites dominated by benthic microalgae would be predicted to have higher rates of GPP, per mg of chlorophyll *a*, than sites dominated by filamentous algae. Even within the same species of unicellular algae, or aquatic macrophytes, variation in chlorophyll *a* concentrations have also been observed due to light availability (e.g. plants in low light environments producing additional chlorophyll *a* to maximise their ability to capture the available light Abal *et al.*, 1994). This relationship between light availability and chlorophyll *a* concentrations is in contrast to the general trend that the total chlorophyll *a* of a streambed will increase as more light becomes available. It is also likely that differences in rates of invertebrate grazing between sites will have a large impact on the algae biomass present at a site, but might have a smaller influence on the primary production rates as this is predominantly controlled by light and nutrient availability (Rosemond, Mulholland & Elwood, 1993).

The relationships between $\delta^{13}C$ values and GPP were in the predicted direction for both plants and sediment, with values increasing (becoming less negative) with increasing rates of GPP. However, R^2 values of 34% and lower for the relationships for data from this study point to variation due to the influence of additional factors. The $\delta^{13}C$ values of aquatic plants can be affected by changes in the rates of organic matter decomposition, respiration and water motion (Farquhar, Ehleringer & Hubick, 1989; France, 1995), so GPP is not expected to be the only influencing factor. In the case of sediment values, $\delta^{13}C$ values are those of the organic carbon component of the sediment. Since sediment organic carbon could originate from multiple possible sources, a tight relationship with GPP would only be expected in streams where detritus from in-stream plant production dominated the organic carbon pool. The

relationships between $\delta^{13}C$ values and GPP in this study were not tight enough to use plant and sediment samples as a surrogate for GPP measurements. It may be the case that $\delta^{13}C$ values are more useful as independent indicators than they would be as surrogates because of the fact that $\delta^{13}C$ values are influenced by multiple aspects of carbon cycling.

Differences at sites with cobble vs. sediment benthic substrate

Differences in the materials enclosed in chambers when deployed at sites dominated by cobbles compared to sites dominated by finer sediments suggest that is appropriate to keep the analyses of these two types of sites separate. In both types of sites, the chambers enclose all the photoautotrophs in the surface area under consideration since photosynthesis can only take place on the upper surfaces. However, chambers inserted into sediment substrates also enclose the microbial community in the sediment to the depth of insertion, which will contribute additional respiration. In contrast, when cobbles are inserted in the chambers, only metabolism of the microbial community associated with the cobble surface is being measured. As a result, higher rates of R_{24} are expected per square meter in sediment sites compared to cobble sites. The differential incorporation of subsurface microbial respiration may be one reason that the model explaining variation in NDM was weak for sediment sites but not for cobble sites.

Conclusions

Stream ecosystem health monitoring has only recently begun to incorporate measures of ecosystem processes (Bunn *et al.*, 1999; Bunn & Davies, 2000; Hill *et al.*, 2000), despite the long history of the importance of these measures to stream ecology

research and their demonstrated effectiveness in assessment of particular impacts on individual systems, such as heavy metal pollution (Crossey & La Point, 1988; Hill *et al.*, 1997). Ecosystem process measures are effective indicators of stream ecosystem health in settings where responses of the processes to the disturbance of interest can be predicted based on an understanding how factors associated with disturbance influence the processes. This study demonstrates the effectiveness of using measures of benthic metabolism to detect a diffuse land-use disturbance gradient in southeast Queensland. The same measures of ecosystem process may not work equally well in different settings, but a similar process of developing conceptual models, identifying features of reference and impacted sites, and evaluating process indicators should be applicable to other systems.

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References

Abal E., Loneragan N., Bowen P., Perry C.J., Udy J.W. & W.C. D. (1994) Physiological and morphological responses of the seagrass Zostera capricorni Aschers. to light intensity. *Journal of Experimental Marine Biology and Ecology* **178,** 113-129.

Addison R.F. & Clarke K.R. (1990) The IOC/GEEP Bermuda workshop. *Journal of Experimental Marine Biology and Ecology* **138,** 1-8.

Bayne B.L., Addison R.F., Capuzzo J.M., Clarke K.R., Gray J.S., Moore M.N. & Warwick R.M. (1988) An overview of the GEEP workshop. *Marine Ecology Progress Series* **46,** 235-243.

Bender M., Grande K., Johnson K., Marra J., William P.J.L., Sieburth J., Pilson M., Langdon C., Hitchcock G., Orchardo J., Hunt C., Donaghay P. & Heinemann K.

(1987) A comparison of four methods for determining planktonic community production. *Limnology and Oceanography* **32,** 1085-1098.

Bott T.L., Brock J.T., Cushing C.E., Gregory S.V., King D. & Petersen R.C. (1978) A comparison of methods for measuring primary productivity and community respiration in streams. *Hydrobiologia* **60,** 3-12.

Bott T.L., Brock J.T., Dunn C.S., Naiman R.J., Ovink R.W. & Petersen R.C. (1985) Benthic Community Metabolism in 4 Temperate Stream Systems: an Inter-Biome Comparison and Evaluation of the River Continuum Concept. *Hydrobiologia* **123,** 3-45.

Bunn S.E. (1995) Biological monitroing of water quality in Australia: Workshop summary and future directions. *Australian Journal of Ecology* **20,** 220-227.

Bunn S.E. & Davies P.M. (2000) Biological processes in running waters and their implications for the assessment of ecological integrity. *Hydrobiologia* **422,** 61-70.

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

Catterall C.P. & Kingston M. (1993) *Remnant Bushland of South East Queensland in the 1990's: its Distribution, Loss, Ecological Consequences and Future Prospects*. Institute of Applied Environmental Research, Griffith University and Brisbane City Council, Brisbane.

Craft J.A., Stanford J.A. & Pusch M. (2002) Microbial respiration within a floodplain aquifer of a large gravel-bed river. *Freshwater Biology* **47,** 251-261.

Crossey M.J. & La Point T.W. (1988) A comparison of periphyton community structural and functional responses to heavy metals. *Hydrobiologia* **162,** 109-116.

Davies P.M. (1994). Ecosystem ecology of upland streams of the northern jarrah forest, Western Australia. Ph.D. thesis. The University of Western Australia.

Dodds W.K., Hutson R.E., Eichem A.C., Evans M.A., Gudder D.A., Fritz K.M. & Gray L. (1996) The relationship of floods, drying, flow and light to primary production and producer biomass in a prairie stream. *Hydrobiologia* **333,** 151-159.

Enríquez S., Duarte C.M., Sand-Jensen K. & Nielsen S.L. (1996) Broad-scale comparison of photosynthetic rates across phototrophic organisms. *Oecologia* **108,** 197-206.

Farquhar G.D., Ehleringer J.R. & Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40,** 503-537.

Finlay J.C. (2001) Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. *Ecology* **82,** 1052-1064.

France R.L. (1995) Carbon-13 enrichment in benthic compared to planktonic algae: Foodweb implications. *Marine Ecology Progress Series* **124,** 307-312.

France R.L. & Holmquist J.G. (1997) Delta super(13) variability of macroalgae: Effects of water motion via baffling by seagrasses and mangroves. *Marine Ecology Progress Series* **149,** 305-308.

Harris J.H. (1995) The use of fish in ecological assessments. *Australian Journal of Ecology* **20,** 65-80.

Hill B.H., Hall R.K., Husby P., Herlihy A.T. & Dunne M. (2000) Interregional comparisons of sediment microbial respiration in streams. *Freshwater Biology* **44,** 213-222.

Hill B.H., Lazorchak J.M., McCormick F.H. & Willingham W.T. (1997) The effects of elevated metals on benthic community metabolism in a Rocky Mountain stream. *Environmental Pollution* **95,** 183-190.

Keithan E.D. & Lowe R.L. (1985) Primary productivity and spatial structure of phytolithic growth in streams in the Great Smoky Mountains National Park, Tennessee (USA). *Hydrobiologia* **123,** 59-68.

Kevern N.R. & Ball R.C. (1965) Primary productivity and energy relationships in artificial streams. *Limnology and Oceanography* **10,** 74-87.

Krause-Jensen D. & Sand-Jensen K. (1998) Light attenuation and photosynthesis of aquatic plant communities. *Limnology and Oceanography* **43,** 396-407.

Lambert W. (1984). The measurement of respiration, p. 413-468. In: Downing J. A.&Rigler F. H. [Eds.], *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters*. Blackwell Scientific Publications.

MacLeod N.A. & Barton D.R. (1998) Effects of light intensity, water velocity, and species composition on carbon and nitrogen stable isotope ratios in periphyton. *Canadian Journal of Fisheries and Aquatic Sciences* **55,** 1919-1925.

Marchant R., Mitchell P. & Norris R. (1984) Distribution of benthic invertebrates along a disturbed section of the LaTrobe River, Victoria: an analysis based on numerical classification. *Australian Journal of Marine and Freshwater Research* **35,** 355-374.

McCreadie J.W. & Colbo M.H. (1991) A critical examination of four methods of estimating the surface area of stone substrate from streams in relation to sampling Simuliidae (Diptera). *Hydrobiologia* **220,** 205-210.

McIntire C.D., Garrison R.L., Phinney H.K. & Warren C.E. (1964) Primary production in laboratory streams. *Limnology and Oceanography* **9,** 92-102.

Morin A., Lamoureux W. & Busnarda J. (1999) Empirical models predicting primary productivity from chlorophyll a and water temperature for stream periphyton and lake and ocean phytoplankton. *Journal of the North American Benthological Society* **18,** 299-307.

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

Mosisch T.D., Bunn S.E., Davies P.M. & Marshall C.J. (1999) Effects of shade and nutrient manipulation on periphyton growth in a subtropical stream. *Aquatic Botany* **64,** 167-177.

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

Naiman R.J. (1983) The Annual Pattern and Spatial-Distribution of Aquatic Oxygen-Metabolism in Boreal Forest Watersheds. *Ecological Monographs* **53,** 73-94.

O'Leary M.H., Madhavan S. & Paneth P. (1992) Physical and chemical basis of carbon isotope fractionation in plants. *Plant, Cell and Environment* **15,** 1099-1104.

Parsons T.R., Maita Y.L. & Lalli C.M. (1984). p. 101-107. In: *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press.

Peterson B.J. & Fry B. (1987) Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics* **18,** 293-320.

Pfeifer R.F. & McDiffet W.F. (1975) Some factors affecting primary productivity of stream riffle communities. *Archiv für Hydrobiologie* **75,** 306-317.

Rapport D.J., Costanza R. & McMichael A.J. (1998) Assessing ecosystem health. *Trends in Ecology & Evolution* **13,** 397-402.

Reid M.A., Tibby J.C., Penny D. & Gell P.A. (1995) The use of diatoms to assess past and present water quality. *Australian Journal of Ecology* **20,** 57-64.

Rosemond A.D., Mulholland P.J. & Elwood J.W. (1993) Top-Down and Bottom-Up Control of Stream Periphyton: Effects of Nutrients and Herbivores. *Ecology* **74,** 1264-1280.

Stebbing A.R.D. & Dethlefsen V. (1992) Introduction to the Bremerhaven workshop on biological effects of contaminants. *Marine Ecology Progress Series* **91,** 1-8.

Steinman A.D. & Lamberti G.A. (1996). Biomass and pigments of benthic algae, p. 295-315. In: Hauer F. R.&Lamberti G. A. [Eds.], *Methods in Stream Ecology*. Academic Press.

Whitton B.A. & Kelly M.G. (1995) Use of algae and other plants for monitoring rivers. *Australian Journal of Ecology* **20,** 45-56.

Wright J.F. (1995) Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology* **20,** 181-197.

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

Table 1 Categories of disturbance gradient descriptors and the specific descriptors chosen for use in generalised linear regression modelling of benthic metabolism indicators. See Storey *et al*. (this issue) for a description of the methods used to quantify the descriptors.

Descriptor category/Descriptor	Explanation
1. Landuse (Catchment scale)	
% Cleared	Percentage of total catchment area cleared
% Crop cover	Percentage of total catchment area cropped
2. Channel Conditions (Reach scale)	
Channel condition	Categorical variable, Scale 1-4, where 1 = Much aggradation/degradation, 4 = None
3. Riparian Conditions (Reach scale)	
Hemiphot cover	Patch scale measure of % riparian canopy cover calculated using fish-eye lens (hemi) photography
Riparian vegetation	Categorical variable, Scale 0-4, where 0 = No riparian vegetation, 4 = Excellent riparian vegetation
4. Water/sediment chemistry (Reach and catchment scale)	
Three water samples were collected at each site: 1) unfiltered for total ionic composition, 2) unfiltered for total concentrations of nutrients, and 3) filtered for concentrations of dissolved nutrients. (See Smith *et al*. for details)	
Ions gradient (PCA 1)	PCA was used to reduce the total number of water chemistry variables. PCA variable 1 explained 53% of the variation in site water chemistry and represented inorganic ions (see Smith *et al*., this issue, for details).
$NO_2 + NO_3$	Dissolved nitrite + nitrate-N concentration (mg l^{-1}) from water sample taken at the time of metabolism measurements
NH_4	Dissolved ammonium-N concentration (mg l^{-1}) from water sample taken at the time of metabolism measurements
TN	Total N (mg l^{-1}) from water sample taken at the time of metabolism measurements
PO_4	Filterable reactive phosphate (mg l^{-1}) from water chemistry sample taken at the time of metabolism measurements
TP	Total phosphate (mg l^{-1}) from water chemistry sample taken at the time of metabolism measurements
Maximum temperature	Maximum water temperature recorded by data logger over 24 hours in open water
Turbidity	Turbidity measured on unfiltered sample in laboratory (NTU).
5. In-stream habitat—none included	
6. Flow related –none included	

Table 2 Regression modelling results for benthic metabolism indicators against catchment and reach scale measures of the disturbance gradient. See Harch et al., this issue, for a description of the modelling process. Total variation explained by each model is shown in the first column, and a break down of that variation into the categories in which the disturbance indices are grouped is shown in subsequent columns.

PROCESS INDICATORS	Approximate R^2 overall model %	Disturbance gradient categories				Number of sites used in analysis
		Landuse %	Channel condition %	Riparian conditions %	Water & sediment chemistry %	
Direct Measures of Benthic Metabolism						
Gross Primary Production (GPP)						
Cobble sites	**89**		9		80	25
Sediment sites	**79**		7	44	28	22
All sites	**63**	4		32	27	47
Respiration (R_{24})						
Cobble sites	**84**	46			38	25
Sediment sites	**85**		5	39	41	22
All sites	**58**				58	47
NDM						
Cobble sites	**90**	18	3		69	25
Sediment sites	**49**	14			35	22
All sites	**38**		1		36	47
P/R						
Cobble sites	**25**				25	25
Sediment sites	**----**[¥]					22
All sites	**10**				10	47
Algal biomass						
Cobble sites	**43**	5	21		17	25
Sediment sites	**81**		6	37	38	21
All sites	**29**					45
Algal growth						
Artificial substrate	**66**	20		9	37	30
Stable Isotopes						
$\delta^{13}C$ (plants)	**60**	15			45	20
$\delta^{13}C$ (sediment)	**49**	35	3		11	43

[¥] = very poor model fit, R^2 not reported

Figure legends

Figure 1 Regression modelling results for gross primary production (GPP) and respiration (R_{24}) at sites with (a & d) cobble or (b & e) sediment substrate and all sites (c & f). Measured values are plotted against the values predicted using a model of disturbance gradient descriptors developed in a Generalised Linear Modelling (GLM) framework using stepwise regression modelling. Square root (GPP + 0.5)) and $\log_{10}(R_{24} + 15)$ transformations were used for modelling. Untransformed units are mg C m^{-2} d^{-1}. Cobble sites are designated with filled circles and sediment sites are designated with open circles.

Figure 2 Relationship between gross primary production (GPP, mg C m^{-2} d^{-1}) and riparian canopy cover as measured using fish eye lens photography and image analysis. Results of regression analysis are shown with a best fit line. Symbols as in Figure 1.

Figure 3 Regression modelling results (GLM) for chlorophyll *a* concentrations on natural substrates at sites with (a) cobble or (b) sediment substrate and all sites (c). The transformation $\log_{10}$(Chl *a* + 1) was used in the model and untransformed units are mg Chl *a* m^{-2}. Other details are as in Figure 1.

Figure 4 Regression modelling results (GLM) for chlorophyll *a* concentrations on artificial substrates. The transformation $\log_{10}$(Chl *a* + 1) was used in the model and untransformed units are mg Chl *a* m^{-2}. Other details are as in Figure 1.

Figure 5 Regression modelling results (GLM) for $\delta^{13}C$ values of aquatic plants (a) and sediment (b). The transformation $\delta^{13}C + 50$ was used for both models and untransformed units are ‰. Other details are as in Figure 1.

Figure 6 Relationships between gross primary production (GPP) and chlorophyll *a* concentrations on natural substrates. Results of regression analysis for cobble and sediment sites are shown with best fit lines. Symbols as in Figure 1.

Figure 7 Relationships between $\delta^{13}C$ values of aquatic plants (a) and sediment (b) and gross primary production (GPP). Results of regression analysis for all sites are shown in (a). Results for cobble, sediment, and all sites are shown with separate best fit lines in (b). Symbols as in Figure 1.

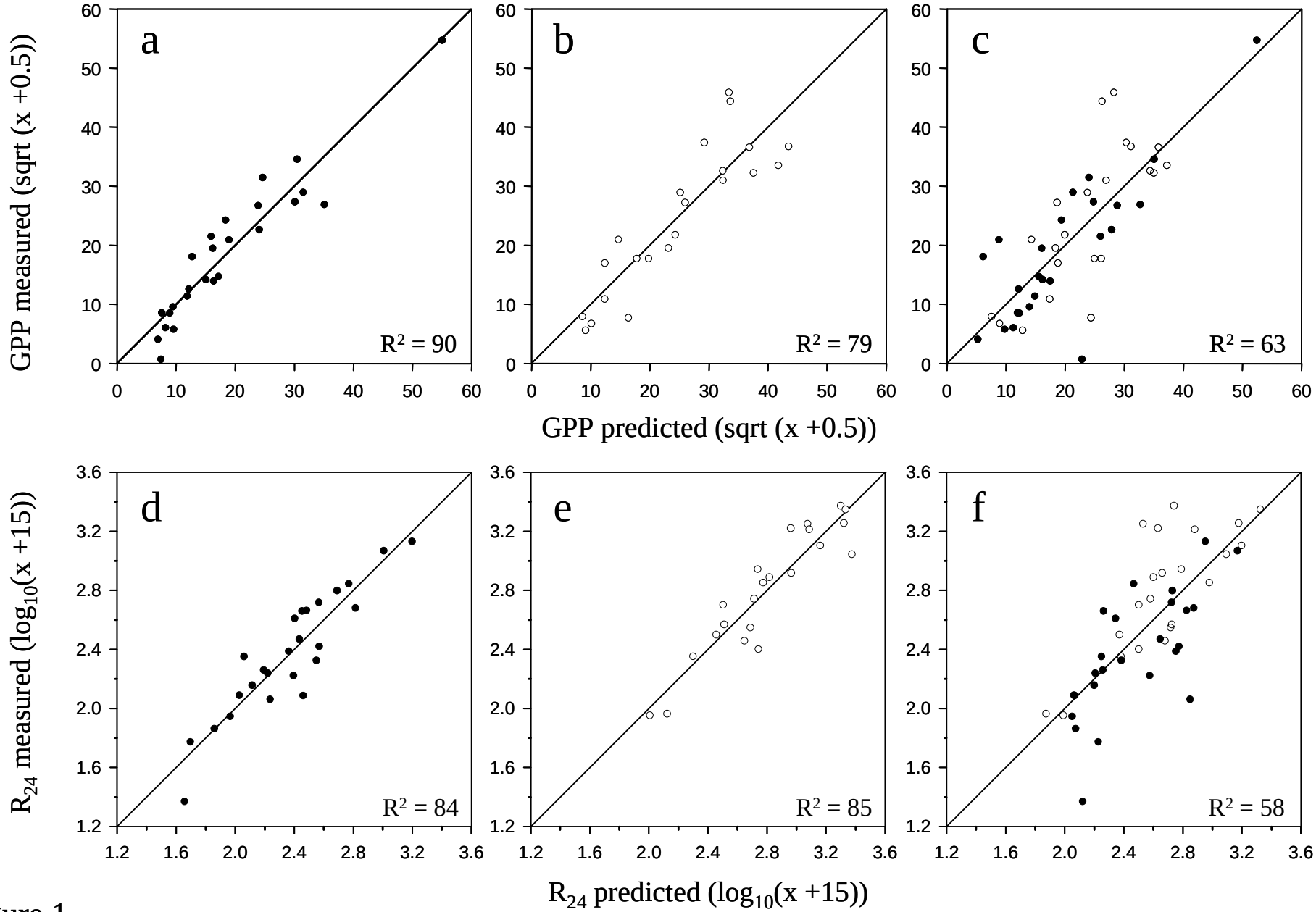

a
b
c
d
e
f
R² = 90
R² = 79
R² = 63
R² = 84
R² = 85
R² = 58
GPP measured (sqrt (x +0.5))
GPP predicted (sqrt (x +0.5))
R24 measured (log10(x +15))
R24 predicted (log10(x +15))

Figure 1

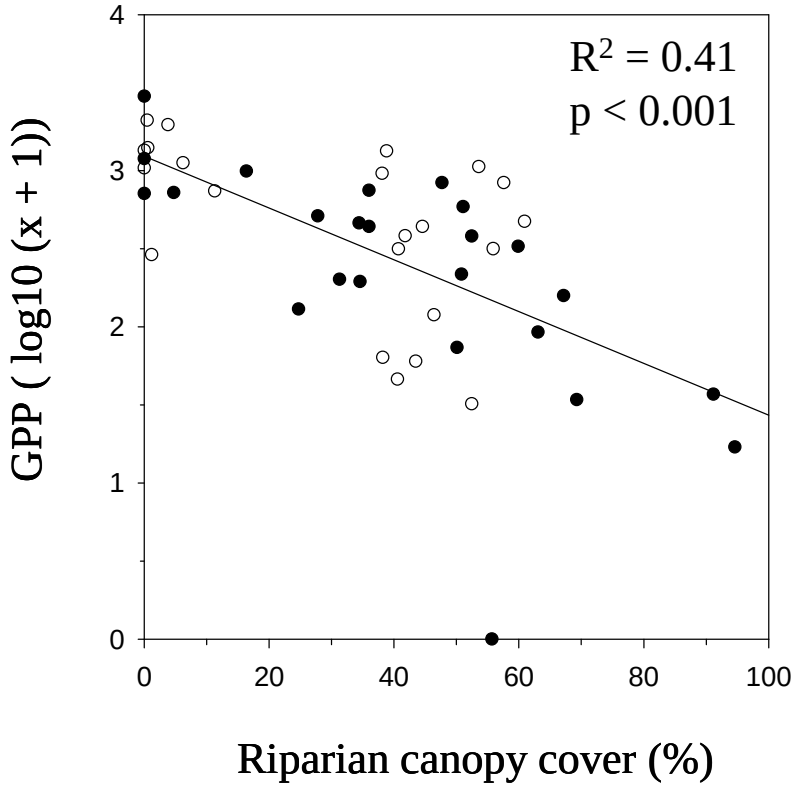

R2 = 0.41
p < 0.001
GPP (log10 (x + 1))
Riparian canopy cover (%)
0
1
2
3
4
0
20
40
60
80
100

Figure 2

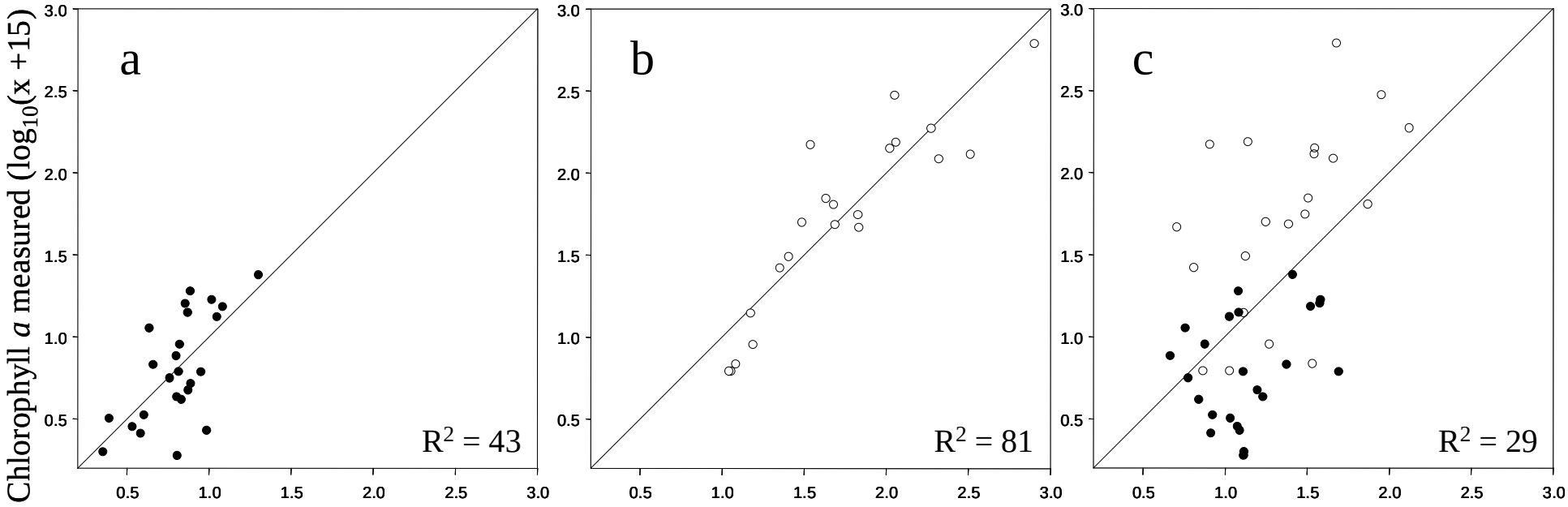

a
b
c
R2 = 43
R2 = 81
R2 = 29
Chlorophyll a measured (log10(x +15)
Chlorophyll a predicted (log10(x +1))
0.5
1.0
1.5
2.0
2.5
3.0

Figure 3

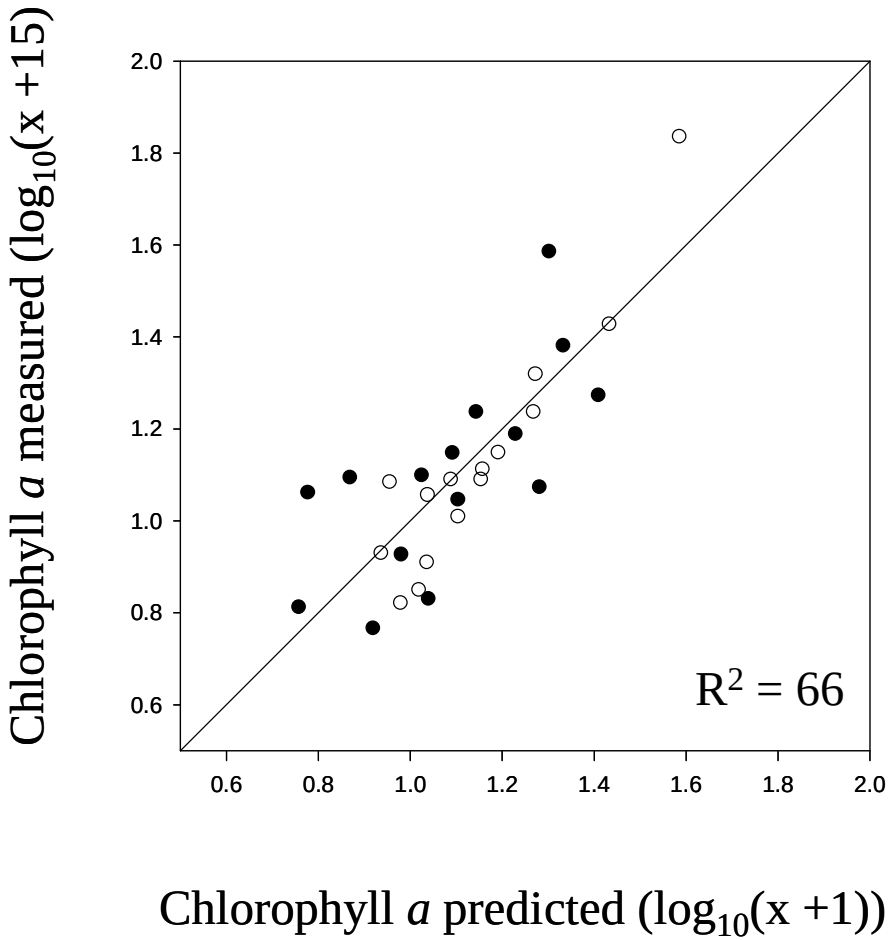
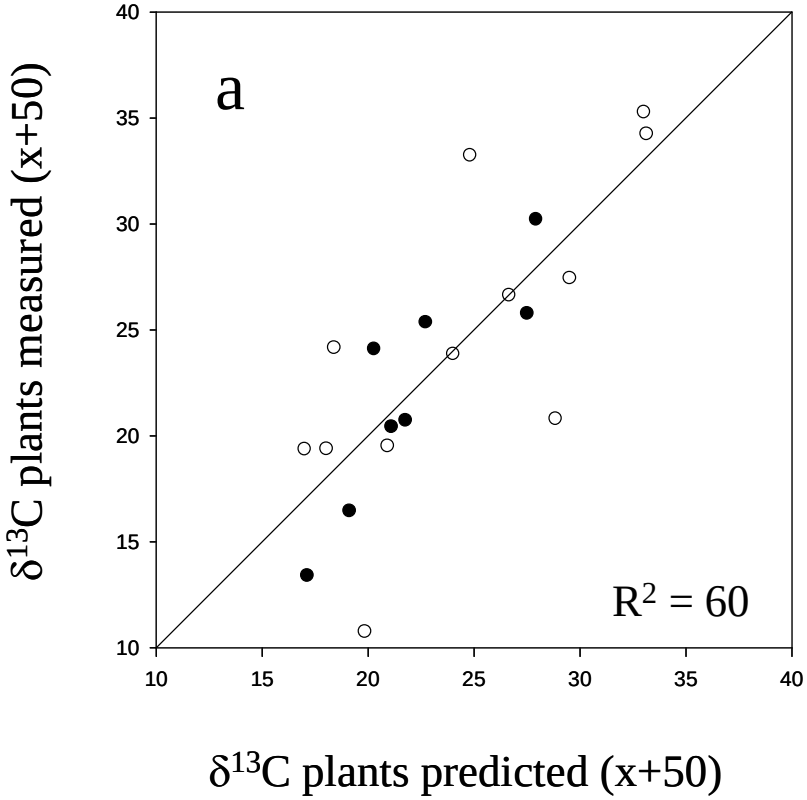

Chlorophyll *a* measured (log_{10}(x +15))
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
R^2 = 66
Chlorophyll *a* predicted (log_{10}(x +1))

Figure 4


a
δ13C plants measured (x+50)
δ13C plants predicted (x+50)
R2 = 60
10
15
20
25
30
35
40

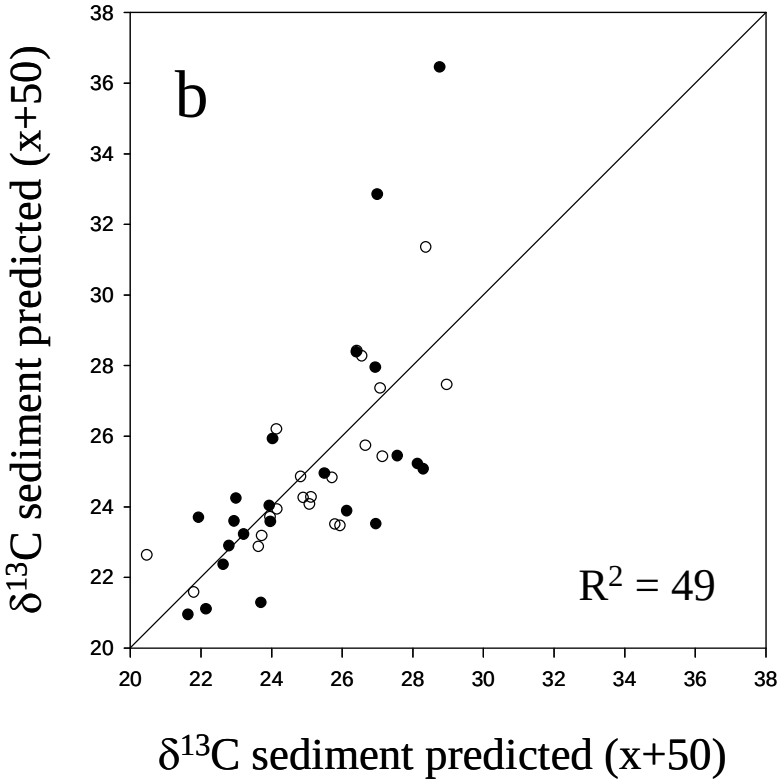

b
δ13C sediment predicted (x+50)
δ13C sediment predicted (x+50)
R2 = 49
20
22
24
26
28
30
32
34
36
38

Figure 5

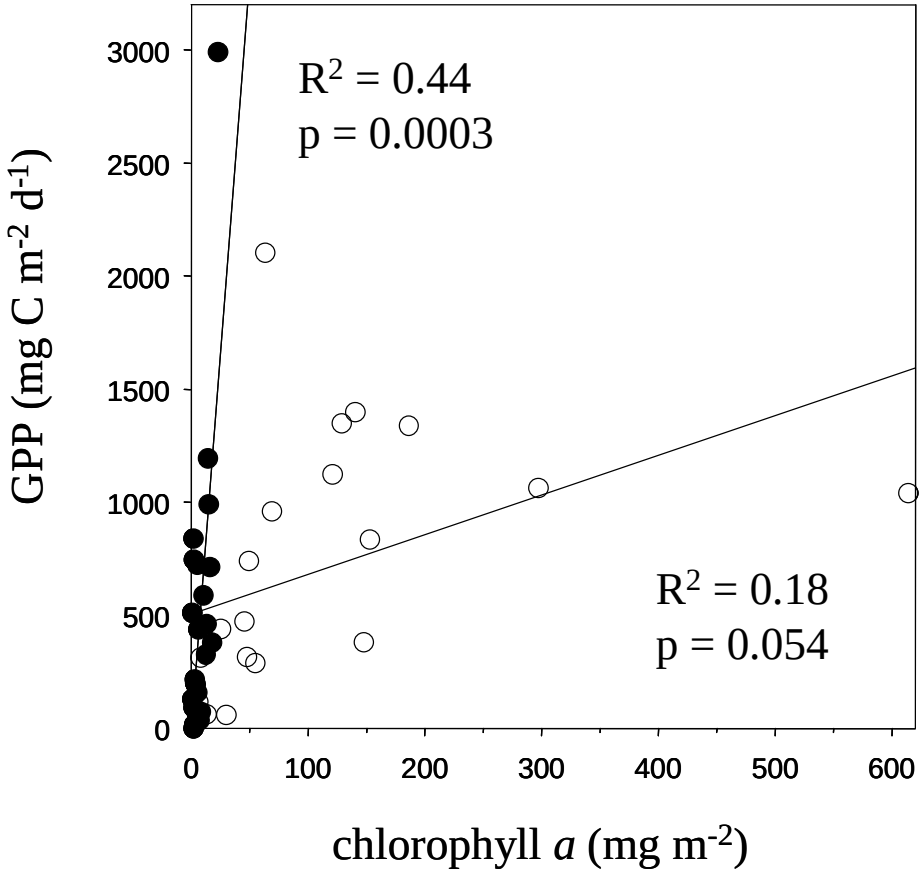

R2 = 0.44
p = 0.0003
R2 = 0.18
p = 0.054
GPP (mg C m-2 d-1)
chlorophyll a (mg m-2)
0
500
1000
1500
2000
2500
3000
0
100
200
300
400
500
600

Figure 6

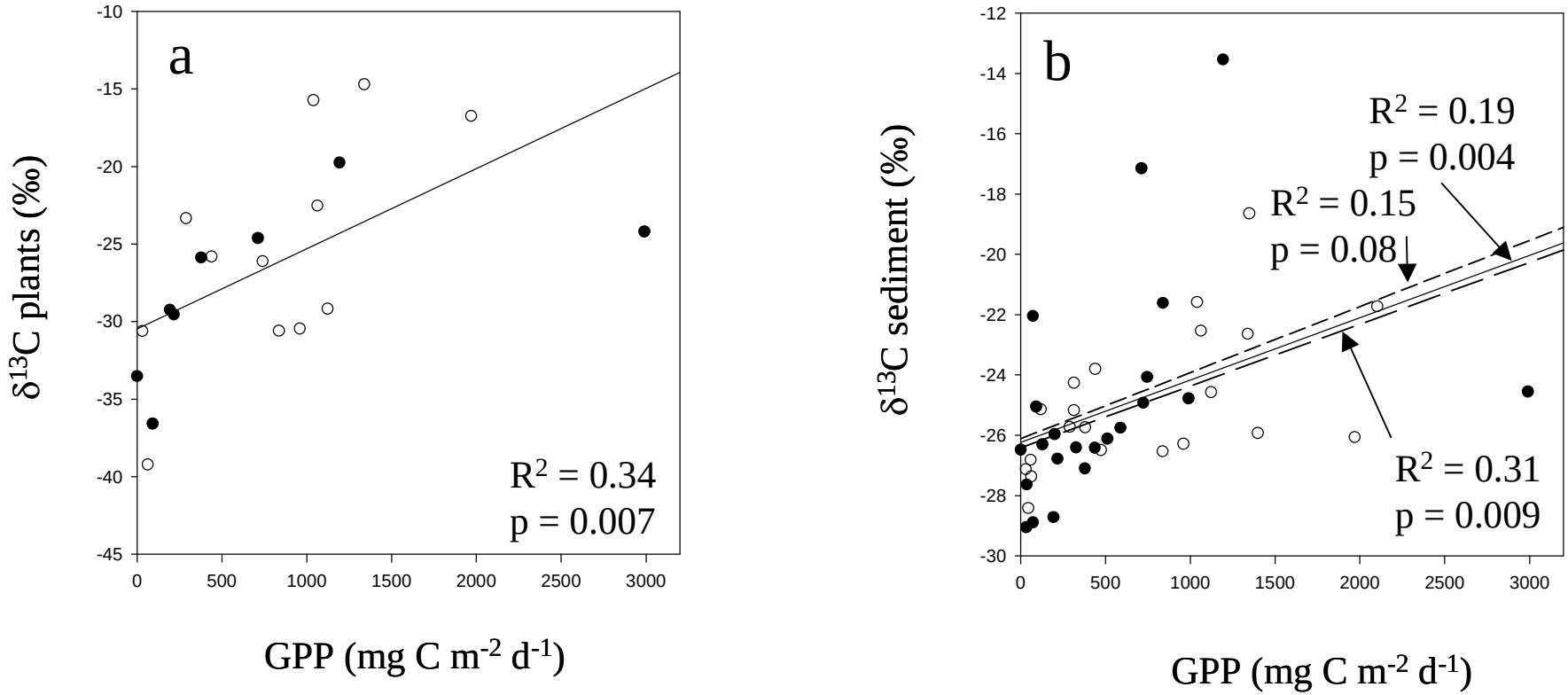

a
b
δ13C plants (‰)
δ13C sediment (‰)
GPP (mg C m-2 d-1)
GPP (mg C m-2 d-1)
R2 = 0.34
p = 0.007
R2 = 0.19
p = 0.004
R2 = 0.15
p = 0.08
R2 = 0.31
p = 0.009

Figure 7