SUMMARY

1. Rates of whole-system metabolism (production and respiration) are fundamental indicators of ecosystem structure and function. Although first-order, proximal controls are well understood, assessments of the interactions between proximal controls and distal controls, such as land use and geographic region, are lacking. Thus, the influence of land use on stream metabolism across geographic regions is unknown. Further, there is limited understanding of how land use may alter variability in ecosystem metabolism across regions.

2. Stream metabolism was measured in nine streams in each of eight regions ($n = 72$) across the United States and Puerto Rico. In each region, three streams were selected from a range of three land uses: agriculturally influenced, urban-influenced, and reference streams. Stream metabolism was estimated from diel changes in dissolved oxygen concentrations in each stream reach with correction for reaeration and groundwater input.
3. Cross primary production (GPP) was highest in regions with little riparian vegetation (sagebrush steppe in Wyoming, desert shrub in Arizona/New Mexico) and lowest in forested regions (North Carolina, Oregon). In contrast, ecosystem respiration (ER) varied both within and among regions. Reference streams had significantly lower rates of GPP than urban or agriculturally influenced streams.

4. GPP was positively correlated with photosynthetically active radiation and autotrophic biomass. Multiple regression models compared using Akaike's information criterion (AIC) indicated GPP increased with water column ammonium and the fraction of the catchment in urban and reference land-use categories. Multiple regression models also identified velocity, temperature, nitrate, ammonium, dissolved organic carbon, GPP, coarse benthic organic matter, fine benthic organic matter and the fraction of all land-use categories in the catchment as regulators of ER.

5. Structural equation modelling indicated significant distal as well as proximal control pathways including a direct effect of land-use on GPP as well as SRP, DIN, and PAR effects on GPP; GPP effects on autotrophic biomass, organic matter, and ER; and organic matter effects on ER.

6. Overall, consideration of the data separated by land-use categories showed reduced inter-regional variability in rates of metabolism, indicating that the influence of agricultural and urban land use can obscure regional differences in stream metabolism.

Keywords: ecosystem respiration, land use, metabolism, primary production, stream metabolism.
assess the influence and interactions of land use on and with these controls. The primary goal of this effort was to improve our understanding of how human activities interact with environmental factors to affect ecosystem functioning across regions. We predicted that agricultural and urban activities would differentially affect stream metabolism across regions via changes in proximal factors. Differential effects were hypothesised to be dependent on distal factors of a given region. For example, changes in light because of clearing of riparian vegetation associated with agricultural and urban activities was hypothesised to affect GPP in forested regions more than in regions with little or low-lying riparian vegetation. Similarly, nutrient loading associated with anthropogenic activities may have a more pronounced effect on stream metabolism in low-nutrient streams relative to streams with inherently higher nutrient concentrations. Because of the interactions between proximal and distal controls on stream metabolism, we hypothesised variation in stream metabolism across land-use categories for a given region would be greatest in low-nutrient, forested ecosystems. We utilised multiple linear regressions to identify independent variables driving GPP and ER separately then applied these variables to confirmatory structural equation models to assess relative strengths of independent variables on overall stream metabolism.

Methods

Study sites

We measured stream metabolism in nine streams in each of eight regions across the United States and Puerto Rico in conjunction with the Lotic Intersite Nitrogen experiment II (LINX II; Table 1), although 2 of the 72 streams are not included in this analysis as explained below. In each region, three streams from three different land-use categories were selected based on the dominant land use adjacent to the study reach. Land-use categories were reference (REF, native vegetation with low human influence), agriculture (AGR, including rangeland, pasture, and row crops), and urban (URB, including low and high density residential, commercial, and golf courses). Land use was also quantified as a continuous variable at the catchment scale using the fraction of each category based on United States Geological Survey

Fig. 1 Primary factors hypothesised to influence stream ecosystem respiration (ER) and gross primary production (GPP). Larger arrows indicate greater influence on rates of metabolism. Land use can influence all proximal factors and alter regional characteristics. Further, land use varies across regions (e.g. row-crop agriculture vs. irrigated pasture).
land-cover classifications (Mulholland et al., 2008). Metabolism measurements were made in conjunction with $^{15}$N-nitrate addition experiments during spring and summer in streams within the continental United States and during winter in Puerto Rican streams; analyses of relationships between metabolism and rates of nitrogen cycling are published elsewhere (see Mulholland et al., 2008; Hall et al., 2009; Mulholland et al., 2009 for additional details and analyses of $^{15}$N addition experiments).

Metabolism calculations

We calculated reach-scale metabolism using the standard technique of diel change in dissolved oxygen ($O_2$) in the open stream channel (e.g. Odum, 1956; Mulholland et al., 2001). Corrections in the $O_2$ budget were made in streams with substantial groundwater input following the methods of Hall & Tank (2005). $O_2$ concentrations and temperature were logged at an upstream and downstream station at 5-15 min intervals for 24-48 h. All sites followed detailed and consistent protocols for calibrating oxygen sensors. Distance between upstream and downstream stations varied and depended on water velocity to achieve a 20-30 min travel time between stations (reach lengths ranged from 50 to 800 m). Rereation rate, a measure of $O_2$ exchange between the stream and atmosphere and expressed as a rate constant (1 d$^{-1}$); was estimated with SF$_6$ or propane releases executed in conjunction with a conservative tracer release, with the latter also providing information on groundwater inputs and water travel time (Wanninkhof, Mulholland & Elwood, 1990; Marzolf, Mulholland & Steinman, 1994).

In 40 of the 72 streams, we were able to calculate metabolism using the 2-station method. In 30 streams, however, we were unable to calculate metabolism using the 2-station method for several reasons (e.g. sensor malfunction or large drift in sensor calibration at one of the two stations). Thus, metabolism estimates in those cases are made using a 1-station calculation, with the downstream sensor (end of the stream reach) being used for calculations in 20 of those streams and the upstream sensor used in 10 sites where downstream sensor data were either lost or of bad quality. Both the 1-station and 2-station estimation methods were randomly distributed among sites with differing land use (i.e. 2-station estimates are not biased by one land-use category or region). At one site (Southwest region, urban stream), we were unable to calculate GPP or ER because of the failure of both sensors. Additionally, in one Wyoming urban stream, we were unable to calculate ER because of particularly high groundwater input.

Metabolism using the 2-station method was calculated as:

$$gO_2m^{-2}min^{-1} = \left(\frac{C_t - C_0}{\Delta t} - kO_2 \times D\right) \times d$$

where: $C$ = change in $O_2$ concentration from the upstream to downstream sensor in milligram per liter at time t and time 0 with t corresponding to water travel time between the two sensors ($M$, min); $k_2$ is the oxygen rereation coefficient (min$^{-1}$) corrected for stream temperature; D is the $O_2$ saturation deficit (i.e. saturation concentration minus average reach concentration) during the time interval for measured stream temperature and atmospheric pressure; $d$ is mean
stream depth (m). We calculated stream depth (d) as follows:
\[
d = \frac{Q}{Vw}
\]
where: Q is stream discharge; w is average stream wetted width from measurements made every 1-2 m along the stream reach; and, V is average water velocity calculated from water travel time between stations. Metabolism using the 1-station method was calculated similarly to the 2-station calculation described above except changes in O2 concentrations were calculated per measurement interval at a single measurement station:
\[
gO_2 m^{-2} min^{-1} = (\frac{C_2 - C_1}{\Delta t_{meas}} - kO_2 + D) \times d
\]
where: C = change in O2 concentration in milligram per litter at time 2 and time 1 at the same location (station). In this case, time is sensor measurement interval (\(\Delta t_{meas}\), min).

Roberts et al. (2007) compared the 2-station and 1-station methods extensively in Walker Branch, Tennessee, and found the two methods gave similar results in streams with high reaeration rates. Streams with short water travel times through the study reach also should have comparable metabolism estimates between 1-station and 2-station calculations (Hall, Thomas & Gaiser, 2007). In our study, for sites where 2-station metabolism estimates were calculated, 1-station estimates were also calculated from both the upstream and downstream sensors. Both estimates were log-transformed then evaluated to assess consistency of methods (Fig. 2). Measures of ER using the 2-station estimation method were not different than those made with the 1-station estimation method (paired t-test, T-value = 0.17, P = >0.1, df = 37; Fig. 2a). Similarly, GPP estimates were comparable when calculated with the 2-station method relative to the 1-station method (paired t-test, T-value = <0.01, P > 0.1, df = 38; Fig. 2b). All analyses performed combined 1- and 2-station log-transformed estimates of GPP and ER.

Ancillary variables that might influence stream metabolism including riparian characteristics, transient storage, and organic matter biomass were measured in all streams using standard methods within 1 week of metabolism measurements (Table 2; Mulholland et al., 2008; Hauer & Lamberti, 2005). For all organic matter biomass estimates, stratified random sampling was conducted based on habitat surveys of each stream. Multiple samples of each organic matter type were randomly collected, and means of standing stock densities (g m\(^{-2}\)) for each organic matter type were weighted by the fractional contribution of that organic matter type to total stream area to calculate whole-stream standing stock. The number of samples per organic matter type was dependent on the relative abundance (e.g. three samples for organic matter types that comprised <10% of the stream reach, 6-8 samples for habitat types that comprised a higher percentage of the reach). Stream nutrient concentrations and photosynthetically active radiation (PAR) were measured concurrently with metabolism. PAR was measured at a location immediately adjacent to the stream channel and characteristic of light availability throughout the channel reach (i.e. shaded stream
Table 2 Summary of measured variables with method of sampling, abbreviation, reference, and statistical transformation used in data analysis

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Abbreviation</th>
<th>Units</th>
<th>Sampling method</th>
<th>Reference</th>
<th>Statistical transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
<td>RE</td>
<td>Categorical m s⁻¹</td>
<td>NA</td>
<td>NA</td>
<td>NA Natural log</td>
</tr>
<tr>
<td>Velocity</td>
<td>VEL</td>
<td>m s⁻¹</td>
<td>NA</td>
<td>Stream Solute Workshop 1990</td>
<td>Natural log</td>
</tr>
<tr>
<td>Discharge</td>
<td>Q</td>
<td>L s⁻¹</td>
<td>Change in conservative tracer from upstream to downstream</td>
<td>Webster &amp; Ehrmann, 1996</td>
<td>Natural log</td>
</tr>
<tr>
<td>Stream temperature</td>
<td>TEMP</td>
<td>Degrees Celsius</td>
<td>Logging Hydrolab or YSI probes deployed in stream channel</td>
<td>Hauer &amp; Lamberti, 2005</td>
<td>None</td>
</tr>
<tr>
<td>Channel width</td>
<td>WID</td>
<td>m</td>
<td>Line transect perpendicular to water flow with a minimum of 10</td>
<td>Ameel et al., 1993; USEPA 1993</td>
<td>Natural log</td>
</tr>
<tr>
<td>Channel depth</td>
<td>DEP</td>
<td>m</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Nitrate concentration</td>
<td>NO₃⁻-N</td>
<td>µg NO₃⁻-N L⁻¹</td>
<td>Collection of filtered water samples (GF/F nominal pore size &lt;0.7 µm) and analysis</td>
<td>Marzolf et al., 1994; Hall &amp; Tank, 2005</td>
<td>Natural log</td>
</tr>
<tr>
<td>Ammonium concentration</td>
<td>NH₄⁺-N</td>
<td>µg NH₄⁺-N L⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Dissolved inorganic nitrogen concentration</td>
<td>DIN</td>
<td>µg N L⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Dissolved organic nitrogen concentration</td>
<td>DON</td>
<td>µg N L⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Soluble reactive phosphorus</td>
<td>SRP</td>
<td>µg P L⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Dissolved organic carbon</td>
<td>DOC</td>
<td>mg C L⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>DO</td>
<td>mg O₂ L⁻¹</td>
<td>Natural log</td>
<td>Hauer &amp; Hill, 1996</td>
<td>Natural log</td>
</tr>
<tr>
<td>Gross primary production</td>
<td>GPP</td>
<td>g O₂ m⁻² d⁻¹</td>
<td>Diel changes in O₂ concentrations corrected for respiration and groundwater input</td>
<td>Marzolf et al., 1994; Hall &amp; Tank, 2005</td>
<td>Natural log</td>
</tr>
<tr>
<td>Ecosystem respiration</td>
<td>ER</td>
<td>g O₂ m⁻² d⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Heterotrophic respiration</td>
<td>CRhet</td>
<td>g O₂ m⁻² d⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Photosynthetically active radiation</td>
<td>PAR</td>
<td>mol quanta m⁻² d⁻¹</td>
<td>Logging light meter deployed adjacent to stream channel; average over 24 h</td>
<td>LiCor 1905A, LI-COR, Lincoln, NB, U.S.A.</td>
<td>Natural log</td>
</tr>
<tr>
<td>Coarse benthic organic matter</td>
<td>CBOM</td>
<td>g AFDM m⁻²</td>
<td>Stratified random sampling and collection of known area of sample</td>
<td>Hauer &amp; Lamberti, 2005</td>
<td>Natural log</td>
</tr>
<tr>
<td>Surface fine benthic organic matter</td>
<td>sFBOM</td>
<td>g AFDM m⁻²</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Deep fine benthic organic matter</td>
<td>dFBOM</td>
<td>g AFDM m⁻²</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Autotrophic benthic organic matter</td>
<td>AUT</td>
<td>g AFDM m⁻²</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Detrital benthic organic matter</td>
<td>dBOM</td>
<td>g AFDM m⁻²</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Dispersion coefficient</td>
<td>D</td>
<td>m² s⁻¹</td>
<td>Transient storage measurements calculated from conservative tracer release and OTIS-P software</td>
<td>Rencala and Walters 1983, Runkel, 1998</td>
<td>Natural log</td>
</tr>
<tr>
<td>Cross sectional area of the channel</td>
<td>A</td>
<td>m²</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Cross sectional area of the transient storage zone</td>
<td>Aₜ</td>
<td>m²</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Aₜ/A</td>
<td>Aₜ/A</td>
<td>m² m⁻²</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Rₜ</td>
<td>Rₜ</td>
<td>s⁻¹</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Fmed200</td>
<td>Fmed</td>
<td>Unitless</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
<tr>
<td>Time spent in the storage zone</td>
<td>RET</td>
<td>s</td>
<td>Natural log</td>
<td></td>
<td>Natural log</td>
</tr>
</tbody>
</table>

© 2010 Blackwell Publishing Ltd, Freshwater Biology, 55, 1874–1890
channels had PAR measurement recorded in similarly shaded locations next to the channel).

Statistics

All statistical analyses were performed on monotonic transformations of data to meet normality assumptions (Table 2). We selected predictor variables based on hypothesised relationships between them and ecosystem metabolism (Fig. 1). We estimated the association between proximal factors and GPP and ER using Pearson correlation analyses. We tested for differences in GPP or ER among regions and land-use categories using two-way analysis-of-variance (ANOVA) followed by Tukey’s post hoc pairwise comparisons. To examine variation in metabolism within regions, we calculated coefficients of variation for each region (experimental unit) by dividing the standard deviation of GPP or ER by the mean of GPP or ER. To compare variation in metabolism across land-use categories within regions, we calculated coefficients of variation for land-use category (n = 3 for each land-use category) with region as the experimental unit (n = 8). Correlation and ANOVA statistics were performed using SAS statistical software (SAS Institute 1999).

Influence of distal factors (region, catchment land use) and GPP and ER and interaction between distal and proximal factors (light, nutrients, organic matter) were determined by developing predictive multiple linear regression models and Akaike’s information criterion (AIC) for statistical inference (Akaike, 1973; Burnham & Anderson, 2002). These multiple regression analyses allowed for inclusion of region as a predictive variable. The coefficients associated with the regional categorical values adjust the intercept of the regression model. Thus, analyses can indicate whether metabolism in one region is higher or lower than another region after accounting for other explanatory variables. Initially, multiple linear regression models predicting GPP or ER (without interaction or higher-order terms) were constructed using all potential predictors of these variables, including proximal and distal factors. To formulate models that balanced predictive ability and parsimony, we used stepwise procedure to add or remove predictor variables to produce models with the lowest scores of AICc (AIC corrected for small sample size) (Burnham & Anderson, 2002). To include a larger pool of explanatory variables, missing data were replaced in a small number of cases (n = 5) with the average value of the other two sites within the same land use and regional classification. While replacing missing data with means can distort estimates of variance, the distribution of missing data was sparse (<3 of 72 sites for any one variable) and distributed among sites, land uses, and regions, suggesting this technique would have minimal influence on the model selection process (McCune & Crace, 2002). When missing data were not included in models, predictive variables were not significantly different. Analyses conducted using estimates for missing data identified additional
predictive variables. Selection of models was conducted with the statistical software R v.2.40 (R Development Core Team, 2006).

To assess proposed relationships among proximal and distal factors and GPP and ER simultaneously, we employed structural equation modelling (SEM) using observed variables and hypothesised causal pathways (Shipley, 2000; Grace, 2006). SEM was conducted on log-transformed data for 64 sites (eight sites were eliminated because of missing data), except that land use, quantified in the model as the sum of percentage urban and agricultural land in the catchment, and was arcsin transformed. Data were modelled using SPSS statistical software equipped with AMOS 17.0 (Amos v. 17.0; SPSS, Chicago, IL, U.S.A.). The original hypothesised model fit the structure of the data poorly, and additional suggested pathways were included incrementally to achieve the best model fit.

Although the purpose of SEM and multiple linear regression techniques are similar, each technique provides unique insight not afforded by the other. Multiple linear regression models allowed for inclusion of categorical regional variables and additional independent variables not afforded by SEM models for assessment of GPP and ER independently. However, multivariate regression compounds measurement error and increases Type I error. Since SEM paths are not additive, but are computed simultaneously, and because error terms are modelled in the analyses, problems with Type I and measurement error are decreased using this technique. We used multiple linear regression models as an exploratory tool whereas the SEM models were used as a confirmatory tool using a priori hypotheses established with exploratory models. SEM takes into account interactions, nonlinearities, correlated independent variables, and measurement error for a more robust analysis relative to multiple linear regressions. Advantages of SEM compared to multiple regression include more flexible assumptions (particularly when associated with multicollinearity), and the ability to test multiple dependent factors (i.e., GPP, ER), simultaneously.

Results

General patterns in stream metabolism

Across all streams, GPP ranged from 0.1 to 23.1 g O$_2$ m$^{-2}$ d$^{-1}$ (Fig. 3). GPP differed significantly among regions (Table 3; Fig. 3a) and land-use category (Table 3; Fig. 3b), and there was no interaction between region and land-use category (ANOVA, $P = 0.32$). Regions with forested riparian vegetation (MA, NC, MI, OR, PR) had lower mean rates of GPP than regions with more open riparian vegetation (KS, WY, SW; Fig. 3a). Overall, reference streams had approximately 30% lower GPP than urban- or agriculturally influenced streams (Table 3; Fig. 3b).

Ecosystem respiration varied almost 100-fold across regions and among land-use categories (Fig. 3c,d). However, unlike GPP, there was a significant interaction between region and land-use category for ER (ANOVA interaction region*land-use category $P = 0.048$). This interaction suggests the response of ER to adjacent land-use practices depends on the regional context of the stream ecosystem. Only some regions had significant differences in ER between land-use categories. For example, the streams in Massachusetts ($P = 0.019$) and Kansas ($P = 0.032$) had higher ER in agricultural streams relative to urban streams, and the North Carolina streams had higher ER in reference relative to urban streams ($P = 0.041$).

All regions in this study, except Kansas, had negative rates of mean net ecosystem production (NEP; Fig. 3c), indicating net heterotrophic metabolism. Massachusetts, North Carolina, and Michigan had significantly lower NEP than other regions. Reference land use had significantly lower NEP than agricultural or urban land use across regions (Fig. 3f). There was no significant interaction between region and land-use category for NEP ($P = 0.37$). Across all sites, log ER co-varied positively with log GPP, but a number of streams had high rates of ER without correspondingly high rates of GPP (Fig. 4).

Factors controlling gross primary production and ecosystem respiration

Gross primary production increased with light availability (Fig. 5a) and total autotrophic biomass (Fig. 5b). Biomass of individual autotroph types (e.g. filamentous green algae, epilithon) did not correlate with any measure of GPP ($P > 0.15$). Although the correlation between GPP and light was strongest when light was measured as PAR, there was also a negative correlation with light measured as percentage
canopy shade in riparian zone (data not shown; \( r = 0.371, P = 0.03 \)). Within land-use categories, GPP was not related to PAR in urban or agricultural streams (Fig. 6). However, a significant linear relationship between GPP and PAR in reference streams across regions suggested that light limitation in reference streams drove the overall relationship; and therefore, light may not have been limiting aquatic primary production in urban and agricultural streams.

The set of multiple regression models selected with AICc scores indicated that several additional variables influenced rates of GPP (Table 3). Specifically, the selected model included region, \( \text{NH}_4^+ \)-N concentrations, PAR, autotrophic biomass, and the fraction of the catchment in urban and reference land-use categories.

Table 3 Summary of multiple regression models for gross primary production (GPP) and ecosystem respiration (ER) selected based on AICc scores. Variables transformed and abbreviated according to Table 2.

| Rank | GPP Model Covariates                                      | AICc  | Δ\( \text{AIc} \) | Likelihood \( [\text{L}(g | x)] \) | wi  | Adjusted \( R^2 \) |
|------|----------------------------------------------------------|-------|------------------|-------------------------------------|-----|-------------------|
| 1    | RE, \( \text{NH}_4^+ \)-N, PAR, AUT, REF, URB           | 12.13 | -2.06           | 2.80                               | 2.80| 0.67              |
| 2    | RE, \( \text{NH}_4^+ \)-N, PAR, REF, URB                | 12.80 | -1.38           | 1.99                               | 1.99| 0.65              |
| 3    | RE, \( \text{NH}_4^+ \)-N, PAR, AUT, REF, URB, DON      | 14.13 | -0.05           | 1.02                               | 1.02| 0.67              |
| 4    | RE, \( \text{NH}_4^+ \)-N, PAR, REF, URB, DON           | 14.18 | 0.00            | 1.00                               | 1.00| 0.66              |

ER model covariates

1. VEL, \( \text{NO}_3^- \)-N, \( \text{NH}_4^+ \)-N, DOC, GPP, CBOM, sFBOM, REF, AGR, URB
2. VEL, TEMP, \( \text{NO}_3^- \)-N, \( \text{NH}_4^+ \)-N, DOC, GPP, CBOM, sFBOM, REF, AGR
3. VEL, TEMP, \( \text{NO}_3^- \)-N, \( \text{NH}_4^+ \)-N, DOC, GPP, CBOM, sFBOM, REF, AGR, URB
4. VEL, TEMP, \( \text{NO}_3^- \)-N, \( \text{NH}_4^+ \)-N, DOC, GPP, CBOM, sFBOM, REF, AGR, URB

© 2010 Blackwell Publishing Ltd, *Freshwater Biology*, 55, 1874–1890
categories as explanatory variables. Variables in the selected multiple regression models were also significant in three alternative models with low AICc values (Table 3). Overall, higher GPP was associated with decreased reference vegetation or urban land use in the catchment (Table 4). The set of multiple linear regression models selected with AICc scores for ER included velocity, temperature, NO$_3^-$-N, NH$_4^+$-N, DOC, GPP, CBOM, surface FBOM, and the fraction of reference, agricultural and urban land use in the catchment (Tables 3 & 4).

Structural equation modelling identified significant causal relationships between stream variables and measured metabolism (Fig. 7). Our model of the controls on stream metabolism was a significant fit to the covariance matrix ($\chi^2$ test $P = 0.161$, df = 13). Significant pathways included land-use effects on SRP, DIN, and GPP; PAR effects on GPP; GPP effects on autotrophic biomass, organic matter, and ER; and organic matter effects on ER. Land use did not affect PAR and DIN, and SRP concentrations did not significantly influence GPP or ER across all regions and land-use categories. Land use had the greatest effect on DIN concentrations, but DIN concentrations did not significantly influence stream metabolism. Catchment land use also significantly influenced GPP, although this must have exerted its influence through a proximal variable not measured in this analysis. When land use was removed, model fit was not consistent with the data (i.e., predictive model did not reproduce the data). ER increased with organic matter which was negatively influenced by GPP. Overall, multiple regression models developed for GPP were stronger than those developed for ER, explaining 67% compared with 28% of the variation, respectively.

Multiple linear regression models selected according to AICc criteria contained similar explanatory variables to the SEM model although GPP and ER response to nutrient concentrations was inconsistent among models. Specifically, multiple linear regression models indicated NH$_4^+$-N and NO$_3^-$-N were important predictors of stream metabolism whereas the SEM model indicated DIN was not a significant proximal or distal influence. This was also true when DIN was modelled as NH$_4^+$-N and NO$_3^-$-N as individual variables in SEM. Separation of DIN into NH$_4^+$-N and NO$_3^-$-N in SEM yielded models that were inconsistent with the data, reducing explanatory power. Adjusted $R^2$ values of multiple regression models (mean $R^2 = 0.66$) for GPP were comparable to $R^2$ calculated for GPP in SEM models ($R^2 = 0.66$). However, adjusted $R^2$ for ER multiple regression models (mean $= 0.28$) were much lower than SEM values ($R^2 = 0.85$), indicating that the SEM model was better at explaining observed variance in ER.

Variation in metabolism
For streams grouped by region, the coefficient of variation (CV) in GPP ranged from 0.5 to 2.0 (Fig. 8a). In comparison, streams grouped by land-use category
SW, KS). It is probable that forested Michigan sites had higher variation in GPP because three of the nine study streams were measured in spring prior to complete leaf-out. Variability in regional ER ranged from 1.2 to 2.3. Variation in ER was greater than variation in GPP in all regions except the Southwest. There was no significant difference in variation of ER among land-use categories (ANOVA P > 0.15).

Discussion

Our data provide one of the most comprehensive analyses of stream metabolism currently available because they span diverse regions and land cover, and they incorporate assessment of distal (region, land use) and proximal (light, nutrients) factors potentially influencing GPP and ER. The stream metabolism rates in this study are within the range reported for many other single and multi-site studies (Minshall et al., 1983;
Urban and agricultural streams had higher values for GPP (middle-third of the distribution and maximum), as would be expected with higher nutrients, increased light because of open canopy, or both. Neither urban nor agricultural streams differed substantially with respect to ER, suggesting that land use more strongly influences autotrophic production than organic matter inputs (amount and type) in our study regions.

Proximal factors influencing gross primary production and ecosystem respiration

First-order controls on stream metabolism identified in this study were consistent with previous studies (Lamberti & Steinman, 1997; Mulholland et al., 2001). Urban and agricultural streams had higher values for GPP (middle-third of the distribution and maximum), as would be expected with higher nutrients, increased light because of open canopy, or both. Neither urban nor agricultural streams differed substantially with respect to ER, suggesting that land use more strongly influences autotrophic production than organic matter inputs (amount and type) in our study regions.
although this analysis provided several novel contributions regarding the relative strength of these proximal controls across geographic regions. For example, even though type and abundance of autotrophic organisms varied widely among our study sites, total autotrophic biomass was correlated with GPP (g AFDM m\(^{-2}\); Fig.5b), and total autotrophic biomass was a significant predictive variable in multiple regression and structural equation models. Interestingly, autotrophs in Puerto Rican streams were dominated by epilithon, and biomass (as indicated by chlorophyll) was low relative to other streams, yet Puerto Rican streams had high GPP relative to available PAR (Fig. 6a). Thus, epilithic autotrophs in these streams appear to have higher photosynthetic efficiencies (Minshall, 1978; Hill, Ryon & Schilling, 1995), and available PAR has less of an effect on ecosystem production.

Multiple regression models indicated NH\(_4\)\(^+-\)N was a proximal factor influencing GPP, and both NH\(_4\)\(^+-\)N and NO\(_3\)\(^-\)N were significant proximal controls identified in ER multiple regression models. However, in SEM models, DIN (sum of NH\(_4\)\(^+-\)N and NO\(_3\)\(^-\)N) was not a significant proximal influence on GPP or ER but was necessary for a model fit consistent with the data (Fig. 8). Although land use can greatly influence DIN concentrations (Mulholland et al., 2008), distal controls (e.g. soil, vegetation, climate) associated with individual regions probably determine the role of DIN in controlling stream metabolism.

Contrary to previous inter-regional comparisons of stream metabolism (Lamberti & Steinman, 1997; Mulholland et al., 2001), we found no relationship between SRP concentration and GPP. Our study incorporated a wider array of stream types from multiple land-use categories than either of the other two studies. The range in SRP concentrations was considerably greater in the current study (0.17-310.5 \(\mu\)g SRP L\(^{-1}\) in this study, 1.8-13.2 \(\mu\)g SRP L\(^{-1}\) in Mulholland et al., 2001), and dissolved inorganic phosphorus may only be a proximal factor controlling GPP at low concentrations, whereas other factors may exert stronger control when phosphorus concentrations are higher. However, the relationship between SRP and GPP was not significant even when only reference sites with lower SRP concentrations were analysed (\(P = 0.70\), data not shown). Thus, our data may indicate that P is not limiting to GPP in most of the study streams and that we selected predominantly N-limited streams (Johnson, Tank & Dodds, 2009). Alternatively, P limitation may not emerge as a key factor because it is masked by other factors (e.g. PAR) that better explain variation across the wide range of systems considered. P availability also may vary temporally, and our single measurement at each stream may have missed preceding periods when it was low. A similar argument may explain why DIN fails to directly link to GPP in the SEM model; single concentration values from a stream may fail to adequately characterise nutrient availability.

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Gross primary production</th>
<th>Ecosystem respiration</th>
<th>Net ecosystem production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper one-third</td>
<td>1.8</td>
<td>9.8</td>
<td>-1.8</td>
</tr>
<tr>
<td>Lower one-third</td>
<td>0.2</td>
<td>3.1</td>
<td>-7.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.1</td>
<td>0.4</td>
<td>-19.6</td>
</tr>
<tr>
<td>Maximum</td>
<td>3.9</td>
<td>23.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Agricultural</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper one-third</td>
<td>3.9</td>
<td>7.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Lower one-third</td>
<td>0.8</td>
<td>4.1</td>
<td>-3.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.1</td>
<td>0.9</td>
<td>-12.6</td>
</tr>
<tr>
<td>Maximum</td>
<td>16.2</td>
<td>15.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Urban</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper one-third</td>
<td>3.3</td>
<td>8.7</td>
<td>-0.3</td>
</tr>
<tr>
<td>Lower one-third</td>
<td>0.7</td>
<td>4.9</td>
<td>-5.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.1</td>
<td>0.5</td>
<td>-17.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>11.9</td>
<td>17.9</td>
<td>7.3</td>
</tr>
<tr>
<td>Dodds, 2006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper one-third</td>
<td>1.8</td>
<td>8.3</td>
<td>-4.2</td>
</tr>
<tr>
<td>Lower one-third</td>
<td>0.4</td>
<td>6.7</td>
<td>-6.7</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.1</td>
<td>2.4</td>
<td>-29.0</td>
</tr>
<tr>
<td>Maximum</td>
<td>15.0</td>
<td>29.0</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table 5 Distributions of whole-stream metabolism rates by land-use category. Rate distributions by trophic status compiled by Dodds (2006) are included for comparison.
Distal factors influencing gross primary production and ecosystem respiration

In comparison with reference streams, urban and agricultural activity increased GPP in most regions (Fig. 3) including 11 individual streams with GPP : ER > 1. This trend suggests that energy sources become increasingly autochthonous in streams with increased urban and agricultural activity in the catchment. Across all sites, GPP was only light limited in reference streams (Fig. 6a) and land-use change associated with agricultural and urban activities probably relieved light limitation in forested regions (MI, NC, OR). Alternatively, agricultural and urban activities may be disconnecting streams from their riparian zones and catchments, ultimately changing the primary energy source (e.g. through modifications such as concrete-lined channels, dredging, and compaction of substrata).

Although we expected land use to significantly influence GPP, we did not expect urban and agricultural activities to yield a similar ecosystem response given differences in pressures applied by these two activities. Across regions, urban and agriculture activities probably influence proximal controls on metabolism similarly (changing light availability, hydrology) resulting in comparable ecosystem responses. Regional agricultural activity (e.g. either row-crop or pasture) can alter proximal factors in a manner that is distinct from regional urban activity (e.g. suburban or urban development) yielding significant differences in ecosystem metabolism among land-use categories within a region. However, this distal effect is muted across regions with variable characteristics.

The study region was a statistically significant variable for models of GPP but not ER (Tables 3 & 4). Thus, distal factors such as climate and catchment vegetation may be as important as proximal factors in driving variation in primary productivity of streams across regions. Further, GPP increased with decreasing fraction of reference land cover, and increasing fraction of agricultural and urban land use in the catchment, probably as a result of the influence of human activities in these land-use categories on proximal factors such as light, nutrients, and organic matter. In a region with little natural riparian vegetation or inherently high nutrient concentrations, changes in these proximal factors would be expected to have minimal effects on GPP. However, no interaction between region and land use was observed indicating that in regions with little riparian vegetation, anthropogenic nutrient enrichment may be alleviated. In contrast, the significant interaction between region and land use effects on ER (Table 3, Fig. 4) suggests that some regional characteristics controlling ER may be influenced by land use.

For our SEM model, land use was quantified as the sum of the fraction of urban and agricultural land use in the catchment and this distal variable significantly influenced GPP but not ER. Thus, agricultural and urban activities evidently influence GPP through mechanisms not identified in this study. For example, changing land use may introduce trace organic contaminants (e.g. pesticides, pharmaceuticals) that influence rates of production through toxicity or as potential carbon sources stimulating heterotrophic activity. Alternatively, reduced stability of benthic substrata may have inhibited algal attachment in agriculturally influenced streams.

Interactions between proximal and distal factors influencing ecosystem metabolism

Comparing models developed using SEM with multiple linear regression models allows for assessment of interactions between proximal and distal controls on stream metabolism. Interactions between proximal and distal controls on stream metabolism were most predominant with regards to the effects of light on GPP and the effects of hydrology on ER. Urbanisation and agriculture tend to reduce light limitation through clearing of riparian vegetation thereby increasing GPP (Young & Huryn, 1999). However, in some regions, particularly those with less compacted soils, agricultural and urban activities may increase light limitation by increasing suspended sediments (Allan, Erickson & Fay, 1997). Similarly, the lack of a relationship between transient storage parameters and ER in this study may be because of the inclusion of these land-use categories across geographical regions. Urban and agricultural activities may decrease transient storage and hydrologic variability through dredging, channelisation, and other activities (Gooseff, Hall & Tank, 2007). Alternatively, the lack of significance of transient storage metrics may indicate ER is primarily controlled by surface organic matter.
with little hyporheic interaction (Fellows, Valett & Dahm, 2001). Overall, multiple regression models developed for GPP were stronger than those developed for ER, explaining 67% compared with 28% of the variation, respectively.

Land use reduced regional variation in stream GPP (Fig. 8). Changes in stream structure associated with agricultural and urban activities were similar across most regions (e.g., stream channelisation, removal of riparian vegetation, etc.). These changes modify regional characteristics (canopy cover, catchment vegetation, hydrology, etc.) that alter ecosystem function via proximal factors (Fig. 1). This homogenisation of ecosystem structure and function has been suggested as a general outcome of urbanisation (Grimm et al., 2008). Anthropogenic simplification of habitats has been and continues to be a global concern (e.g., Cardinale et al., 2001), but the loss of regional variation in ecosystem structure and function in conjunction with this habitat simplification has only been minimally addressed (e.g., Poff et al., 1997; Rahel, 2000). Here, we show that land use can alter abiotic properties, thus overriding regional constraints on stream metabolism. If geographical differences in rates of ecosystem activity are minimised because of land use, differences in stream ecosystem structure among regions may also be threatened. For example, species diversity may decline in conjunction with species having a higher affinity for characteristics associated with a given region. Further, spread of invasive species may be fostered with increased similarity among ecosystems across regions. Future studies and management strategies should strive to assess, identify, and preserve unique regional properties within stream ecosystems to minimise the influence of land-use change on ecosystem structure and function.

Acknowledgments

This work was supported by a US. National Science Foundation grant (DEB-0111410) to PJM, University of Tennessee and additional NSF LTER support at many of the sites. We thank all LINX II site crews for research assistance, private and public landowners and community participants for access to sites and site information, D Cudder and two anonymous reviewers for comments on the manuscript, and BJ Roberts and RJ Bernot for helpful discussions.

References


© 2010 Blackwell Publishing Ltd, *Freshwater Biology*, 55, 1874-1890


McCune B. & Grace J.B. (2002) *Analysis of Ecological Communities*. MJM Software Design, Gleneden Beach, Oregon, USA

© 2010 Blackwell Publishing Ltd, *Freshwater Biology*, 55, 1874-1890


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data Sl. Summary of stream sites and metabolism rates by region.

As a service to our authors and readers, this journal provides supporting information supplied by authors. Such materials are peer-reviewed and may be reorganized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

(Manuscript accepted 14 February 2010)