Appendix S1 for:
Algal assemblage drives patterns in ecosystem structure but not metabolism in a productive river.

Journal

Authors: Alice M Carter[1], Robert O Hall, Jr.[1], Rafael Feijó-Lima[2], Michael DeGrandpre[3], Qipei Shangguan[3,4], H Maurice Valett[2]

[1]Flathead Lake Biological Station, University of Montana, Polson, MT, USA
[2]Divison of Biological Sciences, University of Montana, Missoula, MT, USA
[3]Department of Chemistry, University of Montana, Missoula, MT, USA
[4]Woods Hole Oceanographic Institution

Supplementary Text:

1. Metabolism model fitting and QA/QC.
2. Hierarchical generalized additive model implementation and model checks.

Supplementary Tables:

1. Average metabolism for each site year

Supplementary Figures:

1. Depth by discharge regression fits across sites.
2. K600 by ER covariance across different metabolism model fits.
3. Variability in GPP estimates across different metabolism model fits.
4. Hierarchical GAM goodness of fit metrics.
5. Hierarchical GAM fits to algal biomass measurements.
6. GPP by ER quantile regression fits to calculate autotrophic respiration.
7. Distribution of epilithic and filamentous algal standing crops and net production.

Text S1: Metabolism model fitting and QA/QC

For each site, we fit a single model that incorporated both years of data and tested both normally pooled estimates of K_{600} and estimates binned by discharge across days. We selected a prior probability distribution for K_{600} based on a multi-site relationship (Raymond et al., 2012) using average slope and depth from our sites ($K_{600,mean} \sim Lognormal(2.48, 0.75)$). For P and R, we based the default prior distributions on Hall (2016) with $P \sim N(3.1, 6)$, and $R \sim N(-7.1, 7.1)$.

We found when fitting the Bayesian models, both the normal and binned pooling of K_{600} essentially disregarded any prior information we included for the standard deviation of gas exchange estimates across days ($\sigma_{K_{600}}$). After testing prior values for $\sigma_{K_{600}}$ ranging from 0.005 to 0.2 d^{-1}, we found that the co-variation of K_{600} with P and R remained unacceptably high (SI Figure 2), which indicates that the parameters were poorly identified (Appling et al., 2018b). Given that the discharge within any one of our sites varied little over the time period of measurement, we do not expect large variation in K_{600}. As a result, we decided to model metabolism in a final step, using hand-pooled estimates of K_{600} in a maximum likelihood (mle) model where we fixed K_{600} at the median value estimated by the Bayesian models for each site. The resulting metabolism estimates were not highly sensitive to this change, but the equifinality between K_{600} and R was resolved (SI Figure 3).

We implemented the model in the package *streamMetabolizer* (Appling et al. 2018b, v 0.12.0) in R (R Core Team 2022, v 4.2.2). After testing different model configurations as described above, we fit each model in three steps. First, we fit a Bayesian normally pooled model with the prior for $\sigma_{K_{600}}$ set to 0.01 d^{-1} that we used to visually identify days where the modeled O_2 was a poor fit to the data. Second, we re-ran the same model with those days removed as they prevented pooled estimates. After fitting, we only kept estimates where the Gelman-Rubin $\hat{R}$, a metric indicating the degree to which the model had converged on a stable estimate, was ≤ 1.05 (Appling et al., 2018b). From this subset, we took the median value of daily K_{600} for each site and used this as a fixed parameter in the final mle model runs. After this final model run, we again visually assessed the fit between modeled and measured dissolved oxygen and discarded days where the peaks were offset or the fit was poor (2.4% of estimates).

Text S2: Hierarchical generalized additive model implementation and model checks

We fit hierarchical generalized additive models to the raw biomass sample data in order to interpolate between measurements allowing borrowing of the general pattern across sites. We implemented models in the r package *mgcv* (Wood, 2017) with the following code:

```
biomass_gam <- mcgv::gam(biomass ~ s(doy) + s(doy, site_year, bs = "fs"),
                         data = dat, method = "REML", family = Gamma(link = "log"))
```

where the first smoother, s(doy), is the global function based on day of year (doy) and the second smoother allows for variation in the curves at the level of each site year. We ran a total of four hierarchical models where biomass represented either epilithon or filamentous algae quantified in units of either ash free dry mass and chlorophyll *a* mass on a log transformed data. We checked model fits using the recommended tests in Pedersen et al. (2019), with plots shown in Figure S4

Site	Year	GPP x ER correlation	GPP (g O_2 m_2 d^{-1})	ER (g O_2 m_2 d^{-1})	NEP (g O_2 m_2 d^{-1})	P:R
Perkins	2020	0.83	9.3 ± 0.6	-10.3 ± 0.4	-1.0 ± 0.3	0.89 ± 0.04
Perkins	2021	0.61	12.7 ± 0.5	-12.4 ± 0.5	0.30 ± 0.5	1.03 ± 0.03
Deer Lodge	2020	0.88	6.5 ± 0.7	-6.9 ± 0.6	-0.40 ± 0.3	0.93 ± 0.05
Deer Lodge	2021	0.89	7.6 ± 0.7	-7.2 ± 0.5	0.46 ± 0.3	1.05 ± 0.04
Garrison	2020	0.87	9.2 ± 0.6	-7.6 ± 0.3	1.54 ± 0.3	1.19 ± 0.04
Garrison	2021	0.64	9.4 ± 0.6	-8.0 ± 0.3	1.34 ± 0.4	1.17 ± 0.05
Gold Creek	2020	0.91	8.3 ± 1.2	-9.5 ± 1.5	-1.18 ± 0.3	0.86 ± 0.04
Gold Creek	2021	0.78	10.0 ± 0.5	-8.3 ± 0.2	1.65 ± 0.3	1.19 ± 0.03
Bear Gulch	2020	0.96	8.3 ± 1.0	-7.1 ± 0.7	1.19 ± 0.4	1.12 ± 0.06
Bear Gulch	2021	0.89	10.3 ± 0.9	-11.9 ± 1.3	-1.6 ± 0.3	0.86 ± 0.02
Bonita	2020	0.91	5.9 ± 0.9	-8.3 ± 0.3	-2.39 ± 0.6	0.69 ± 0.09
Bonita	2021	0.92	10.5 ± 1.6	-11.4 ± 1.5	-0.95 ± 0.5	0.89 ± 0.05

Table 1: Means and standard errors of metabolism at each site during 2020 and 2021. Standard errors are corrected for autocorrelation (Bence, 1995)

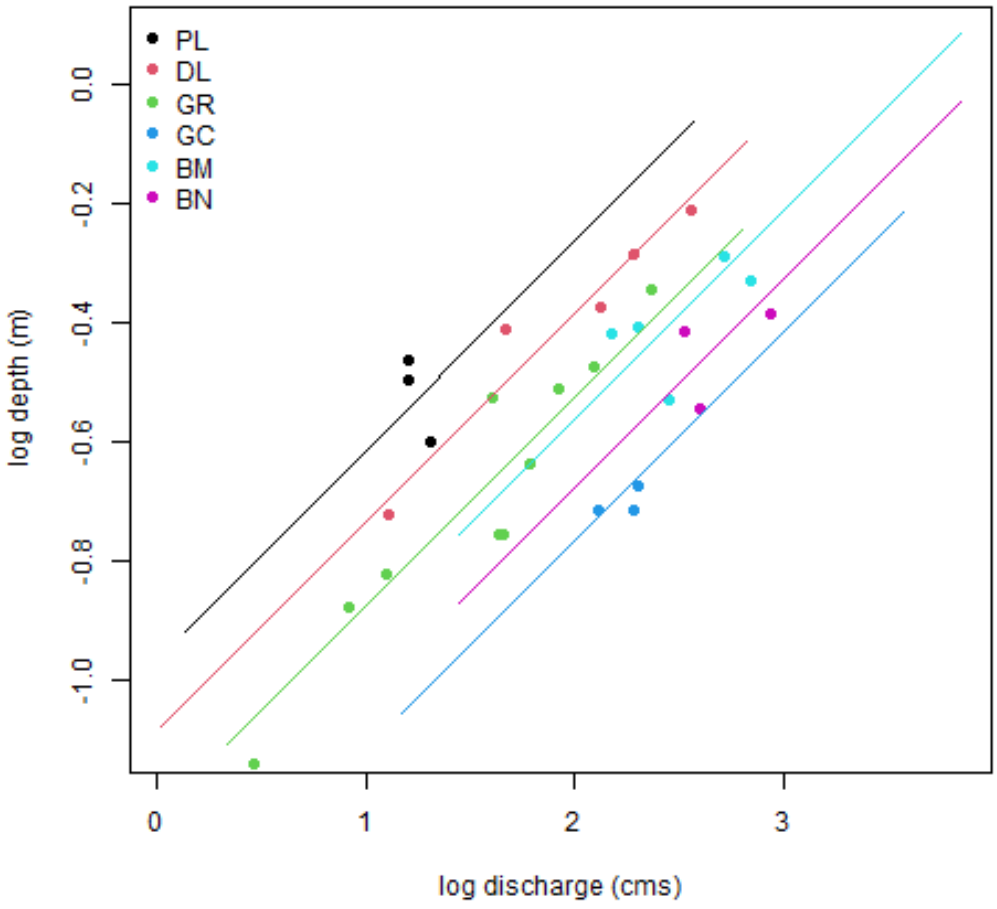

Figure S1: Model of average stream depth as a function of discharge pooled across all six sites.

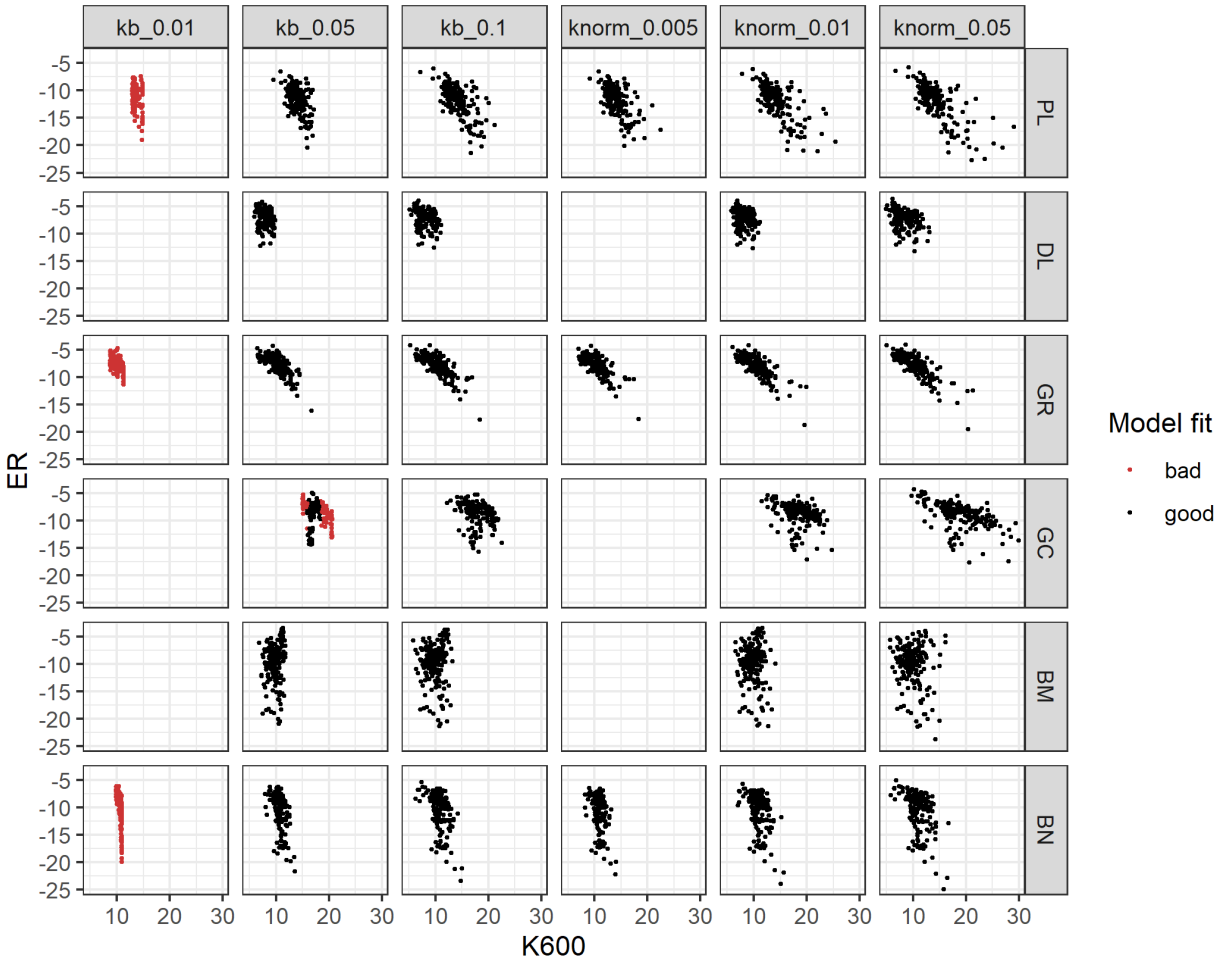

Figure S2: K600 by ER relationships across all days for each site using partially pooled model fits where K600 values were fit to different bins of discharge values (kb), normally pooled model fits where a single distribution of K600 values was estimated for all discharge values (knorm). For each of these model types, we tested different values of the hyperparameter $K600_{\sigma_\sigma}$ shown after the underscore on the plot labels. This hyperparameter determines how much dispersion around a mean value of K600 for a given discharge bin is allowed by the StreamMetabolizer package when estimating metabolism (Appling et al., 2018a). Smaller values indicate less day to day variation in gas exchange, but can make the model more difficult to fit. Days where the model fit was poor (Rhat *geq* 1.1) are indicated by red points. In modeling metabolism, the goal is to eliminate covariation between ER and K600 in the posterior estimates as high covariation can indicate that your model may be equifinal and produce poor estimates (Appling et al., 2018b).

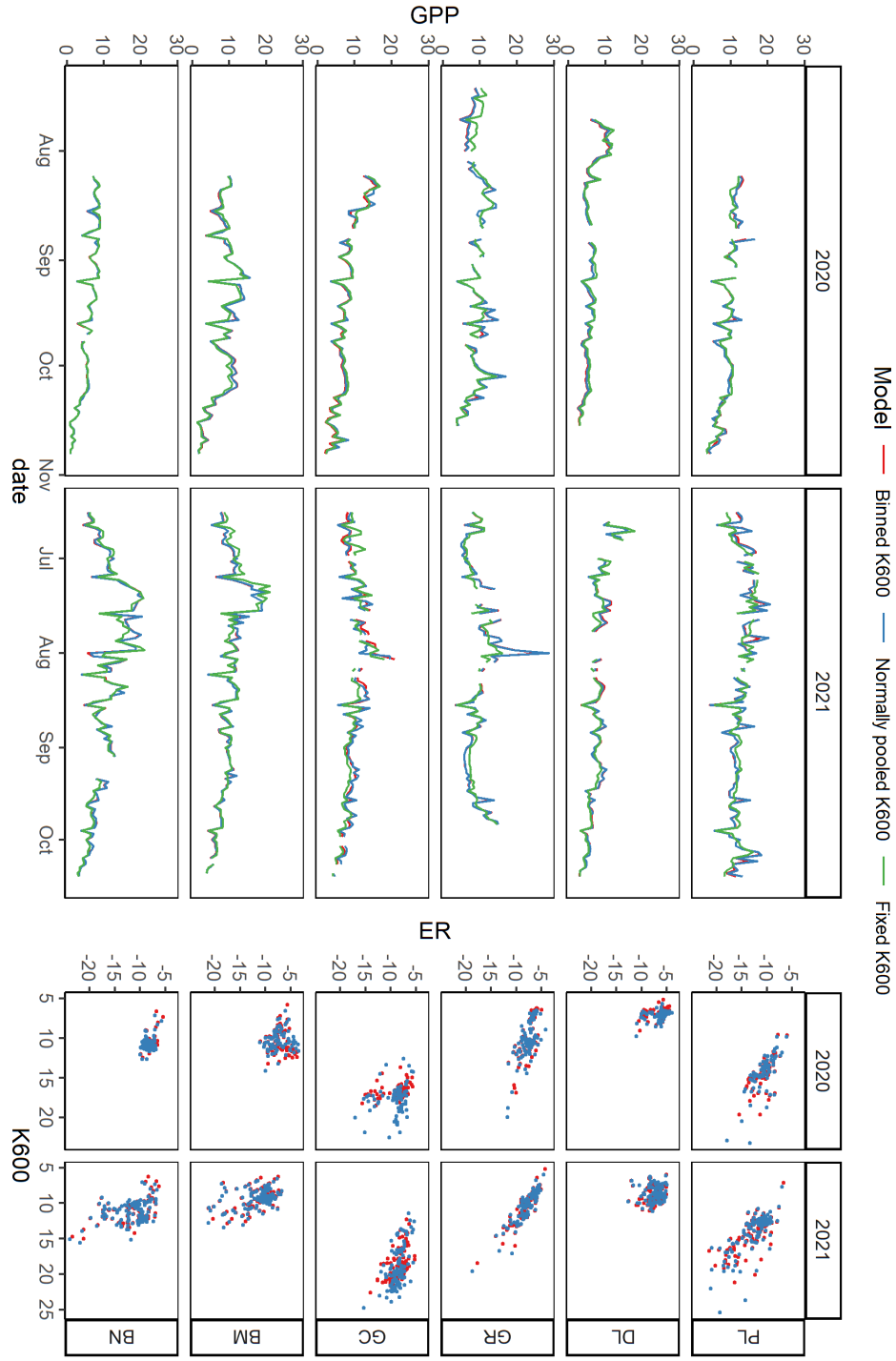

Figure S3: Comparison of model metabolism estimates across the six sites and two growing season for different model fits comparing a model with K_{600} estimates binned by discharge, K_{600} estimates normally pooled across all discharges, and K_{600} fixed, or hand pooled across the whole dataset. The results show that (a) different models had minimal impact on the productivity estimates (b) the equifinality between K_{600} and R remained unacceptably high in the binned and normally pooled models, justifying our choice of using a fixed K_{600}.

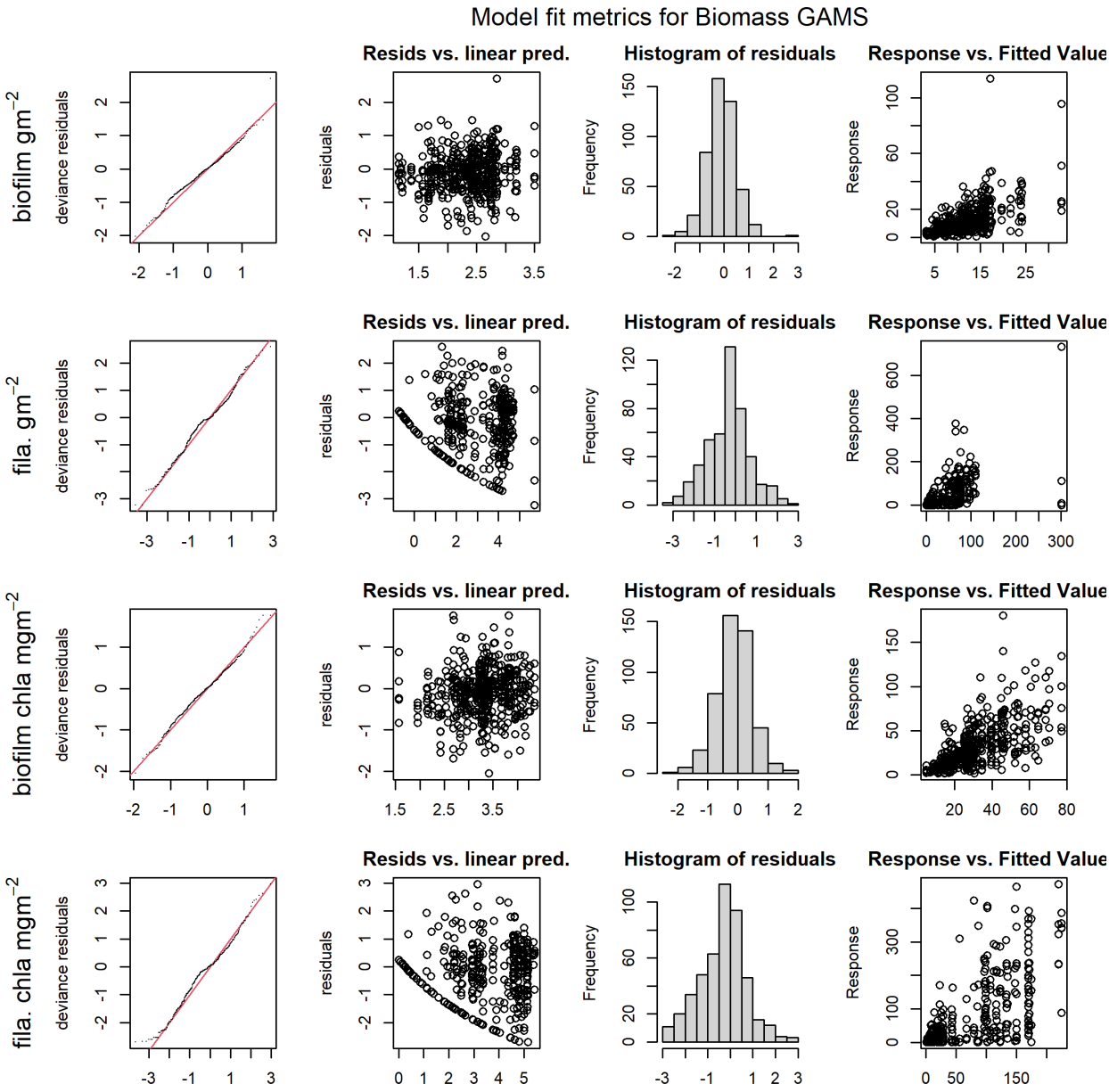

Figure S4: Goodness of fit test for Hierarchical GAM fits to algal biomass data showing that the models with the log link function met the assumptions that the residuals were normally distributed and showed minimal pattern in the residuals vs linear predictor outside of the boundary condition where algal biomass was zero (Pedersen et al., 2019).

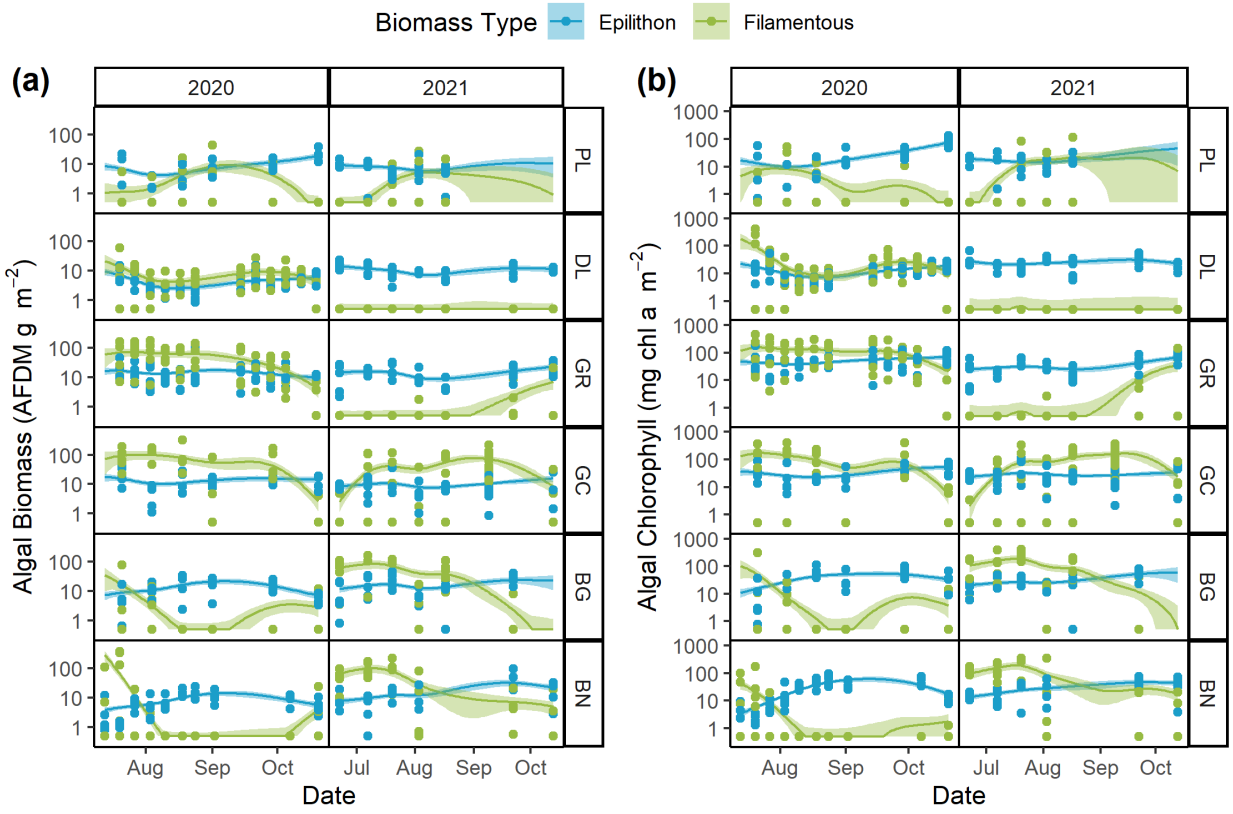

Figure S5: Model fits to (a) algal biomass, g AFDM m^{-2} and (b) chlorophyll, mg chl a m^{-2}. Points show measurements and solid lines show daily mean GAM fits (± standard error shaded) for the epilithic (blue) and the filamentous algae (green). The sites are labeled as in Figure 1.

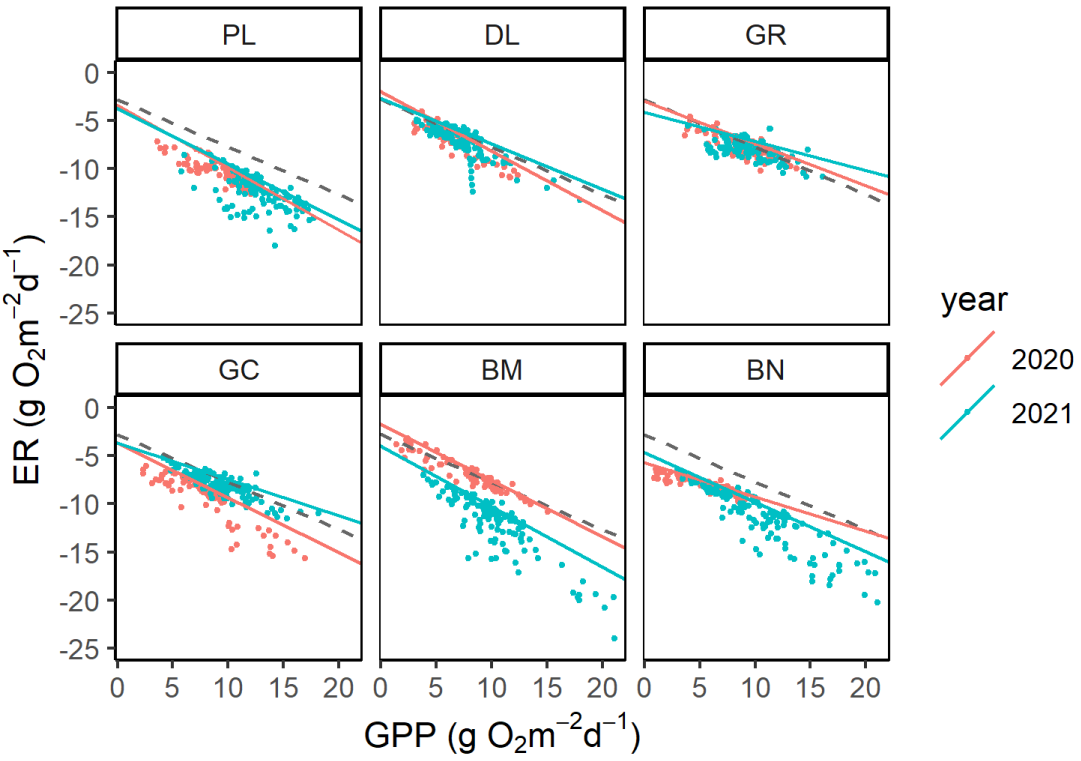

Figure S6: Multilevel quantile regression fits of GPP conditioned on the 90% quantile of ER for all site years of metabolism estimates. A 1:1 dashed line is shown in grey for reference.

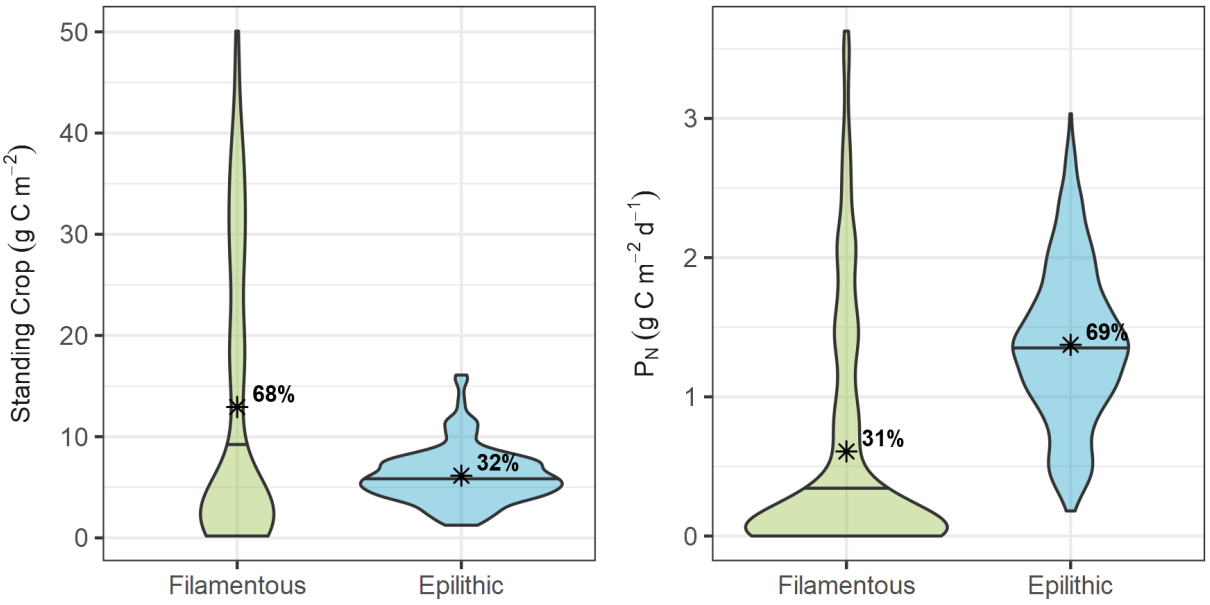

Figure S7: The distribution of daily values of standing crop and net production (P_N) for the two algal forms showing the median (horizontal lines) and means ($*$). Percents indicate the average contribution of each form to the totals.

References

Appling, A. P., R. Hall, M. Arroita, and C. B. Yackulic, 2018a. streamMetabolizer: Models for Estimating Aquatic Photosynthesis and Respiration. R package.

Appling, A. P., R. O. Hall, C. B. Yackulic, and M. Arroita, 2018b. Overcoming equifinality: Leveraging long time series for stream metabolism estimation. *Journal of Geophysical Research: Biogeosciences* **123**:624–645.

Bence, J. R., 1995. Analysis of short time series: correcting for autocorrelation. *Ecology* **76**:628–639.

Hall, R. O., 2016. Metabolism of Streams and Rivers: Estimation, Controls, and Application. Estimation, Controls, and Application. Elsevier Inc.

Pedersen, E. J., D. L. Miller, G. L. Simpson, and N. Ross, 2019. Hierarchical generalized additive models in ecology: An introduction with mgcv. *PeerJ* **7**:e6876.

R Core Team, 2022. R: A language and environment for Statistical Computing. R Foundation for Statistical Computing.

Raymond, P. A., C. J. Zappa, D. Butman, T. L. Bott, J. Potter, P. Mulholland, A. E. Laursen, W. H. McDowell, and D. Newbold, 2012. Scaling the gas transfer velocity and hydraulic geometry in streams and small rivers. *Limnology and Oceanography: Fluids and Environments* **2**:41–53.

Wood, S., 2017. Generalized Additive Models: An Introduction with R. Chapman and Hall/CRC, second edition.