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CONTROLS ON AUTOTROPHIC RESPIRATION, HETEROTROPHIC RESPIRATION, AND DECOMPOSITION IN NORTHERN FORESTED RIVERS

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CONTROLS ON AUTOTROPHIC RESPIRATION, HETEROTROPHIC
RESPIRATION, AND DECOMPOSITION IN NORTHERN FORESTED RIVERS

By

Renn Schipper

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Biological Sciences

MICHIGAN TECHNOLOGICAL UNIVERSITY

2021

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This thesis has been approved in partial fulfillment of the requirements for the Degree of
MASTER OF SCIENCE in Biological Sciences.

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Abstract

Autotrophic respiration (AR), heterotrophic respiration (HR), and decomposition are important contributors to the carbon cycle in streams. It is important to understand how different environmental factors, such as canopy cover and dissolved organic carbon (DOC), influence these processes. DOC concentrations in northern forested streams are increasing, which may affect light and carbon availability. To examine the effects of DOC and canopy cover on these processes we measured gross primary production, ecosystem respiration and decomposition at 8 sites in 4 streams in the Upper Peninsula of Michigan and used quantile regression to estimate AR and HR. Among sites, AR and decomposition showed no relationship with canopy cover using Spearman's correlation ($p = 0.33$), while neither respiration process nor decomposition showed a relationship with DOC concentrations ($p = 0.75$). The results do indicate potential regional and temporal variation in AR and HR; however the quantile regression approach is insufficient to examine this.

1 Introduction

A unique aspect of stream ecosystems is that they are fundamentally controlled and constrained by the environment that surrounds them (Hynes 1975, Jankowski & Schindler 2019). Terrestrial environments supply large amounts of dissolved and particulate organic carbon (C) to rivers and streams (Battin et al. 2009), which in turn fuels C cycle processes like metabolism and decomposition (Hynes 1975, Vannote et al. 1980, McLaughlin & Kaplan 2013, Vachon 2020). Metabolism is an important set of processes in streams, including primary production that transforms inorganic C into an organic form, and respiration that transforms organic C back into an inorganic form. A related process is decomposition, or the breakdown of particulate organic C, often supplied from adjacent terrestrial ecosystems as leaf litter, which fuels respiration for some heterotrophic stream organisms. Due to the prevalence and importance of these processes, stream ecologists have studied how they vary along a stream continuum and which characteristics influence them (Meyer & Edwards 1990, Hynes 1975, Atkinson et al. 2008, Mulholland et al. 2001, Lamberti & Steinman 1997, Webster & Benfield 1986, Minshall et al. 1983).

The River Continuum Concept (RCC) (Vannote et al. 1980) predicted how the terrestrial environment influences northern forested streams and rivers from headwaters to outlet. Since the headwaters of these forested streams are narrow, the canopy of terrestrial plants reduces the amount of light that reaches the stream surface, which then limits the amount of gross primary production (GPP) that can be performed by in-stream autotrophs, such as algae and plants (McCabe 2010). Therefore, the RCC predicts that

forested headwater streams will be reliant on organic matter that enters from the terrestrial environment to support the energy requirements of heterotrophs like microbes, invertebrates and fishes that comprise the food web (Minshall 1978, Minshall et al. 1985). This terrestrial material enters into the food web through decomposition performed by microbes and invertebrates, which the RCC predicted would have a reversed gradient in relation to primary production, with higher decomposition in headwaters due to the organisms being better adapted to break down organic material that enters the stream (Vannote et al. 1980). As the streams widens downstream, light availability increases due to less canopy covering the stream, allowing for more GPP and shifting from a reliance on allochthonous C, which originates outside of the stream, to autochthonous C that originates inside of the stream (Meyer & Edwards 1990, Minshall et al. 1985).

Respiration has also been studied along a stream continuum, but patterns of rates and controlling characteristics are not as clear as for GPP and decomposition (Minshall et al. 1983, McTammany et al. 2003). This may be due to most studies examining respiration as ecosystem respiration (ER), or the total respiration that is being performed in the system. However, ER is actually composed of two processes: autotrophic respiration (AR) and heterotrophic respiration (HR). While they are similar processes at a cellular level, they are different at a larger scale as they are performed by different groups of organisms, where AR is the respiration that is performed by organisms that can also perform primary production, and HR is the respiration that is performed by organisms that cannot fix their own C from an inorganic source. Part of the complication is that HR and AR cannot be easily parsed by common techniques for measuring ER in the field, but

examining these two separately would allow for better understanding of what factors control them. For example, Solomon et al. (2013) examined the effect of dissolved organic carbon (DOC) on ER in lakes, and there was no significant relationship. However, they hypothesized that this was due to autotrophic activity decreasing as a response to higher light limitation while heterotrophic activity increased with DOC supply.

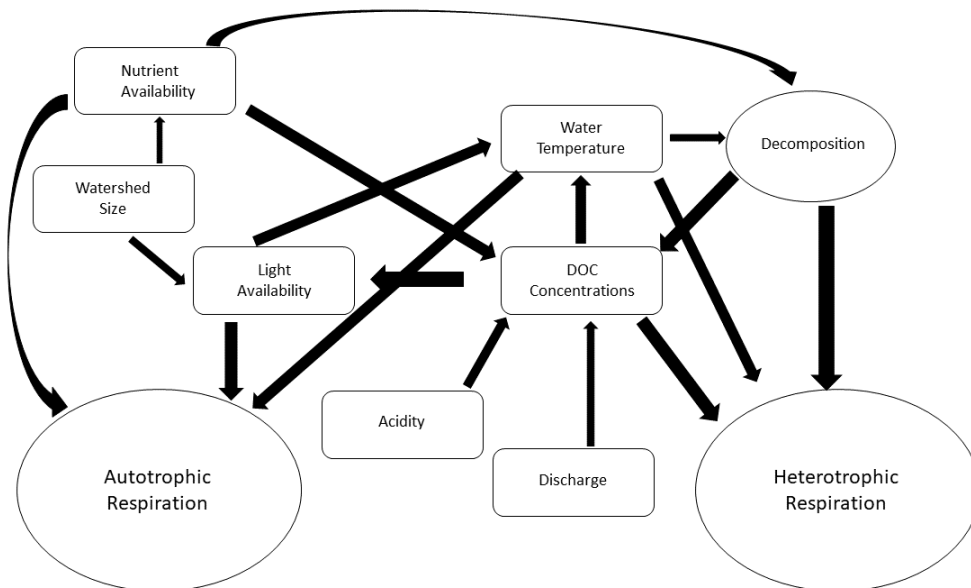


Figure 1. Direct and indirect effects of environmental characteristics on autotrophic and heterotrophic respiration. The arrows point from which characteristic is causing the effect to which is affected.

Understanding AR and HR independently in streams is important because the controls on the rates of these processes are likely to differ based on organism characteristics (Figure 1). Rates of AR should be closely tied to rates of GPP because both processes are performed by autotrophs. Therefore, AR should be affected by the

controls that are important for primary producers, such as light, nutrient availability, and watershed size (Mulholland et al. 2001, Manning et al. 2018, Lamberti & Steinman 1997). Lamberti and Steinman (1997) found that watershed size was one of the best predictors of primary production, and therefore there is the potential that watershed size can also be a predictor of AR. Although watershed size may only be an indirect control on AR, it is a coarse-grain measure that encapsulates many different factors that could actually be the controlling factor, such as an increase in nutrient inputs from the terrestrial environment (Lamberti & Steinman 1997). In their work on the RCC, Vannote et al. (1980) discuss how light availability is one of the larger controls on primary production and a major component determining if a river is autotrophic or heterotrophic, so it has the potential to be a large control on AR. Because autotrophs perform both primary production and AR, this would support the idea that light availability also plays a role in controlling AR. Phosphorus and nitrogen concentrations have also been found to be a major influence on ER in rivers (Mulholland et al. 2001, Benstead et al. 2009). The concentrations of both of these nutrients are likely to influence both AR and HR due to the fact they are limiting nutrients, and if more is added to a system, more growth and biotic activity is able to occur. Higher nutrient concentrations fuel higher rates of decomposition (Benstead et al. 2009), which increases HR rates, and also increases carbon sequestration by algae (Rosemond et al. 2015), which would lead to higher rates of AR.

HR should not be as closely related to light and primary production as AR, because heterotrophs can use organic C that enters the river from the terrestrial environment (Hynes 1975), which is abundant in forested streams like those in the Upper

Peninsula of Michigan. As a result, HR should be influenced by factors that affect the availability of the organic carbon, such as acidity and discharge (Tank et al. 2010, Demars 2019). Niyogi et al. (2002) found that primary production in streams was not correlated to pH, however, waters that were more acidic with pH <5.7 were found to have slower decomposition (Mulholland 1997) which has the potential to limit the availability of organic C due to the C not being in a usable form for heterotrophs (Tank et al. 2010). In addition, streams and rivers with a higher base flow were found to have higher ecosystem respiration (Demars 2019). Demars (2019) explains that rivers that have higher base flow are more likely to have soil porewater more connected to the river, which would lead to more DOC being available, which could then fuel HR due to an increase in microbial activity. Another major contributor to the respiration of an ecosystem is the hyporheic zone (Mulholland et al. 1997, Edwards & Meyer 1987). Respiration rates in streams were shown to be higher at sites that had deeper hyporheic zones (Mulholland et al. 1997), which may be due to a larger microbial community present in the subsurface zone. While there are groundwater interactions through snowmelt recharge via snowmelt in streams in Michigan's Keweenaw Peninsula, this interaction is most prevalent leading up to and during peak snowmelt (Stottlemeyer & Toczydlowski 1991) and many of the streams in the Keweenaw Peninsula are bedrock constrained with minimal subsurface exchange after snowmelt. Therefore, it is important to study and understand AR and HR separately to predict their potential responses to ongoing and future global change.

The largest pool of organic C in streams is DOC (Allan 1995), and ongoing changes in DOC in rivers could have important consequences for rates and contributions

of AR and HR. The DOC concentrations in northern rivers have been increasing globally, believed to be due to decreases in sulfur deposition, increased temperatures, and increased precipitation (Roulet & Moore 2006, de Wit et al. 2016, Meingast et al. 2020). In rivers in Northern Michigan and throughout the upper Midwestern US, wetlands are a large source of DOC (de Wit et al. 2016, Yallop et al. 2010), and wetland inputs may be increasing due to increased temperature caused by climate change (Monteith et al. 2007). DOC leads to the brown appearance or “staining” common in rivers draining forests and wetlands, and therefore increased DOC concentrations could lead to a decrease in light penetration (de Wit et al. 2016). Decreased light could decrease primary production, which could also affect rates of AR. On the other hand, HR may increase, since heterotrophs could use the increased organic C to fuel their own processes. However, DOC increases could also be associated with altered water temperatures and nutrient supplies, such that the responses of AR and HR to these changes may be complicated and requires detailed quantification of both processes in the field.

Similar to AR and HR, decomposition may also be influenced indirectly by light availability and DOC concentrations. Water temperature is a primary control on decomposition, with higher water temperatures leading to higher rates of decomposition (Martinez et al. 2014). Water temperatures could increase if the water is exposed to, or absorbs, more light, and light availability could be influenced by both canopy cover and DOC concentrations. Water browning has been shown to lead to increased surface water temperatures in lakes due to decreased water clarity and increased light absorption (Pilla et al. 2018, Williamson et al. 2015, Read & Rose 2013). Understanding how the DOC increases affect both respiration and decomposition can help us understand how river

ecosystems may change as a result of climate change due to increasing DOC concentrations and increased temperatures (Roulet & Moore 2006).

We designed two studies to examine how different environmental factors were related to metabolism and decomposition rates in streams of the Upper Peninsula of Michigan. The first, which we will refer to as “the longitudinal study”, examined the variation in ER, HR, AR and decomposition along a river continuum, while the second, the “DOC gradient study”, examined the variation and controls of the same processes between streams with different DOC concentrations. We hypothesized that ER (AR + HR) rates would be higher in downstream sites than in headwater sites, due to an increase in rates of AR with increasing light availability at downstream sites, but no similar change in HR. We also hypothesized that decomposition would be lower in downstream sites relative to headwater sites because, following the predictions made in the RCC, headwater sites should have organisms that are better able to perform decomposition since there is a higher influx of terrestrial C (Vannote et al. 1980). Among streams, we hypothesized that ER rates would not be affected by DOC concentrations, due to the offsetting effects of HR and AR. We hypothesized that higher DOC concentrations would stimulate HR but also result in more browning of the water (de Wit et al. 2016) which would limit light availability and cause AR to decrease. Finally, we hypothesized that decomposition rates would be faster at sites with warmer water temperatures, and that water temperatures would be influenced by differences in canopy cover and DOC among sites.

2 Methods

2.1 Study Area

For the longitudinal study, five sites were chosen along the Pilgrim River, located in Houghton county, Michigan. The watershed is 62.9 km² with a total reach length of 21.1 km and an average annual discharge of 0.98 m³/s (Michigan Department of Environmental Quality 2012a, USGS 2020). The sites ranged from 1 km upstream of the mouth of the river where it enters the Keweenaw Waterway to the headwaters, 15-16 km upstream (Table 1). The terrestrial vegetation is dominated by hardwood sugar maple and yellow birch forest (Michigan Department of Environmental Quality 2012a).

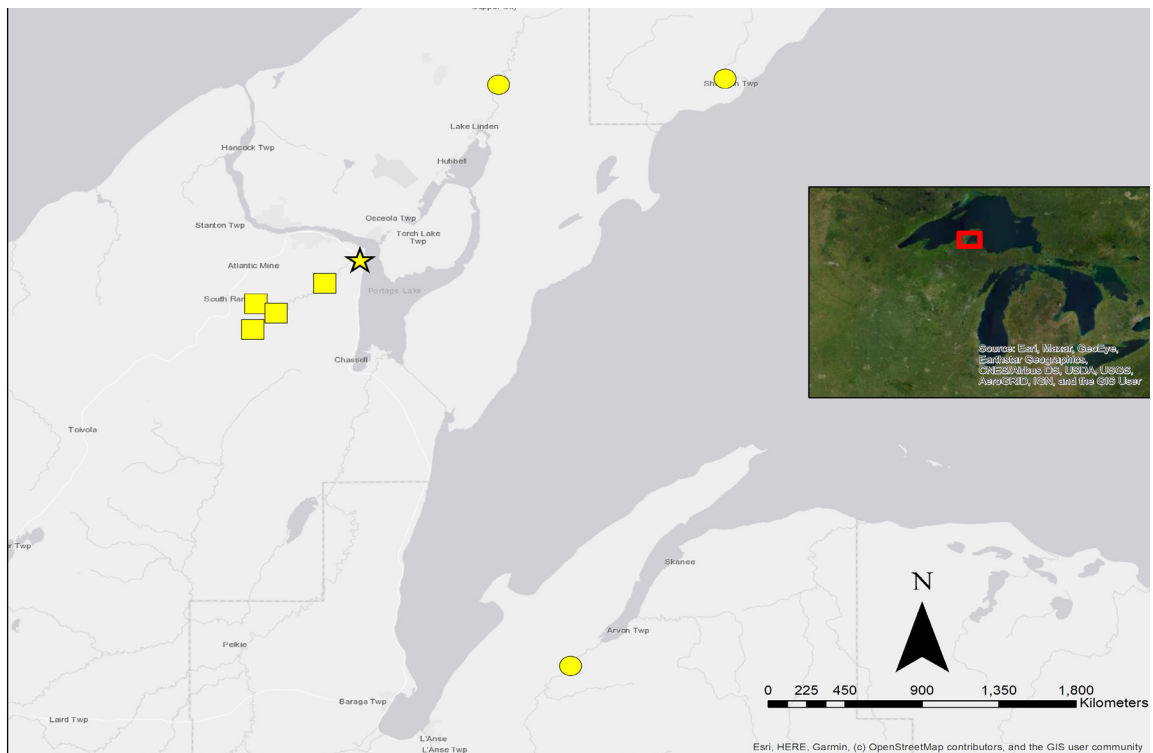


Figure 2. The distribution of study sites within the area around Houghton in the Upper Peninsula of Michigan. The squares represent the sites used along the Pilgrim River for study 1, the circles represent the sites for study 2, and the star represents Pilgrim River Km 1. (ESRI 'Light Gray Canvas' and ESRI 'Topographic')

Table 1: Mean environmental characteristics for each site. The (*) means that the characteristic was not measured at that site. Nutrient concentrations measured were total dissolved phosphorus (TDP), ammonium (NH₄), dissolved organic carbon (DOC), nitrate (NO₃), soluble reactive phosphorus (SRP) and total dissolved nitrogen (TDN).

<i>Site</i>	<i>South Fork Pilgrim River Km 16</i>	<i>North Fork Pilgrim River Km 15</i>	<i>Pilgrim River Km 14</i>	<i>Pilgrim River Km 6</i>	<i>Pilgrim River Km 1-2019</i>	<i>Pilgrim River Km 1 - 2020</i>	<i>Tobacco River</i>	<i>Silver River</i>	<i>Trap Rock River</i>
<i>Location (latitude, longitude)</i>	47.05, -88.619	47.0693, -88.5169	47.0621, -88.5979	47.0843, -88.5507	47.1013, -88.5172	47.1013, -88.5172	47.233, -88.17	46.8, -88.32	47.229, -88.391
<i>Deployment Period</i>	14 May 2019 – 06 Sep 2019	14 May 2019 – 06 Sep 2019	11 May 2019 – 06 Sep 2019	20 May 2019 – 06 Sep 2019	14 May 2019 – 06 Sep 2019	06 Jun 2020 – 20 Oct 2020	29 Jul 2020 – 20 Oct 2020	06 Jun 2020 – 20 Oct 2020	06 Jun 2020 – 20 Oct 2020
<i>Width (m)</i>	2.75	3.3	5.5	8.8	7.5	8.3	9.5	14.6	10.5
<i>DOC (mg C/L)</i>	5.35	15.05	7.15	5.30	6.35	8.05	16.63	11.33	6.66
<i>Canopy Cover (%)</i>	96.4	3.5	90.9	13.4	19.7	11.7	6.0	27.4	9.4
<i>Discharge (m³/s)</i>	0.07	0.00	0.21	0.21	0.39	0.74	0.51	1.51	0.79
<i>Temperature (°C)</i>	12.75	16.93	14.31	13.68	13.14	14.64	14.88	14.54	13.87
<i>NH₄ (µg/L)</i>	6.92	6.95	7.42	9.19	7.95	7.54	19.43	8.68	5.29
<i>TDN (µg N/L)</i>	370	260	320	280	270	360	490	330	330
<i>NO₃ (µg /L)</i>	280	*	230	170	140	230	77	870	210
<i>SRP (µg /L)</i>	11	*	11	7	8	11	5	7	10
<i>TDP (µg P/L)</i>	9.4	1.9	8.4	14	10	11	6.8	8.1	7.0

The DOC gradient study included the Pilgrim River km 1 site (USGS gauge 04043016), as well as sites in three other rivers: the Trap Rock River, the Tobacco River, and the Silver River. The Trap Rock River site was located 6 km upstream from the mouth of the river into Torch Lake, at USGS gauge 04043050 near Lake Linden, Michigan with a watershed of 111.37 km², average annual discharge of 1.29 m³/s, and land cover of deciduous forest, mixed forest, and woody wetlands (USGS 2020, Michigan Department of Environmental Quality 2012b). The Silver River is located 4 km upstream of the mouth of the river into Huron Bay of Lake Superior, at USGS gauge 04043150 near L'anse, Michigan, with a watershed of 178.71 km², average annual discharge of 2.44 m³/s, and watershed land cover of 92% hardwood forest (Weaver et al. 2010, USGS 2020). The Tobacco River is located near Gay, Michigan with land cover consisting of majority forests with some scrub palustrine wetlands (Keweenaw Planning Commission 2017). The study site was located 2 km upstream from the mouth of the river into Keweenaw Bay of Lake Superior with a watershed size of 137.15 km² (Michigan Department of Environment Great Lakes and Energy 2021). Environmental characteristics, such as water chemistry, canopy cover, and stream width were measured and summarized in Table 1.

2.2 Environmental Characteristics

To characterize the physical and chemical conditions at each study site, we measured canopy cover and concentrations of nutrients in the water following procedures described in Eberhard et al. (2018). Canopy cover was measured using a densiometer

next to where the sensors were deployed. Stream water was collected from each site to measure the nutrient concentrations. The stream water was filtered using Millipore 0.45 μm nitrocellulose membrane filters into plastic Nalgene bottles. These samples were stored frozen until analysis was performed for nitrate (NO_3^-), ammonium (NH_4^+), total dissolved phosphorous (TDP), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and soluble reactive phosphorous (SRP). Water chemistry samples and canopy cover measurements were collected once a month for the longitudinal study, and once every two weeks for the DOC gradient study. The DOC and TDN concentrations were measured using Shimadzu TOC-VCSN with a total N module TNM-1 (Shimadzu Scientific Instruments, Columbia, Maryland). The NH_4^+ concentrations were measured using a fluorometric procedure (Holmes et al. 1991; Taylor et al. 2007) on a Turner Aquafluor (Turner Designs, Palo Alto California). The TDP concentrations were measured using acid-persulfate digestion and molybdenum-antimony colormetric determination methods with a Thermo Scientific 10 s UV–Vis spectrophotometer (Ameel et al 1993; APHA 2005; Nydahl 1978; Valderrama 1981). SRP was analyzed on a SEAL AQ2 discrete analyzer (SEAL Analytical, Mequon, Wisconsin) based on USEPA method 365.1 revision 2.0 (USEPA 1993a) and APHA method 4500- P F (APHA 2005). NO_3^- was analyzed on a SEAL AQ2 discrete analyzer (SEAL Analytical, Mequon, Wisconsin) based on USEPA method 353.2 revision 2.0 (USEPA 1993b) and APHA method 4500 NO_3^- (APHA 2005).

2.3 Metabolism Modeling

Dissolved oxygen and temperature were measured at one location each using O₂ MiniDOT sensors (PME Instruments, Vista CA) at 10-minute intervals. These sensors were deployed at each site from May until September 2019 for the longitudinal study and May until October 2020 for the DOC gradient study, except at the Tobacco River where the sensor was not deployed until August due to waiting for landowner permission. Discharge was measured either manually or using USGS gauge data, The Pilgrim River USGS gauge data was used for the Tobacco River site, as that river did not have an associated gauge, after performing a linear regression comparing the manually measured Tobacco River discharge with the Pilgrim River discharge and confirming they were closely related ($p < 0.05$, $r^2 = 0.94$, $F = 32.36$, $df = 2$). Discharge and MiniDOT data was used to estimate rates of GPP and ER using the *streamMetabolizer* package in R (Appling et al. 2018, R Core Team 2020). This package estimates rates of GPP, ER, and the gas exchange rate (k_{600}), normalized for temperature and molecular properties to a Schmidt number of 600, with a Bayesian model that examines the change in O₂ concentrations. The single station method assumes stream homogeneity, or that the whole stream above the point of measurement experiences similar change in oxygen (Odum 1956).

This model examines the change in oxygen concentration over time, and fits predictions for the rates of GPP, ER and k_{600} . ER is assumed to be constant throughout the day, and since there is no light for GPP, the change in O₂ at night is attributed to ER. Then GPP is estimated for the daytime periods while accounting for ER and the gas

exchange rate. The equation that is used in the model to estimate rates of production, respiration, and k_{600} is:

$$1) \frac{dO_t}{dt} = P_t + R_t + D_t$$

where dO_t/dt is the rate of change of O_2 at time t , P_t is the production, R_t is respiration, and D_t is the gas exchange. It can be further partitioned as:

$$2) P_t = GPP * \frac{1}{Z_t} * \{(t_1 - t_0) * \frac{PPFD_t}{\int_{u=t_0}^{t_1} PPFD_u du}\}$$

$$3) R_t = ER * \frac{1}{Z_t}$$

where z is the mean water depth, PPFD is the photosynthetic photon flux density ($\mu\text{mol photons m}^{-2} \text{ d}^{-1}$), and u is continuous time within a day (Appling et al. 2018). The gas exchange can be calculated with:

$$4) D = KS = zkS$$

where D is the rate of diffusion, S is the saturation between the atmosphere and the water, K is the gas transfer coefficient (corrected for volume it is k) and z is the depth (Odum 1956). It can improve model performance to directly measure gas exchange (Nifong et al. 2020), however due to time, weather, and COVID restraints, we did not measure k_{600} for either study and instead used the estimates that were made by the *streamMetabolizer* package. The value of k_{600} is estimated with the equation:

$$5) \text{Gas Exchange} = k_{600} * \left(\frac{S_A + S_B T_t + S_C T_t^2 + S_D T_t^3}{600} \right) S_E$$

where T_t is the water temperature in °C, the Schmidt number coefficients are $S_A=1568$, $S_B=-86.04$, $S_C=2.142$, and $S_D=-0.0216$, and the scaling exponent $S_E=-0.5$ (Appling et al. 2018). Once the rate modeling was complete, we performed linear regressions in R (R Core Team 2020) comparing ER and k_{600} to look for equifinality in the model. Equifinality is where many different combinations of estimated values for GPP, ER, and k_{600} are able to fit different O_2 records, or large values of all three are just as likely to fit the data as ecologically realistic values (Appling et al. 2018). Additionally, ER is reported as a negative value, with larger negative values indicating higher rates. This is due to respiration being measured as the consumption of oxygen, and this is also true for AR and HR rates described below.

2.4 Autotrophic and Heterotrophic Respiration

A quantile regression approach was used following the procedure of Hall and Beaulieu (2013) to estimate rates of AR using the *quantreg* package in R (Koenker 2020, R Core Team 2020). This approach uses the 90th percentile slope of ER vs GPP because AR is believed to be the minimum amount of ER on any day above the base HR (Hall & Beaulieu 2013). The equation that is used to estimate AR is:

$$6) AR = (AR_f)(GPP)$$

Where AR_f is the slope of the quantile regression, and which is estimated to be the fraction of primary production that is immediately respired by autotrophs. The AR estimate is then used to estimate HR with the equation:

$$7) HR = ER - AR$$

Because this is a statistical approach to estimating these different rates, there are strict assessments that must be made. In order to assess the quantile regression estimates we followed the recommendations given by Hall and Beaulieu (2013). No estimates were used where the confidence interval of the quantile regression slope was > 0.4 . The correlation between HR and GPP was also examined, and we did not use any sites that showed a correlation of 0.3 or higher as that was shown to decrease or increase the ARf estimates by 0.15, depending on the sign of the correlation. Also, any sites that had low variation in GPP were found to have unrealistic estimations of rates, so they were excluded from further analyses.

2.5 Decomposition

Cotton strip assays were deployed at each site following the procedure outlined in Tiegs et al. (2013). This method was used because it provides a more standardized approach of measuring decomposition than the more commonly used leaf packs (Boyero et al. 2016, Follstad-Shah et al. 2017), which have more variables that could influence decomposition rates like the quality of the leaf litter, nitrogen: phosphorus ratios, and lignin concentrations (Webster & Benfield 1986, Gusewell & Gessner 2009, Boyero et al. 2016). Five cotton strips were deployed at each site and were incubated for 27-33 days. Upon collection the strips were washed with 90% ethanol, then dried at 40°C and placed stored in a desiccator until the tensile-strength measurements. Tensile strength is the amount of pulling force a material can receive until it breaks, and decomposition will weaken the cotton strips, resulting in a loss of tensile strength. The tensile strength of the incubated strips as well as control strips were measured by pulling at a rate of 2 cm/min

to measure maximum tensile strength using an Instron 4206 load frame (Instron, Norwood, MA) with an MTS Renew controller and 100 lbs load cells (MTS Systems, Eden Prairie, MN). The tensile loss per day was calculated using the equation in Tiegs et al. (2013) and standardized across streams for temperature by dividing the tensile loss rates by degree-days. This was done because temperature has a large effect on rates of decomposition, and correcting for degree-days allows the examination of decomposition in relation to factors other than temperature (Pozo et al. 2011).

2.6 Statistical Analysis

The 95% confidence intervals (CI) were calculated for all rates of ER, AR, and HR and were used to infer if the rates were significantly different between sites in both studies. If any sites had overlapping CI, they were determined to be not significantly different. The data sets from both studies were combined to examine the hypotheses that ER would be unaffected by DOC concentrations and a negative relationship with canopy cover. The combined data set was also used to examine the prediction that AR would decrease with increasing DOC concentrations and canopy cover. A Spearman's correlation test was also performed between HR and DOC concentrations, to test the relationship between rates of HR and DOC concentrations. While there is the potential for pseudo replication between the sites in the Pilgrim River influencing the results, all the sites showed variation in the environmental characteristics. To test the hypothesis that decomposition rates would be lower in

downstream sites compared to headwater sites, rates of decomposition were tested for normality and homoscedasticity using the Shapiro-Wilks test and Levene's Test respectively, and then analyzed using analysis of variance (ANOVA) with distance from the mouth of the river as the factor, followed by a Tukey's honest significance difference (HSD) *post hoc* test to examine the differences between the mean rates at each site for both studies. Additionally, to test the hypothesis that decomposition rates would be higher at sites with warmer water temperatures, the data sets from the two studies were again combined to perform Spearman's correlation tests following a Shapiro-Wilks test for normality to test the relationship between decomposition rates, canopy cover and water temperature. Finally, Spearman's correlation tests were performed to examine the relationship between mean water temperature with both canopy cover and DOC concentrations, which was decided after testing both for normality with the Shapiro-Wilks test. All tests were performed in R (R Core Team 2020) using a significance level of $p < 0.05$. Additionally, while all respiration rates are reported with negative values, they were made positive for statistical analysis, so if there is a positive relationship, the results of the test reflected that.

3 Results

3.1 Environmental Characteristics

All of the sites showed a wide variation in their environmental characteristics (Table 2). Canopy cover ranged from almost completely open, with 3.54% at the North Fork Pilgrim River Km 15, to almost completely covered, with 96.36% at the South Fork Pilgrim River Km 16. Additionally, the DOC concentrations ranged from 5.30 mg/L the Pilgrim River Km 6 site to 16.63 mg/L the Tobacco River. Similar to our hypotheses, spearman correlation tests showed that water temperature was positively correlated to DOC concentrations ($\rho = 0.92$, $p < 0.001$) and negatively correlated with canopy cover ($\rho = -0.7$, $p = 0.04$; Fig 3.) TDP and NH_4 also both showed large variation between the study sites (1.90 to 13.55 $\mu\text{g/L}$ and 5.29 to 19.43 mg/L respectively), while SRP and NO_3 concentrations did not vary as widely (Table 2).

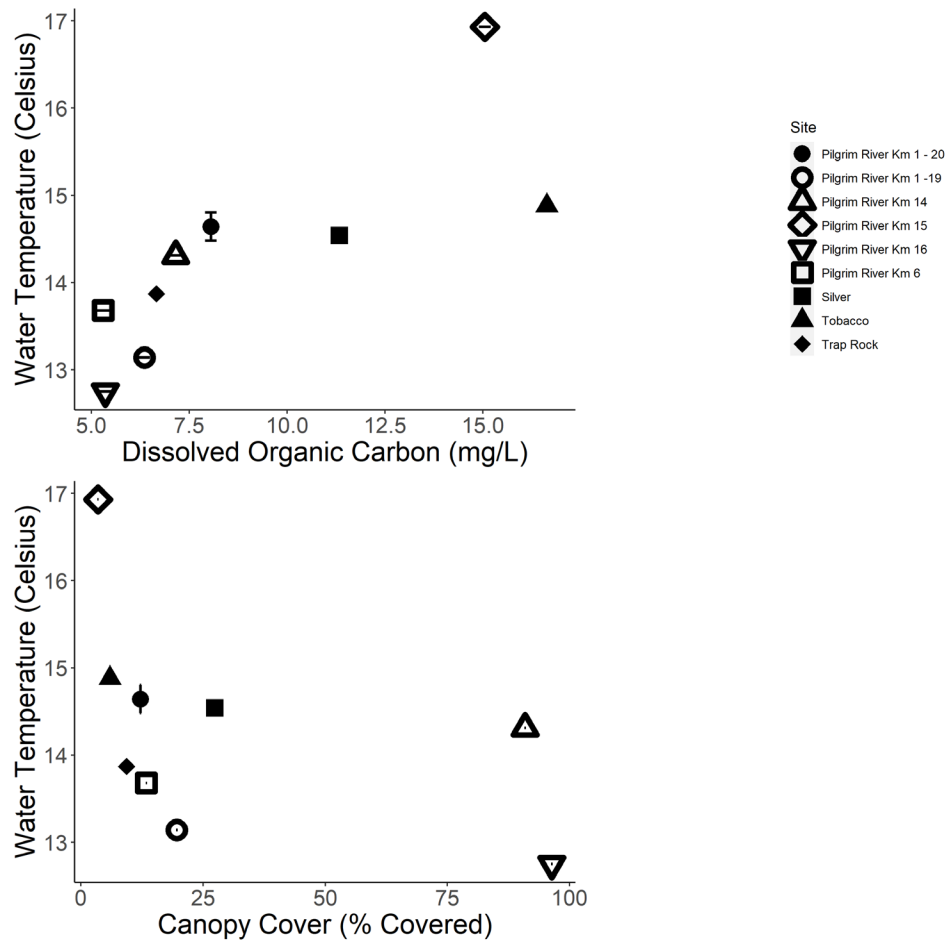


Figure 3. Plots of average water temperature, averaged over the whole study period, compared to dissolved organic carbon concentrations (Top) and canopy cover (Bottom). Error bars represent the 95% confidence intervals of the water temperature. The unfilled symbols represent sites from the longitudinal study, filled are DOC gradient study sites.

3.2 Gross Primary Production and Ecosystem Respiration

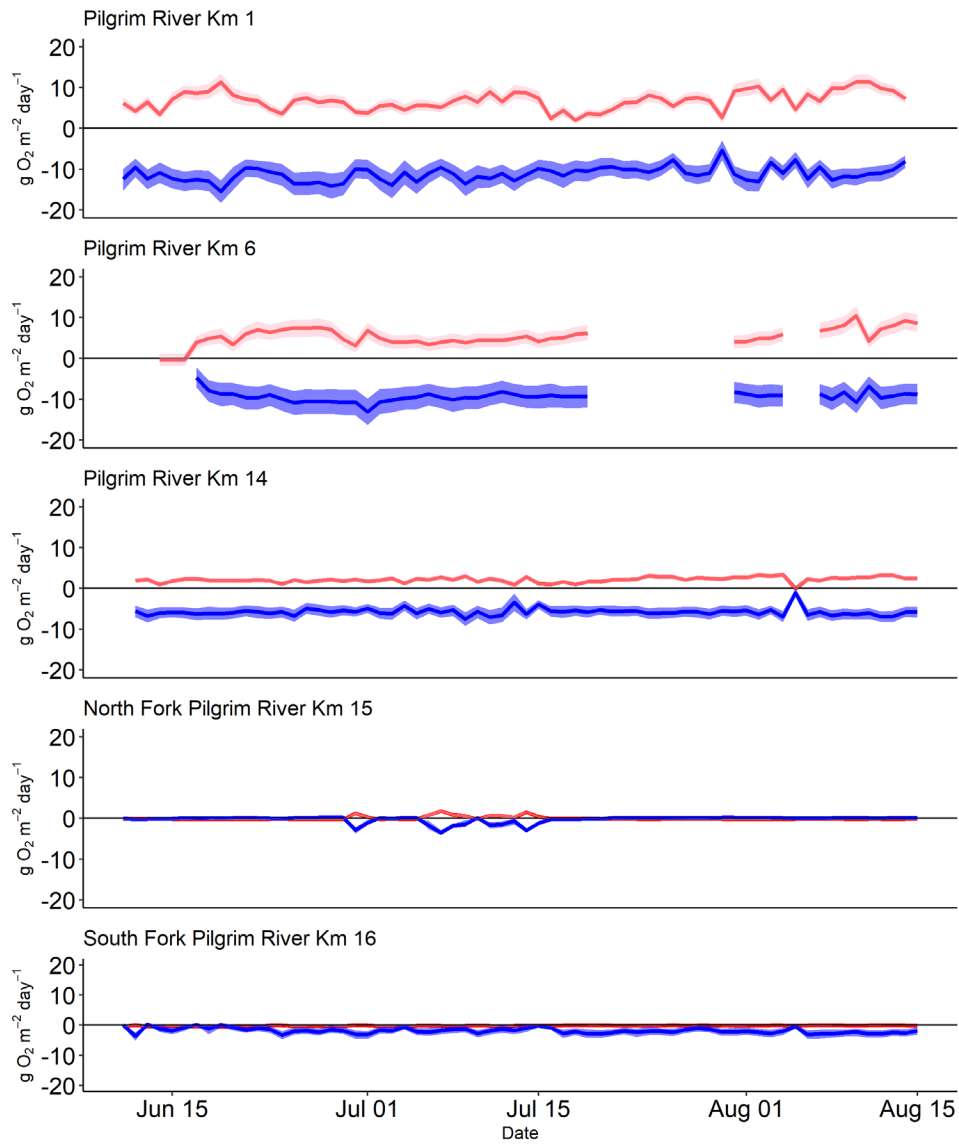


Figure 4 Time series plots of GPP (red) and ER (blue) at each site for the longitudinal study in summer 2019. The 95% confidence interval from the model is indicated with the lighter shades of each color. The GPP confidence intervals are narrow enough to not be distinguishable at all sites but Pilgrim River Km 1 and River Km 6. The gaps in Pilgrim River Km 6 are from times when the DO_2 sensor was buried in sediment, preventing estimation of metabolism on these dates.

The three downstream sites from the longitudinal study showed differences in the rates of both GPP and ER. The rates of GPP at these sites all differed significantly based on non-overlapping 95% CI, but the rates were not greater upstream relative to downstream as hypothesized. Pilgrim River Km 6 had the highest rate of GPP, with a mean rate of $5.82 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI 5.28 to 6.36), while Pilgrim River Km 1 had a mean rate of $3.15 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI 2.69 to 3.61), and the Pilgrim River Km 14 had a mean rate of $2.09 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI of 1.95 to 2.23). The headwater sites, North Fork Pilgrim River km 15 and South Fork Pilgrim River km 16, had GPP rates near zero ($-0.04 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$, 95% CI -0.10 to 0.02, and $-0.16 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$, 95% CI -0.19 to -0.12, respectively) (Fig. 4). There was strong correlation between ER and k_{600} for these two sites (North Fork Pilgrim River km 15 $r^2 = 0.98$ $n = 115$ days, South Fork River km 16 $r^2 = 0.89$ $n = 115$ days; Appendix A), which can indicate poor model performance (Appling et al. 2018). Poor model performance can then lead to inaccurate estimates of k_{600} as a result of equifinality, which can lead to higher error in GPP estimates and higher estimates of ER rates than if k_{600} was measured directly (Nifong & Taylor 2020). Therefore, the model is unable to confidently fit fluxes due to atmospheric interactions vs. biological activity, and the GPP and ER data from the two headwater sites (km 15 and km 16) were left out of further analyses.

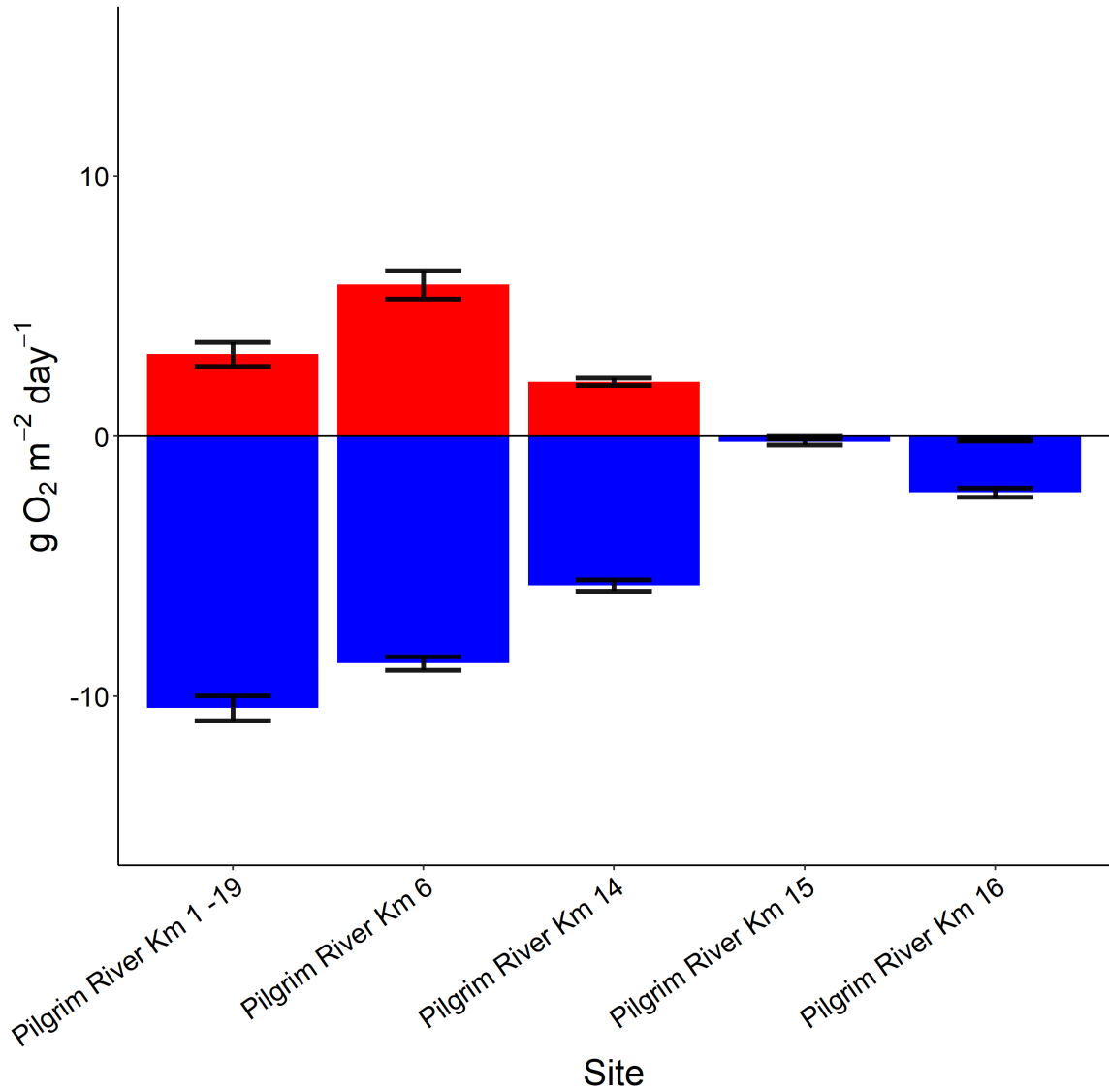


Figure 5 Bar plots of GPP (red) and ER (blue) at each site for the longitudinal study in 2019. The 95% confidence interval is indicated with the black bars.

The rates of ER at the three downstream sites in the longitudinal study did differ according to our predictions based on non-overlapping 95% CI. Mean ER rates were higher upstream than downstream, as revealed by River Km 1 having the highest average of -10.46 g O₂ m⁻² day⁻¹ (95% CI -9.99 to -10.94), Pilgrim River Km 14 having the lowest

average rate of ER of $-5.74 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI -5.53 to -5.95) and Pilgrim River Km 6 intermediate at $-8.74 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI -8.48 to -9.00) (Fig. 5).

For the DOC gradient study, the only site with a significantly different average GPP rate was the Tobacco River, which had a rate of $1.09 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI 0.07 to 2.11). The remaining three sites all had higher rates of GPP, but were not different from each other based on overlapping 95% CI (Fig. 6). The Silver River and Trap Rock River had the highest average rates of ER ($-11.56 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$, 95% CI -10.48 to -12.64 , and $-12.18 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$, 95% CI -11.54 to -12.83 respectively), the Pilgrim River Km 1 site had the lowest average rate of ER, $-5.49 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI -4.75 to -6.22), and the Tobacco River was intermediate at $-8.56 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI -7.90 to -9.22).

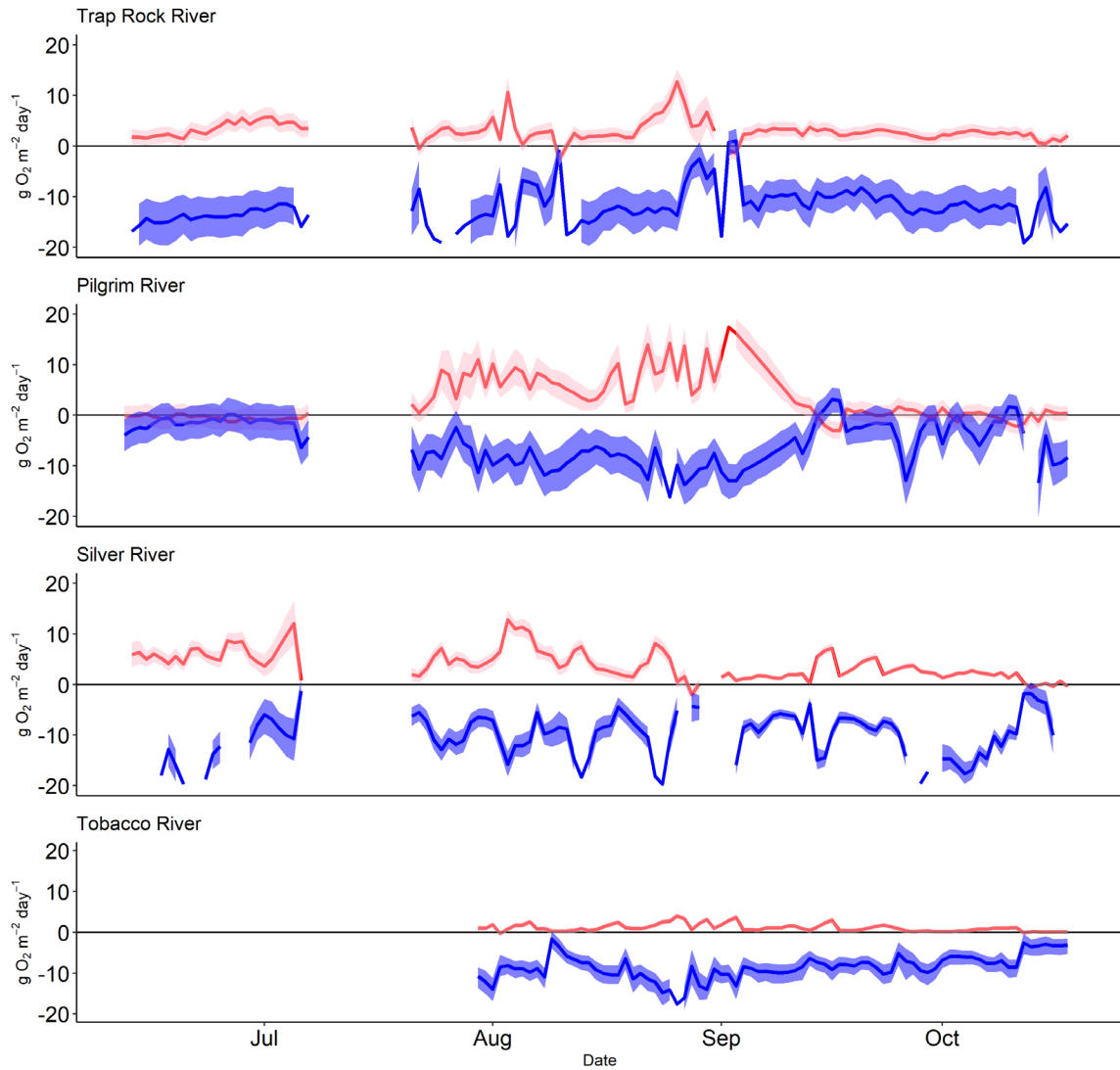


Figure 6 Time series plots of GPP (red) and ER (blue) at each site for the longitudinal study in summer 2019. The 95% confidence interval is indicated with the lighter shades of each color. The GPP confidence intervals are narrow enough to not be distinguishable. The sites are arranged from lowest DOC concentration (Top) to highest (Bottom).

3.3 Autotrophic and Heterotrophic Respiration

The values of AR_f were estimated for all sites across both studies with variable success (Appendix B). Four sites produced realistic estimates based on the criteria of Hall and Beaulieu (2013) ($AR_f < 1$, $CI < 0.4$; Table 2). Two sites had low variation in GPP

rates (Tobacco River, South Fork Pilgrim River Km 16, Pilgrim River Km 14), and therefore produced unrealistic estimates based on these same criteria. Additionally, the Trap Rock River HR rates were correlated with GPP rates ($r = 0.52$), which lead ARf to have a $CI > 0.4$, not meeting the criteria for inclusion in further analyses.

Table 2: Estimates of the fraction of gross primary production that fuels autotrophic respiration, ARf. These values were calculated as the slope of a quantile regression of gross primary production and ecosystem respiration, using the 0.9 quantile. Confidence Interval (CI is also included), any sites with $CI > 0.4$ are unusable.

Site	<i>Pilgrim River Km 14</i>	<i>Pilgrim River Km 6</i>	<i>Pilgrim River Km 1 – 2019</i>	<i>Pilgrim River Km 1 -2020</i>	<i>Tobacco River</i>	<i>Silver River</i>	<i>Trap Rock River</i>
ARf	-1.33	-0.39	-0.62	-0.70	-2.52	-0.70	-0.51
CI	-1.69 to -1.12	-0.44 to -0.37	-0.85 to -0.48	-0.76 to -0.68	-2.83 to -2.09	-0.78 to -0.58	-0.56 to 0.09

We estimated AR and HR rates for the 4 sites where we had reliable estimates of ARf (Figure 7). AR at the Pilgrim River Km 1 during the longitudinal study was the highest with an average rate of $-2.09 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (Fig.7 A.) and a 95% CI of -1.62 to -2.8, while the remaining sites were lower and had overlapping 95% CI. The Silver River had the highest mean rate of HR, $-9.04 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI -8.02 to -10.06), while the Pilgrim River Km 1 site from the DOC gradient study had the lowest mean rate of HR of $-3.28 \text{ g O}_2 \text{ m}^{-2} \text{ day}^{-1}$ (95% CI -2.72 to -3.83). The other two sites were not significantly different due to overlapping 95% CI (Fig. 7 B).

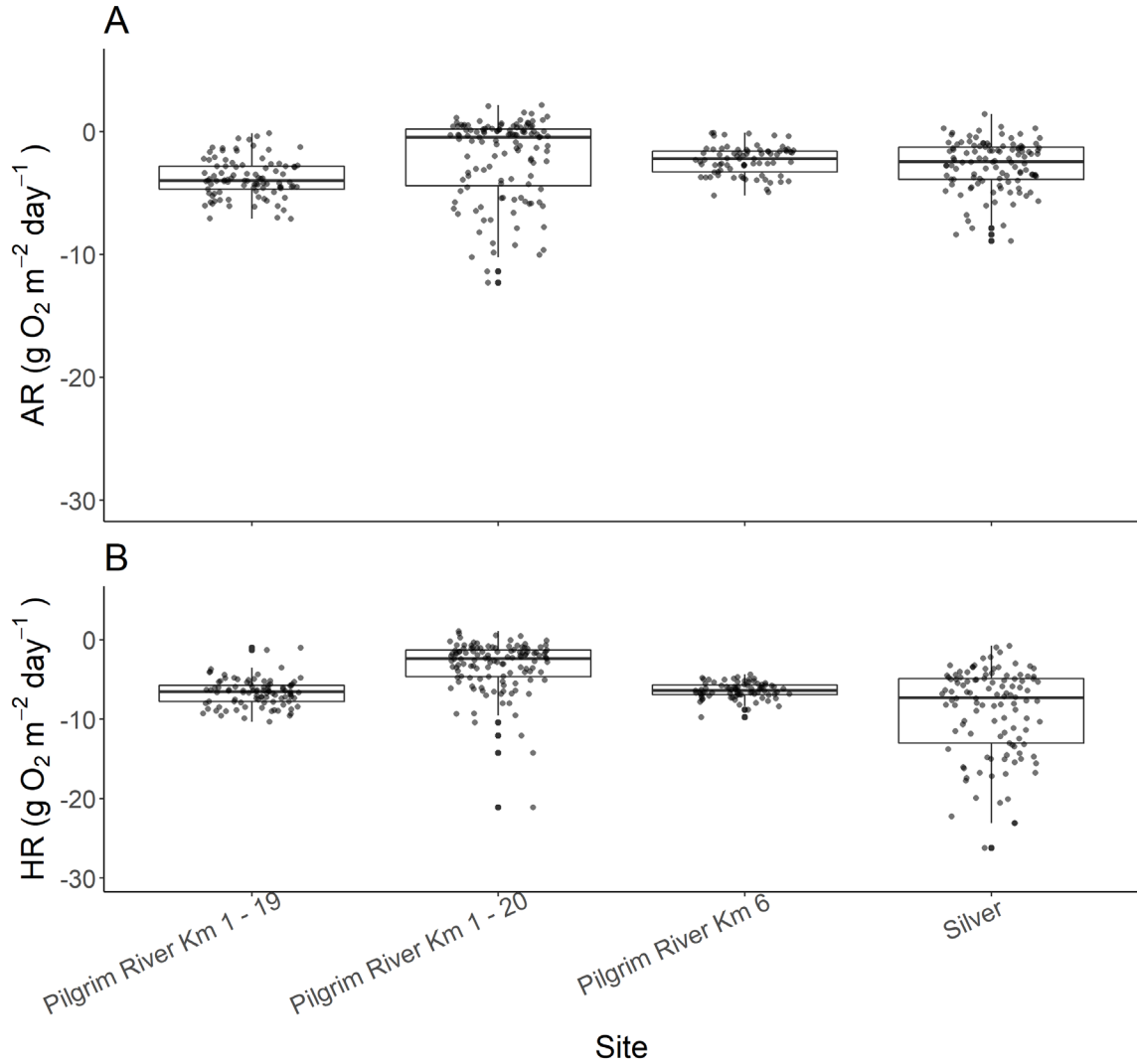


Figure 7 Boxplots comparing the rates of both autotrophic respiration (AR, figure A) and heterotrophic respiration (HR, figure B) among the different sites. The edges of the boxes represent both the 0.25 and 0.75 quantiles, while the lines extending out are the outliers.

3.4 Decomposition

Average tensile loss corrected for degree days was significantly different among sites in the longitudinal study (ANOVA $F = 44.1$, $df = 4, 20$, $p < 0.001$), but despite what

we predicted, the North Fork Pilgrim River Km 15 site had the lowest average rate of 0.01 % tensile loss (degree day)⁻¹, while the Pilgrim River Km 14 and South Fork Pilgrim River Km 16 had the highest mean rates at 0.06 % and 0.04 % tensile loss (degree day)⁻¹, respectively (Fig. 8). Additionally, there was a significant difference in average tensile loss among the sites in the DOC gradient study, both corrected (ANOVA $F = 6.9$, $df = 3$, 12 , $p = 0.006$) and not corrected for degree days (ANOVA $F = 6.3$, $df = 3$, 12 , $p = 0.008$). The Tukey HSD *post hoc* test showed that the rate of tensile loss at the Pilgrim River Km 1 site was the highest among all the sites, which were not significantly different from each other (Fig. 8 C and D).

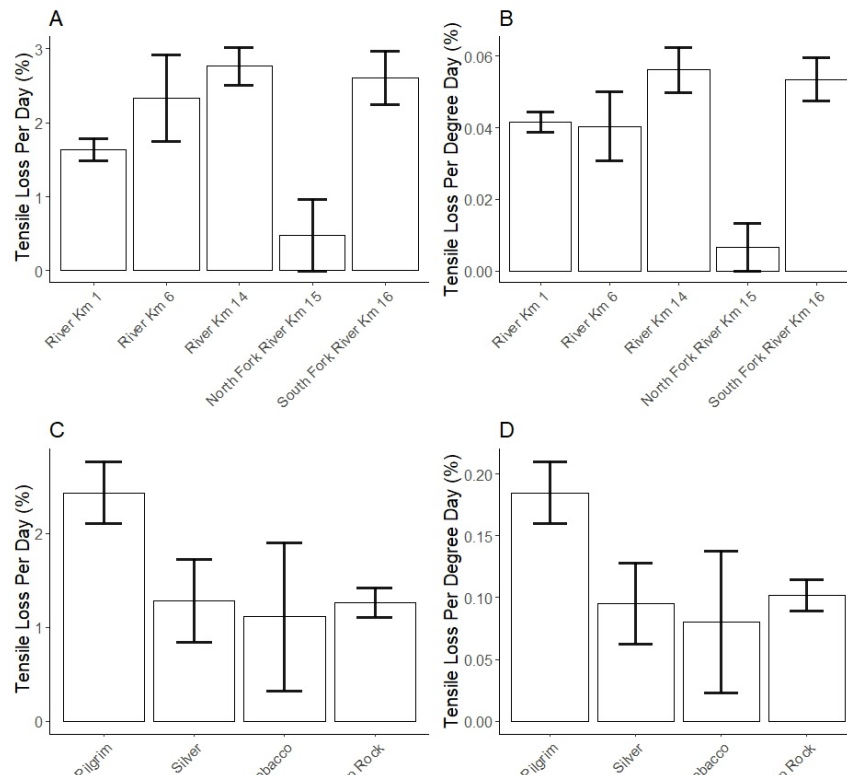


Figure 8 Comparison of tensile loss per day (A and C) and tensile loss per degree day (B and D). The error bars represent standard error. A and B are from the longitudinal study while C and D are from the DOC availability study.

3.5 Relationship between Environmental Characteristics and Process Rates

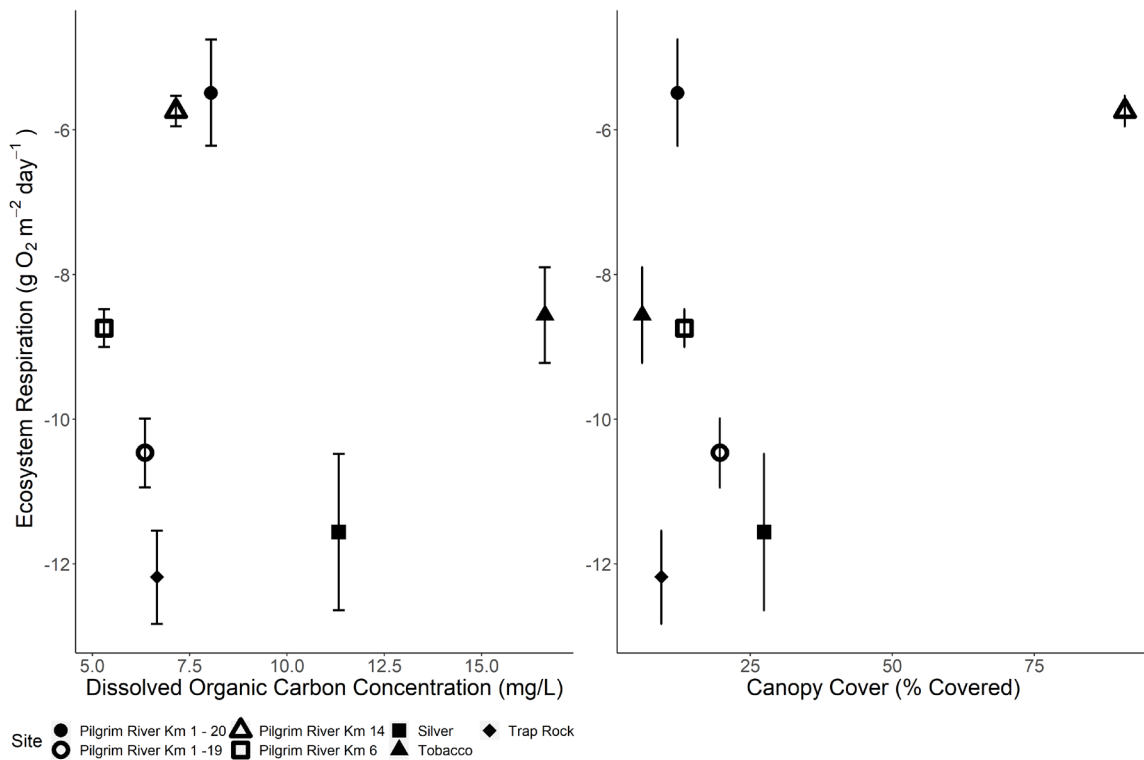


Figure 9 Comparison of average ecosystem respiration rates and dissolved organic carbon concentrations (Left) and canopy cover (Right). The error bars represent the 95% confidence interval of ecosystem respiration. The unfilled symbols represent sites from the longitudinal study, filled DOC gradient study sites.

The sites from both studies were combined to examine the effects of different environmental characteristics on ER, AR, HR and decomposition rates. Similar to our initial prediction, ER was not significantly related to DOC concentrations ($p = 0.59$, $\rho = -0.25$). ER was also not significantly related to canopy cover ($p = 0.96$, $\rho = -0.03$).

contradictory to our prediction (Fig. 9). When partitioned into AR and HR, neither showed relationships that matched our hypotheses. AR rates were not significantly related to canopy cover ($p = 0.33$, $\rho = 0.8$ or DOC concentrations ($p = 1$, $\rho = 0$) and HR was not significantly correlated with DOC concentrations ($p = 0.75$, $\rho = 0.9$) (Fig 10, Table 3). Additionally, there was no significant relationship found between water temperature and ER ($p = 0.30$, $\rho = -0.46$), AR ($p = 0.42$, $\rho = 0.6$), or HR ($p = 0.33$, $\rho = 0.8$).

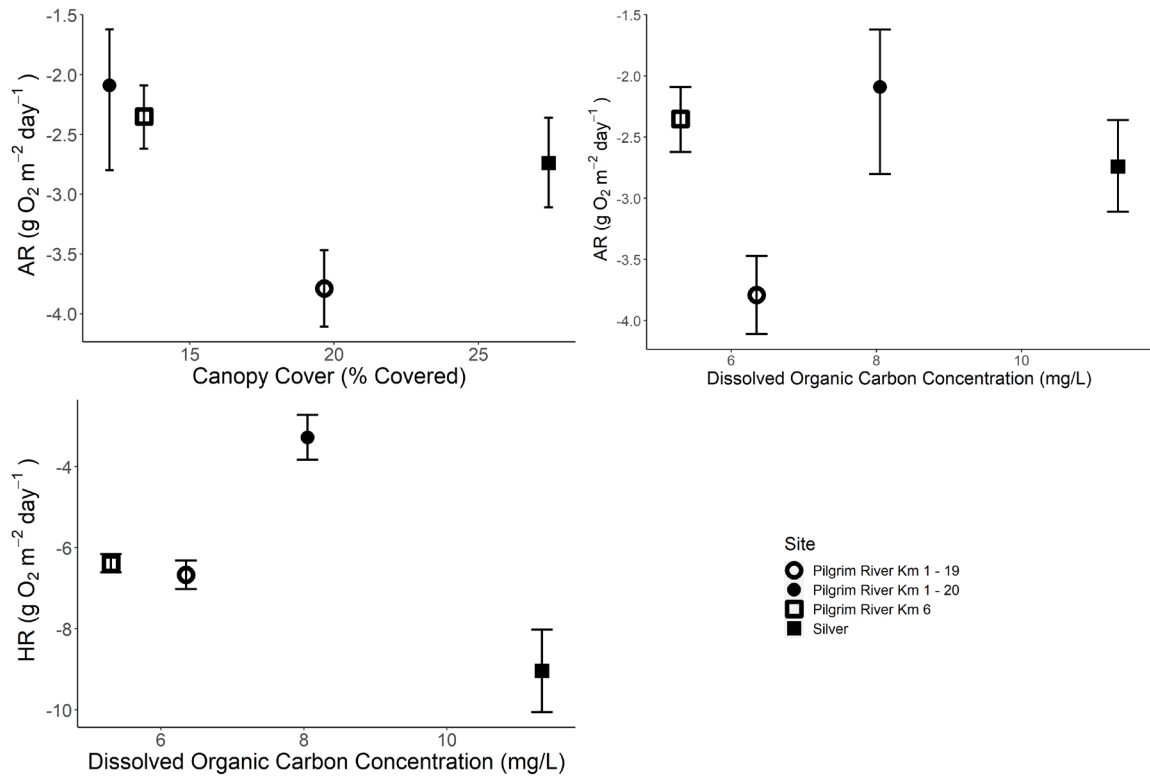


Figure 10 Comparison of average autotrophic respiration (AR) rates and canopy cover (Top) and dissolved organic carbon concentrations (middle), and average heterotrophic respiration (HR) with dissolved organic carbon concentrations. The error bars represent the 95% confidence interval of AR and HR. The unfilled symbols represent sites from the longitudinal study, filled are DOC gradient study sites.

Decomposition rates were not significantly related to canopy cover when corrected for degree-days ($p = 0.90$, $\rho = -0.5$), but were positively related when not corrected for temperature ($p = 0.01$, $\rho = 0.82$) (Fig 11). Decomposition also did not have a relationship with temperature when not corrected for degree-days ($p = 0.91$, $\rho = -0.05$), but did have a significant negative relationship when corrected ($p < 0.01$, $\rho = -0.93$).

Table 3: Summaries of the Spearman's Correlation tests performed. The respiration rates were all made positive, this change was made to indicate if the rates were increasing, a positive relationship would be shown, and if decreasing then a negative relationship would be shown. Significant relationships are bolded.

Relationship Tested	p-value	rho
ER vs DOC concentrations	0.6	-0.3
ER vs Canopy Cover	1.0	-3.0×10^{-2}
ER vs Water Temperature	0.3	-0.5
AR vs Canopy Cover	0.3	0.8
AR vs DOC concentrations	1	0
AR vs Water Temperature	0.4	0.6
HR vs DOC Concentrations	0.8	0.4
HR vs Water Temperature	0.3	0.8
Decomposition (Degree Day Corrected) vs Canopy Cover	0.9	-0.1
Decomposition (Not Degree Day Corrected) vs Canopy Cover	0.0	0.8
Decomposition (Degree Day Corrected) vs Water Temperature	2.4×10^{-4}	-0.9
Decomposition (Not Degree Day Corrected) vs Water Temperature	0.9	-0.1

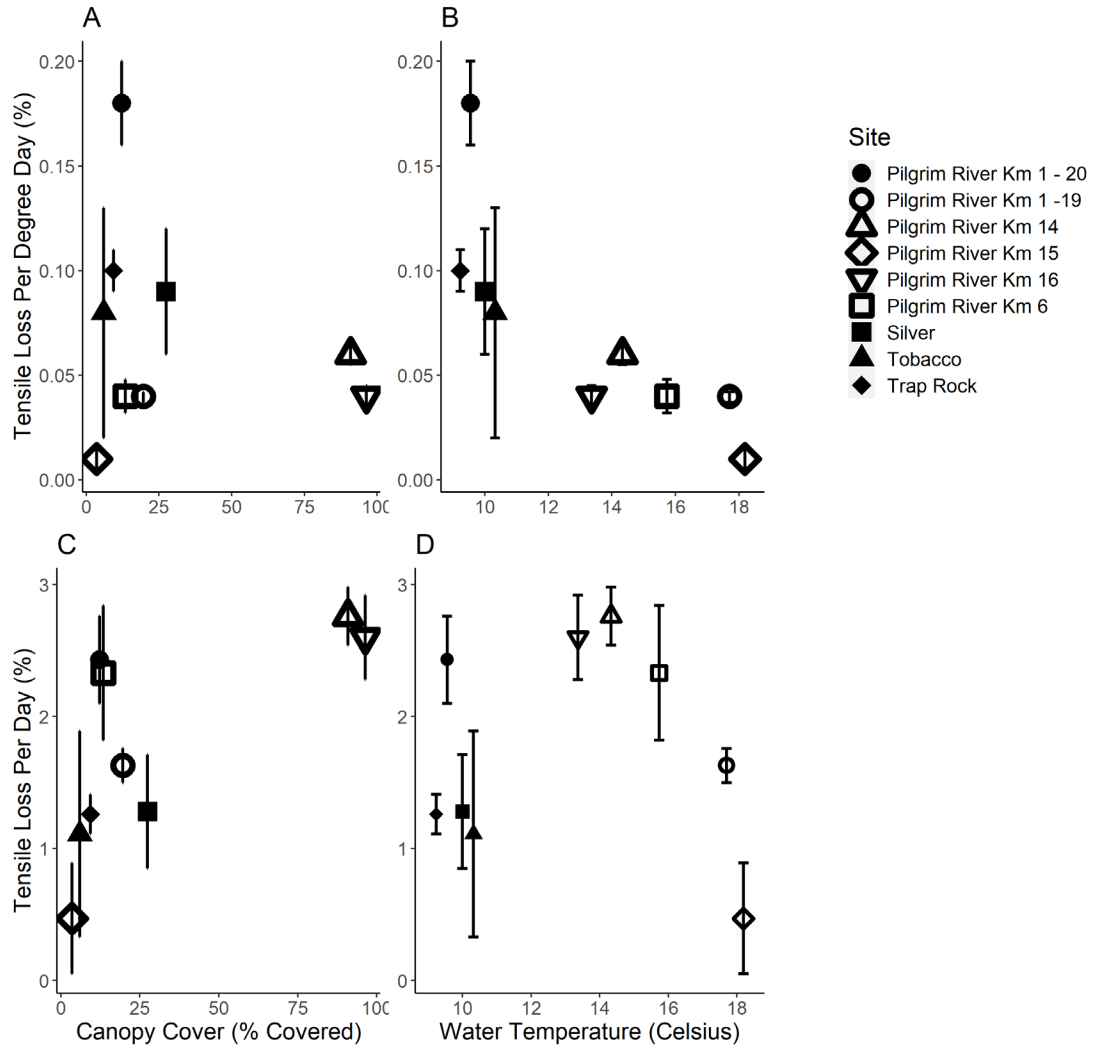


Figure 11 Comparison of average tensile loss per degree- day (A and B) and per day (C and D) with canopy cover (A and C) and water temperature (B and D) The error bars represent 95% confidence intervals of the tensile loss. The unfilled symbols represent sites from the longitudinal study, filled are DOC gradient study sites.

4 Discussion

It is important to know and understand which factors influence AR, HR, and decomposition, as these processes are important contributors to the carbon cycle in streams and will be affected by environmental change due to anthropogenic effects. One large source of environmental change is the increasing DOC concentrations in northern forested streams, which has the potential to limit light (de Wit et al. 2016) and provide a carbon source to heterotrophs. However, our results indicated that DOC and light availability were not significantly related to AR or HR, and although canopy cover was related to decomposition rates, DOC concentrations were not. Our results do suggest that there may be regional variation to AR and HR when compared to previous studies that have partitioned out these rates, and better understanding the controls and influences on these processes will help us better predict how they will change due to climate change.

The results support our predictions that downstream sites would exhibit higher rates of ER, and, similar to the findings of Solomon et al. (2013), there was no correlation between ecosystem respiration and DOC concentration. However, there was no significant relationship between DOC and either HR or AR rates at our sites, which does not support our hypothesis of an offset between AR and HR as DOC increased. This may be the result of the methods used to estimate AR and HR, as we were not able to separate these processes statistically at all of the sites.

The method developed by Hall and Beaulieu (2013) to partition AR and HR rates is still relatively new with few studies having quantified rates (Arriota et al. 2019). Hall and Beaulieu (2013) estimated rates for 13 different streams, from Idaho, Ohio, Tennessee,

and Northern Spain. While the rates of GPP were similar among our sites and the sites used by Hall and Beaulieu (Marcarelli et al. 2010, Beaulieu et al. 2013, Roberts et al. 2007, Izaguirre et al. 2008), the four sites where we were able to estimate AR_f had a much greater portion of the primary production being used to fuel autotrophic respiration. The largest AR_f value, which is the fraction of GPP that is estimated to be immediately respired by autotrophs and closely associated heterotrophs, estimated by Hall and Beaulieu (2013) was 0.69, while the majority ranged from 0.11-0.47. Three of our streams had values between 0.62 and 0.70, and while the Pilgrim River Km 6 site had the lowest value of 0.39, this could indicate there is regional variation in how much GPP goes into AR, as the highest estimate by Hall and Beaulieu (2013) was also in the Midwest of the United States at Shepherd Creek, in Ohio. There may also be temporal variation of autotrophic and heterotrophic respiration. Pilgrim River Km 1 was used for both studies and the rates of both AR and HR were significantly different from each other. Rates of both AR and HR were lower in the DOC gradient study, May-Oct 2020, than the longitudinal study, May-Aug 2019, and the DOC gradient study showed a larger fraction of GPP fueling AR. However, while there may be regional and temporal trends, the quantile regression method is insufficient to study them, as this method does not work for all stream types.

One type of stream that this approach fails in estimating the rates are streams with low GPP variability. Blackwater rivers have lower rates of GPP in comparison to non-blackwater rivers sites, though with higher rates of ER than would be expected of non-blackwater rivers with the same productivity (Meyer & Edwards 1990). We found that the metabolism rates we estimated for the Tobacco River sites were consistent with other

black water rivers (Meyer & Edwards 1990, Naiman 1983). The Tobacco River site, a blackwater river in our DOC gradient study, showed comparable rates of ER to the rest of the sites in the rest in the DOC gradient study, in addition to the highest concentration of DOC and the lowest rates of GPP. Since very little primary production occurred at this site, it could also be inferred that there is little AR happening here, so the majority of ER is likely a result of HR. However, because the quantile regression approach (Hall & Beaulieu 2013) does not perform well at sites with little variation in GPP, the actual rates cannot be quantified, which indicates that this approach of partitioning AR and HR is not adequate for blackwater rivers. Additionally, while the *streamMetabolizer* package (Appling et al. 2018) is able to estimate rates of k_{600} , it is still beneficial for streams that are shallow and have low rates of primary production to have direct measurements of reaeration (Nifong & Taylor 2020). As seen in Appendix A, both the North Fork Pilgrim River Km 15 and South Fork River Km 16 sites in the longitudinal study had high correlation between k_{600} and ER rates which produced unreliable results of primary production. If k_{600} was measured directly for these as well as the other sites, k_{600} could have been fixed in the metabolism model, which may have allowed alleviated equifinality. Another limitation of the quantile regression approach is that the rates of AR are inflated due to the difficulty of separating out rates of closely associated heterotrophs (Hall & Beaulieu 2013, Hotchkiss & Hall 2015).

Our decomposition results were comparable to, but in the low range of rates, reported values for other rivers that flow through northern forests (Tiegs et al. 2019), which range from 0% to 20% loss in tensile strength per day. The results from the longitudinal study do show some support for our prediction of lower decomposition rates in downstream

sites in comparison to headwater sites, except for the North Fork Pilgrim River Km 15 site, which was a headwater site and had the lowest rates. One explanation for this could be because of the difference in canopy cover; the North Fork Pilgrim River Km 15 site was almost completely open canopy, while the two sites with the highest decomposition rates, Pilgrim River Km 14 and South Fork Pilgrim River Km 16, were almost completely closed. Mosele Tonin et al. (2018) found similar results, where closed canopy streams had decomposition rates 1.4 to 6.6 times faster than in open canopy streams in southern Brazil. Additionally, the correlation results indicate a positive relationship between decomposition when uncorrected for temperature with canopy cover, but no relationship when corrected for temperature, and no relationship with DOC concentrations. While canopy cover had a weak relationship with uncorrected decomposition rates, it was negatively related to water temperature. Additionally, DOC concentrations were positively related to water temperature, similar to our predictions. Further work needs to be done to assess whether those environmental characteristics actually have a strong influence on the water temperature, or if there is another mechanism that relates them, such as the contribution of groundwater interactions (Tague et al. 2007, Brown et al. 2007).

While temperature is a well-known driver of both decomposition and respiration (Webster & Benfield 1986, Boyero et al. 2016, Follstad-Shah et al. 2017, Yvon-Durocher et al. 2010, Griffiths et al. 2013), our results do not support our prediction that decomposition would be higher in sites with warmer temperatures, nor was there a relationship between temperature and ER, HR, or AR. The lack of a relationship between temperature and these processes are potentially due to low variation in temperature

between the sites, and because other environmental factors may contribute more to the among site variation in the current study. Another potential explanation for the lack of relationship between decomposition and temperature is the use of cotton strips instead of leaf packs. Cotton strip assays do not account for the decomposition activity of invertebrates (Tiegs et al. 2019). The cellulose that the cotton strips are made of may not be as labile as other carbon sources found in these streams. Other studies used material from plants in the area surrounding the streams, so the biological communities may be more proficient to breakdown that material, so typical relationships found between decomposition and environmental characteristics may not be as prevalent with cotton strips. Additionally, Follstad-Shah et al. (2017) found that detritivore decomposition rates were influenced by temperature and Boyero et al. (2011) found that higher latitude streams had detritivore activity contributing a larger portion to the decomposition rates. This would suggest that using cotton strips may not account for an important contributor to decomposition in northern streams.

Overall, the results of this study suggest that respiration rates were not significantly related to either light availability or DOC concentrations, nor were decomposition significantly related to water temperature in the northern forested streams we studied. However, the results do suggest that there is temporal variation in rates of both HR and AR, and decomposition may be influenced by canopy cover along a longitudinal gradient at these sites. Additionally, regional differences may exist in how much GPP is consumed by AR, although the quantile regression approach to estimate AR is limited to streams that have a wide variation in GPP. Additionally, the rates of AR are inflated because it is unable to partition out the fraction of GPP that is respired by closely associated

heterotrophs and estimates of HR are lower (Hall & Beaulieu 2013, Hotchkiss & Hall 2015)). A more accurate and inclusive method is needed so we can have a better understanding of the local and regional factors that influence the different rates of respiration. Understanding the controls on both respiration and decomposition will be important to help predict how river ecosystems may change as a result of climate change and provide important data to better integrate inland aquatic ecosystems into global climate models.

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A Ecosystem Respiration and K_{600} Comparison

Estimates of ecosystem respiration and gas exchange were compared for each site. This was done to evaluate the effectiveness of the *streamMetabolizer* model (Appling et al. 2018) performance.

A.1 Longitudinal Study

Ecosystem respiration and k_{600} at the Pilgrim River Km 1 and 6 sites had a weak relationship ($r^2 = 0.30$ and $r^2 = 0.22$ respectively), the Pilgrim River Km 14 site had a stronger relationship ($r^2=0.68$) while the head waters had the strongest (North Fork Pilgrim River Km 15 $r^2= 0.91$, South Fork River Km 16 $r^2= 0.95$)

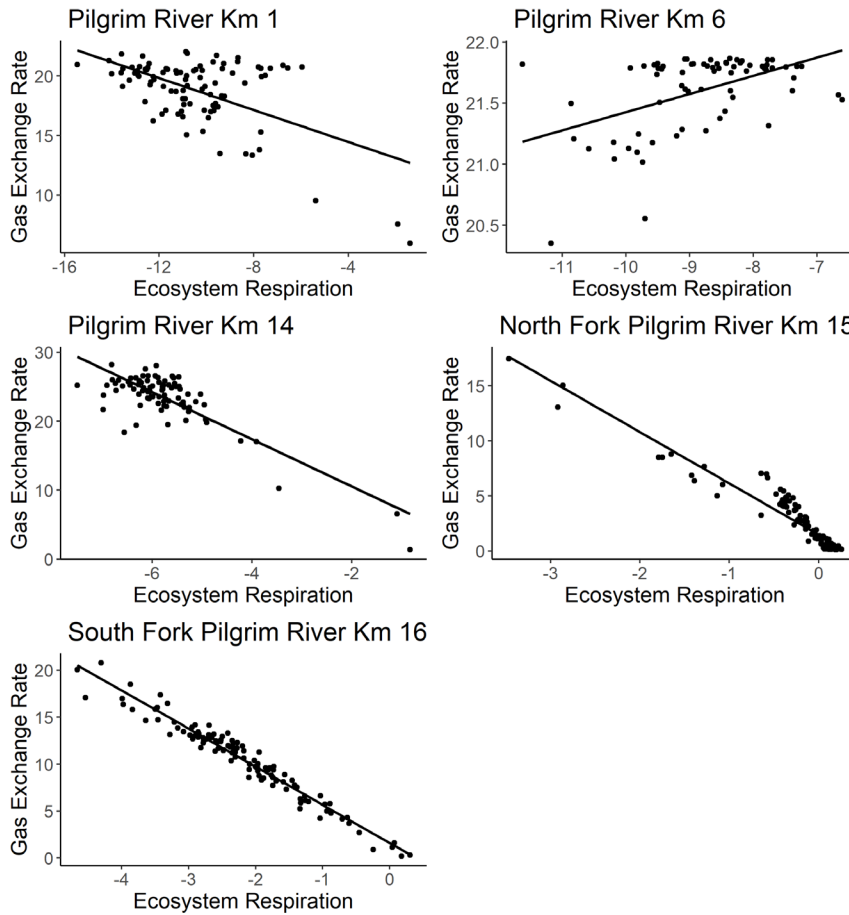


Figure A.1. Comparison of the gas exchange rate and ecosystem respiration for each site in the longitudinal study. Rates of ecosystem respiration are in $\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$, while the gas exchange rate is reported as the coefficient k_{600} .

A.2 DOC gradient Study

All the sites had relatively similar relationship between ecosystem respiration and k_{600} , however the Tobacco River site had the weakest relationship ($r^2 = 0.29$). The Silver River and Trap Rock River sites had similar relationships ($r^2 = 0.66$ and $r^2 = 0.64$ respectively), while the Pilgrim River Km 1 site was slightly lower ($r^2 = 0.51$).

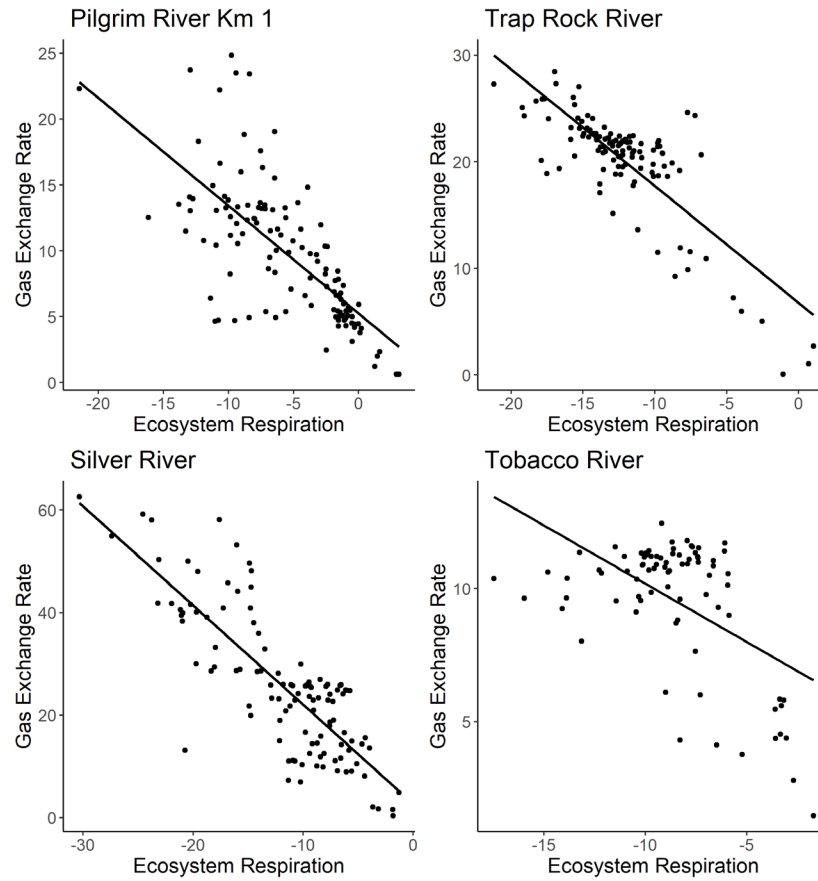


Figure A.2. Comparison of the gas exchange rate and ecosystem respiration for each site in the DOC gradient study. Rates of ecosystem respiration are in $\text{g O}_2 \text{ m}^{-2} \text{ day}^{-1}$, while the gas exchange rate is reported as the coefficient k_{600} .

B Gross Primary Production and Ecosystem Respiration Quantile Regressions

B.1 Longitudinal Study

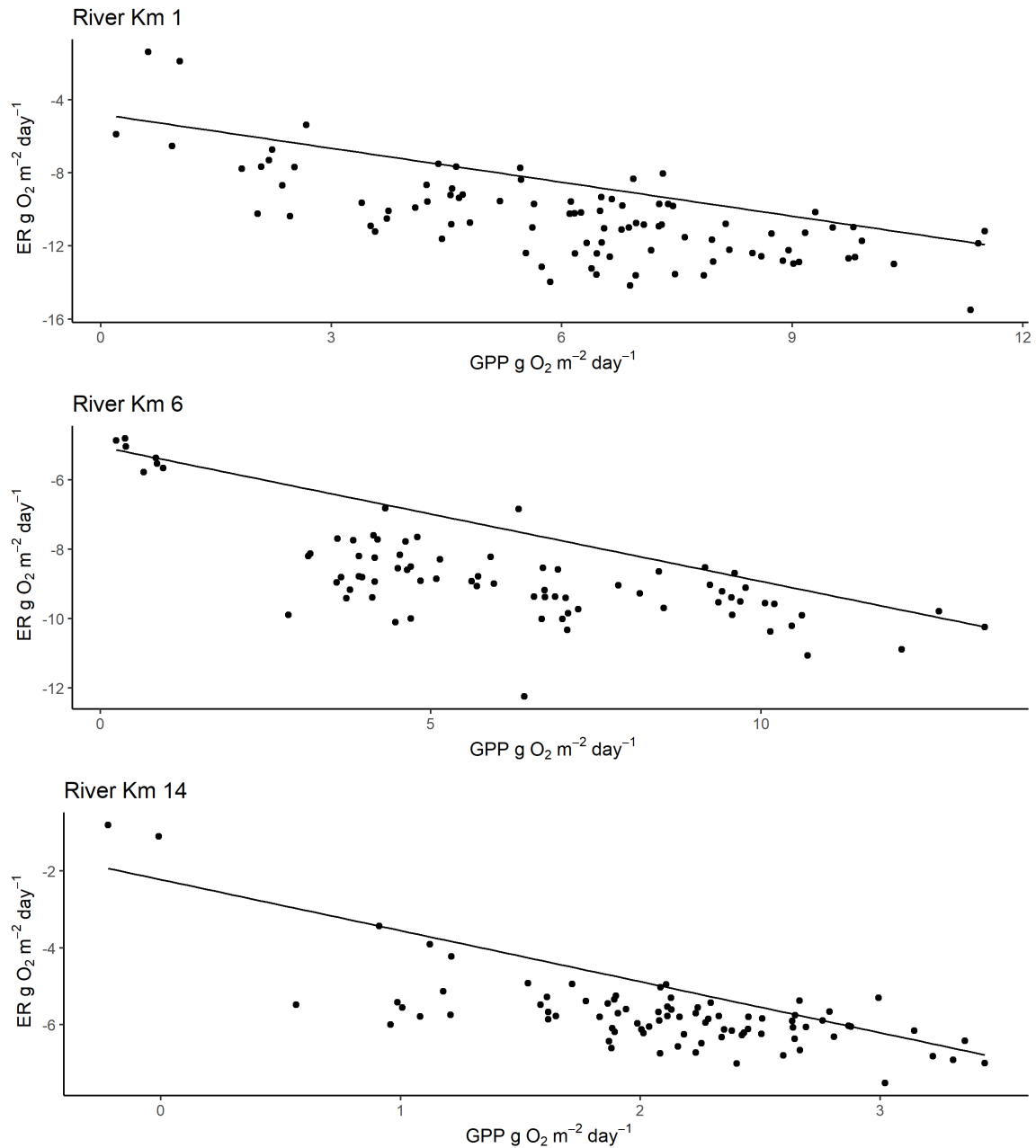


Figure B.1 The quantile regression plots for the three downstream sites in the longitudinal study. The line is the slope for the 90th quantile, which is also ARf.

B.2 DOC Gradient Study

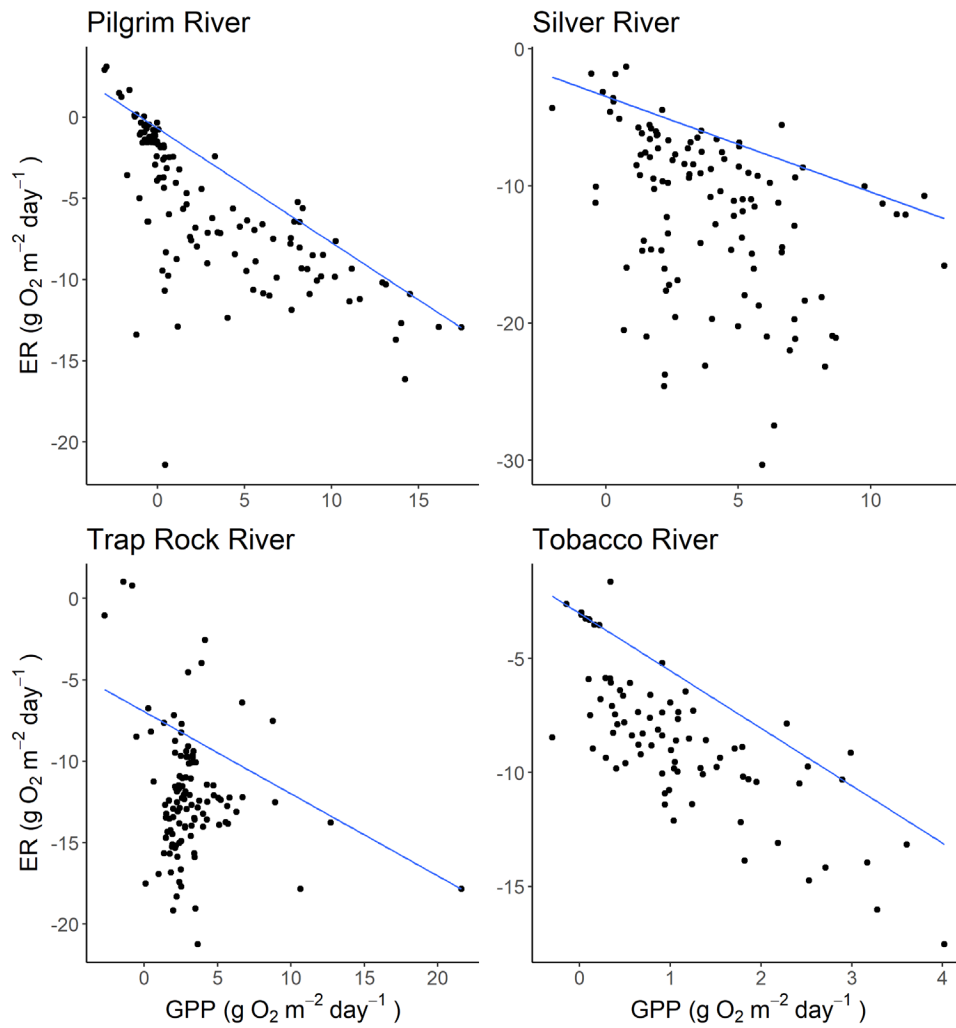


Figure B.2 The quantile regression plots for each site in the DOC gradient study. The line is the slope for the 90th quantile, which is also ARf.