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Sensitivity of Aquatic Organic Matter Degradation To Changing Temperature and Nutrient Conditions in a Coastal Watershed

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SENSITIVITY OF AQUATIC ORGANIC MATTER DEGRADATION TO CHANGING TEMPERATURE AND NUTRIENT CONDITIONS IN A COASTAL WATERSHED

By

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ABSTRACT

The degradation of organic matter (OM) within inland waters plays a pivotal role in the global carbon cycle and quantifying carbon budgets. Here, measurements of dissolved oxygen (DO) decay rates were used to infer the extent and kinetics of OM degradation under variable conditions. The goal of the investigation was to quantify how OM samples within the Waccamaw River watershed, South Carolina, respond to changes in temperature and nutrient availability as a function of their source location and lability. Samples were collected from urbanized stormwater detention ponds and undeveloped upland forested wetland drainages to provide contrasting and distinct OM sources to the river, as each possesses different degrees of OM lability. To explore the temperature sensitivity of OM degradation, samples from the summer of 2020 were incubated within three temperature regimes (20, 27.5, and 35°C) and temperature sensitivity was quantified as Q_{10} (relates to how a biological rate would change with 10 degrees of warming). Results indicate that temperature-driven increases in DO decay rate experienced in the more refractory, forested wetlands were more than double those experienced in the Waccamaw River and stormwater detention ponds. In summer of 2021, the potential for synergistic interactions between warming water temperatures and increasing nutrient loading from coastal development was investigated through a series of experimental enrichment experiments conducted at two temperature regimes (ambient and ambient + 5°C). Results indicated that nutrient loading produced a significant increase in DO decay rates, relative to a treatment control at ambient temperatures, approximately 42% of the time, but upon combining nutrients and warming, approximately twice as many stations (83%) yielded significant increases in DO decay rates. Additionally, the labile carbon amendments showed a significant response across the board (100%). Overall, our results suggest that naturally OM-rich systems are more susceptible to ongoing climate change, as warming temperatures allow for relatively greater OM degradation of more refractory, natural sites, even in the absence of increased urbanization. These systems will likely experience even greater DO decay rates when warming temperatures and nutrient loading increase simultaneously, which is predicted with ongoing warming and stronger, flashier storm events in the future.

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LIST OF ABBREVIATIONS

CDOM..Chromophoric Dissolved Organic Matter

Chl-a..Chlorophyll-a

DIN ....Dissolved Inorganic Nitrogen

DIP ....Dissolved Inorganic Phosphorus

DO...Dissolved Oxygen

DOC..Dissolved Organic Carbon

MTE ....Metabolic Theory of Ecology

OM ....Organic Matter

PC...Particulate Carbon

PN ....Particulate Nitrogen

Q_{10}...Q_{10} Temperature Coefficient

TDN ....Total Dissolved Nitrogen

TDP ....Total Dissolved Phosphorus

TMDL ....Total Maximum Daily Load

CHAPTER 1

INTRODUCTION

The U.S. has been undergoing substantial urbanization, with the southern region of the country experiencing the greatest increase in regional population growth of ~11.5 million people between 2000-2008 and an 80% increase in developed land (U.S. Census Bureau 2008, U.S. Department of Agriculture 2009, O'Driscoll et al. 2010). Increasing the spatial extent of developed land affects the physical and chemical conditions within coastal waters, primarily through the expanding coverage of impervious surfaces, essentially increasing stormwater runoff inputs (Gold et al. 2019). Along with altered land use via extensive urbanization, the world is also getting warmer due to ongoing climate change. The IPCC currently projects a 2.7 to 4.4°C global average temperature increase by the end of the century (IPCC 2021). As the world continues to change, it is important for scientists to understand how different processes may vary in response. The degradation of aquatic organic matter (OM) within inland waters plays an important role in carbon dynamics, and this may be influenced by the changing patterns noted above.

Here, we examined the Waccamaw River, located along the South Carolina coast in Horry and Georgetown counties, as it is characterized by historically impaired dissolved oxygen (DO) although a total maximum daily load (TMDL) has been in place since the late 1990s-early 2000s (SCDHEC, 1999). It's important to note that the Waccamaw River is only one example of the many organic-rich systems classified as a "blackwater" body within the Southeastern U.S., and provides an ideal location to analyze the influence of nutrient loading and warming temperatures on OM degradation. The watershed of the Waccamaw River is comprised of a number of distinct organic matter sources including: stormwater detention ponds in the urbanizing Myrtle Beach region, undeveloped, natural upland forest wetlands, and cypress drainages. Investigating the influence of nutrients and temperature on the DO decay rates of these individual sources in unison with the Waccamaw River proper will allow us to determine the sensitivity of OM degradation as a function of its source and lability in a warming climate, as well the mechanisms influencing overall DO impairment in this economically important river.

CHAPTER 2

YEAR 1 – TEMPERATURE SENSITIVITY

2.1 ABSTRACT

Degradation of inland water organic matter (OM) is an important component of global carbon budgets. Here, the rate and extent of OM degradation was investigated in response to warming (35˚C) in the dissolved oxygen (DO) impaired, blackwater Waccamaw River, South Carolina. Our experiment utilized 5-day DO decay rates under three incubation temperature regimes in waters from the Waccamaw River and two distinct OM sources: natural, refractory OM from forested wetlands and urbanized, labile OM from stormwater detention ponds. Average increases in DO decay rate, relating to IPCC temperature predictions, for wetlands (~24 to 41%) were more than double river (~12 to 19%) and pond (~10 to 17%) sites, indicating wetlands possess significantly larger temperature sensitivities (Q_{10} = 2.14) than the river (1.49) and ponds (1.41). Results suggest naturally OM-rich systems are more susceptible to ongoing climate change, exacerbating DO impairment through greater OM degradation, even in the absence of urbanization.

2.2 INTRODUCTION

Unraveling the intricacies of OM degradation within inland waters is necessary for understanding global carbon dynamics, as recent estimates predict that of the 5.1 Pg C y^{-1} delivered from terrestrial sources to inland waters, approximately 75% is processed and eventually released into the atmosphere as CO_2 (Drake et al. 2018). Inland waters do not receive a homogeneous input of OM. Rather, these systems are comprised of a heterogeneous mix of OM compounds derived from sources that represent a range of habitats and land uses. Shifting land uses associated with urbanization, in particular, alter OM composition and promote nonpoint source inputs of readily degradable OM (Hosen et al. 2014). As oxygen is consumed during OM degradation, shifts in OM lability associated with stormwater inputs from developed landscapes can alter dissolved oxygen (DO) dynamics of inland waters (Gold et al. 2020). Widespread DO impairment is an increasing threat to aquatic resources (Saari et al 2018, Breitburg et al. 2018) such that exploring connections between increasing urbanization, OM sources and lability, and DO dynamics is needed for understanding both global carbon biogeochemistry and water quality. Increasing urbanization in a warming world further necessitates the need to simultaneously understand the influence of temperature on OM lability and DO dynamics.

Increasing temperatures promote enhanced planktonic microbial respiration within aquatic systems, and given their generally low efficiencies, this occurs at a higher rate relative to production (Vázquez-Domínguez et al. 2007), resulting in DO loss throughout the water column (Yvon-Durocher et al. 2010). It is widely accepted that biologically mediated degradation rates increase with temperature (Lloyd and Taylor 1994). The metabolic theory of ecology (MTE) further suggests that the rate of all biological processes largely possess a similar temperature sensitivity, commonly expressed as a Q_{10} temperature coefficient of 2.0 (Gillooly et al. 2001; Brown et al. 2004). In soils, however, while microbial respiration increases with temperature, the exact relationship varies and is related, in part, to the source and quality of the OM degraded (Sierra 2012; Tang et al. 2017). Within the soil sciences, several studies have suggested that low-quality and more recalcitrant OM results in elevated Q_{10} temperature coefficients and thus higher temperature sensitivities (Davidson and Janssens 2006; Vanhala et al. 2007; Conant et al. 2008). Similar studies in aquatic systems are largely lacking, although Lønborg et al. (2019) recently determined that metabolic responses to temperature varied relative to DOM bioavailability in tropical waters. Indeed, changes in OM degradation relative to temperature may account for variability observed across different ecosystems (Jankowski et al. 2014). Using a universal Q_{10} temperature coefficient in modeling studies may thus lead to significant inaccuracies in OM degradation with global warming.

This study used 5-day DO decay rates determined in incubations of sample water collected from differing sources and exposed to varying temperature to explore how OM source and lability influences the temperature sensitivity of aquatic OM degradation. The study focused on the Waccamaw River, a 'blackwater' river located in coastal South Carolina, USA (SCDHEC 1999; SCDHEC 2014a). OM inputs to the river proper are primarily in the form of refractory material from largely unaltered, forested wetlands (hereafter "wetlands") and labile material from stormwater detention ponds (hereafter "ponds") created to manage stormwater associated with areas of rapidly increasing residential development. The Waccamaw River is also an example of the widespread prevalence of blackwater coastal waters undergoing DO impairment (Whitworth et al. 2010). Continuous monitoring data from USGS stations within the mainstem of the Waccamaw River suggest both periodic DO impairment (< 4.0 mg O_2 L^{-1}) during the summer (Fig. 2.1) and a trend of declining DO saturation with increasing temperature (Fig. 2.2), suggesting a potential relationship between increased microbial respiration rates and warming. As such, the Waccamaw River serves as an ideal study site to explore the potential influences of a warming world on DO impairment in a system rich in OM inputs of varying quality.

2.3 METHODOLOGY

2.3.1 STUDY LOCATION AND SAMPLING DESIGN

The Waccamaw River is a typical coastal plain river of the southeastern United States located within the greater Pee Dee Basin. Its watershed is dominated by extensive wetlands (36.9%) that export vast quantities of highly chromophoric dissolved OM (CDOM) and give the river its characteristic 'blackwater' designation (SCDHEC 2007). Although blackwater rivers are naturally prone to low DO concentrations, increasing residential and peri-urban development (10.5%) in the Greater Myrtle Beach, SC, area contributes substantial additional anthropogenic OM. A characteristic feature of residential development in this region is the construction of a large number of ponds used as best management practices for stormwater management (Smith et al. 2018). Ponds are increasingly suspected of producing and exporting large quantities of highly labile OM that contributes to DO demand in downstream waters (McCabe et al. 2020).

To characterize OM decomposition rates and temperature sensitivities, OM from contrasting watershed sources was collected during summer of 2020 (June 5 – August 5) from replicate wetlands (n=3) and ponds (n=5). Wetlands were all located in Hobcaw Barony, a protected undeveloped tract of land in the lower Waccamaw watershed. All stormwater pond samples were collected from medium density residential developments located in Georgetown and Horry counties, SC. Replicate samples were also collected from three locations within the lower mainstem Waccamaw River, where DO impairment is greatest.

2.3.2 EXPERIMENTAL INCUBATIONS

For each sampling event, surface water samples from each location were returned to the laboratory and split into replicates that were equilibrated overnight to one of three different experimental temperatures: 20°C, the standard incubation temperature for the determination of 5-day biochemical oxygen demand (BOD_5; Eaton et al. 2005); 27.5°C, the average summer temperature observed in the Waccamaw River; and 35°C, several degrees above highest ambient summer temperatures, allowing an inference of the impacts of potential climate change within the region. Temperature-equilibrated samples were subsequently partitioned into three, 300 mL borosilicate glass BOD bottles prior to further incubation. Measurements of DO in each BOD bottle were made approximately every 12 hours over the course of a 5-d dark incubation using a Wiltrox 1 (Loligo Systems, Denmark) chemiluminescent oxygen meter for mini sensors (accuracy ± 0.4% / precision ± 0.1%). This instrument utilizes fiber optic technology and an internal sensor membrane within each individual bottle, preventing the need for invasive sampling by opening the bottle. Consumption of DO was measured and reported as both the absolute BOD_5 and as the relative exponential decay rate calculated from a curve fit of the time-series of DO measurements (see below).

2.3.3 ANCILLARY MEASUREMENTS

Prior to equilibrating water samples to experimental temperature, particulate subsamples were collected using combusted, 25 mm diameter, 0.7 μm pore size, glass fiber filters (GF/F) and analyzed for concentrations of particulate carbon (PC), nitrogen (PN), total particulate phosphorus (TPP), particulate inorganic/organic phosphorus (PIP/POP), and chlorophyll-*a* (Chl-*a*). The filtrate (dissolved fraction) was analyzed for concentrations of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP) and DOM absorbance.

TDN and TDP were analyzed on a SEAL Analytical AA3 nutrient auto analyzer following Standard Methods SM 4500-N C (Eaton et al. 2005). DOC samples were acidified to pH 2 with 10% hydrochloric acid (HCl) prior to analysis utilizing high-temperature combustion (720°C) on a TOC-VCPN Shimadzu Analyzer, as described by Benner and Strom (1993). DOM absorbance spectra was measured from 250-550 nm with a Shimadzu UV-2450 UV-Vis dual beam spectrophotometer and then converted to absorption coefficients at 355 nm (a_{355}) with units of m^{-1}.

Chl-*a* was analyzed on a Turner Trilogy Laboratory Fluorometer following the U.S. EPA method 445.0 (Arar and Collins 1997). PC and PN were analyzed on a PerkinElmer Series II CHNS/O Analyzer 2400. Previous work showed that less than 10% of PC is comprised of particulate inorganic carbon in this system, thus total PC is assumed to be equal to POC (Schroer et al. 2018). Total particulate P and PIP analyses were measured using the ash/hydrolysis method (Aspila et al. 1976, Benitez-Nelson et al. 2007). POP was calculated as the difference between TPP and PIP.

2.3.4 STATISTICAL AND DATA ANALYSIS

All statistical analyses were completed using R (version 4.0.3). To determine how the Waccamaw River varied with its source material, wetlands and ponds, one-way analysis of variance (ANOVA) was used. If significant differences were identified, a Tukey HSD post hoc comparison was also conducted. The difference between initial and final DO concentrations after 5 d is termed BOD_5. Decay rates of DO were calculated using the slope from natural log transformed decay trends, and triplicates averaged. Temperature coefficients (Q_{10}) were calculated following the van't Hoff equation:

$$Q_{10} = (\frac{R2}{R1})^{\frac{10}{(T2-T1)}}$$

where R1 and R2 correspond to the DO decay rate over the 5 d incubation at a given temperature (T) treatment, T1 (lower T) and T2 (higher T).

2.4 RESULTS

2.4.1 DISSOLVED OXYGEN AND TEMPERATURE SENSTIVITY

Average BOD_5 for ponds (2.65 ± 1.03 mg O_2 L^{-1}) was significantly greater ($p = 7.12x10^{-3}$) than the Waccamaw River (1.26 ± 0.60 mg O_2 L^{-1}), with wetlands falling in the middle (2.07 ± 0.50 mg O_2 L^{-1}). For each temperature treatment, there were significant differences in DO decay rates across site types (one-way ANOVA, $p < 0.05$). Decay rates increased, albeit to differing degrees, between temperature treatments for each system (Fig. 2.3), with the biggest shifts between the rate at 20°C and 35°C occurring within wetlands (0.05 to 0.18 d^{-1}), followed by ponds (0.07 to 0.16 d^{-1}), and the Waccamaw River (0.03 to 0.08 d^{-1}). Ponds showed the largest variability among site types at each of the three temperature regimes (Fig. 2.3). Values of Q_{10} did not differ across site types (Fig. 2.4) for the lower temperature range (20 to 27.5 °C; $p = 0.169$). In contrast, for the upper range of temperatures (27.5 to 35 °C), wetlands Q_{10} values (2.14 ± 0.41) were significantly higher than those measured in either the Waccamaw River (1.49 ± 0.36; $p = 1.56x10^{-2}$) or ponds (1.41 ± 0.21; $p = 4.73x10^{-3}$).

2.4.2 ORGANIC MATTER CHARACTERISTICS OF SITE TYPE

Ancillary measurements of water quality (Table 2.1) were used to help characterize differences between the Waccamaw River, wetlands and ponds. Concentrations of Chl-a were significantly different among the three site types ($p = 0.046$), with highest average Chl-a concentrations measured within ponds. Although selection of sites was designed to establish contrasting degrees of organic matter quality, PC:PN ratios did not significantly differ among site types nor did PC or PN concentrations. Concentrations of TPP were low across sites, with average values of TPP in wetlands (0.44 ± 0.13 mM) about three-times lower ($p = 1.43x10^{-2}$) than ponds (1.15 ± 0.55 mM). Concentrations of PIP and POP shared trends, lowest within wetlands (0.16 ± 0.06 and 0.28 ± 0.08 mM) and highest within ponds (0.50 ± 0.26 and 0.65 ± 0.31 mM).

The average absorption coefficient of the dissolved fraction, a_{355}, varied significantly ($p = 6.87x10^{-8}$) and was more than ten-times greater for wetlands (166.6 ± 38. 5 m^{-1}) relative to ponds (13.7 ± 5.1 m^{-1}). The Waccamaw River had an average a_{355} between the end members (45.8 ± 21.8 m^{-1}). Whole and filtered water samples yielded similar trends for nutrient concentrations. Briefly, ponds contained significantly less average TDN (30.8 ± 9.9 mM) than wetlands (86.2 ± 24.8 mM; $p = 2.16x10^{-5}$) and the Waccamaw River (66.8 ± 9.7 mM; $p = 1.14x10^{-3}$). Similarly, the Waccamaw River contained significantly more TDP (1.90 ± 0.32 uM) than either wetlands (0.51 ± 0.32 uM; $p = 3.35x10^{-3}$) or ponds (0.58 ± 0.26 mM; $p = 1.54x10^{-2}$). Despite no trends in PC, the dissolved fraction showed that DOC significantly differed ($p = 6.30x10^{-8}$) with highest average DOC concentrations within wetlands (3790 ± 852 mM) compared to lower values in the Waccamaw River (1091 ± 519 mM) and ponds (543 ± 152 mM).

2.5 DISCUSSION

2.5.1 WACCAMAW RIVER TRENDS

Ambient monitoring of the Waccamaw River at Hagley Landing (USGS site number: 2110815) from 2010 to 2020 showed that minimum DO concentrations less than 4.0 mg L^{-1} continued to occur (Fig. 2.1) despite implementation of total maximum daily load (TMDL) regulations for this region of the river (SCDHEC 2014a). Although the data are highly seasonal, a Mann-Kendall Trend confirmed that DO concentrations have declined ($p = 1.28 \times 10^{-6}$; $\tau = -0.0538$), while temperatures increased ($p = 1.59 \times 10^{-3}$; $\tau = 0.0349$) over this period. Percent DO saturation versus temperature is significantly linearly correlated ($p = 2.20 \times 10^{-16}$, Fig. 2.2), suggesting a link between intensifying DO impairment and increasing warming, likely due to increased respiration rates. Our findings are consistent with predictions across the Southeastern United States that suggest increases in community respiration with rising temperatures (Mulholland et al. 1997).

2.5.2 TEMPERATURE SENSITIVITY AND SITE CHARACTERISTICS

The Waccamaw River, ponds and wetlands vary in their OM composition as evidenced by average absorption coefficients, DOM, and dissolved nutrient concentrations. Measurements of a_{355} show that wetlands have the highest absorption coefficients, consistent with the humic-rich nature of these waters. In contrast, ponds are characterized by much lower a_{355} value, suggesting less aromatic, lower molecular weight OM (Wear et al. 2015). This pattern is similar to previous results for the southeastern U.S., which showed that a_{355} declines with increasing percent impervious surface coverage (White 2014). Further, ponds were highest in Chl-a, which is not surprising since they are sources of substantial autochthonous algal production (e.g., Lewitus et al. 2008). Combined, these results support the notion that autochthonous pond material is likely comprised of more labile OM relative to terrestrial wetland OM.

Here, the rate of exponential decay in DO concentration over a 5-d incubation was used as the operational definition of OM lability, analogous to the many studies that have used time series decay rates of DOC to define OM lability (e.g., Holmes et al. 2008). Patterns in DO decay rates were consistent with presumed labilities based on source (e.g., higher DO decay rates were associated with ponds). Decay rates of DO were also more rapid with increasing temperature (Fig. 2.3). The sensitivity to increasing temperature (Q_{10}, Fig. 2.4) was not uniform, however, and argues against a single universal value for thermal dependence of metabolic rates (e.g, Gillooly et al. 2001; Brown et al. 2004). As such, modeling studies that rely on a universal temperature sensitivity are greatly oversimplified (Huey and Kingsolver 2011). Results further suggest that such variations in temperature sensitivity likely reflect differences in OM source and lability. In the Waccamaw River watershed, wetland sites, with the most refractory OM, had a significantly higher average Q_{10}, relative to the river or pond sites.

The influence of source on the temperature sensitivity of OM degradation has been the subject of many previous studies in the soil science literature (e.g., Davidson and Janssens 2006; Vanhala et al. 2007; Conant et al. 2008) although specific relationships with lability remain poorly resolved (Sierra 2012; Tang et al. 2017). In contrast, little attention to temperature sensitivity has occurred in the aquatic science literature (but see Lønborg et al. 2019). While results to-date are limited, these shared findings suggest that warming induced from climate change can differentially affect both terrestrial and aquatic systems relative to OM composition. In the case of the Waccamaw River, although continued urbanization may have negative impacts on water quality, natural wetland systems are more sensitive to warming, within the upper temperature range (Fig. 2.4).

A mechanistic understanding of OM temperature sensitivity remains allusive. Work in the soil literature indicates that more refractory OM possesses a higher temperature sensitivity due to alleviation of the high activation energies required for decomposition of more complex substrates (Bosatta and Ågren 1999; Davidson and Janssens 2006). Davidson and Janssens (2006) describe that even with relatively constant substrate accumulation, increasing temperatures can rapidly elevate substrate availability for enzymatic reactions, based on Michaelis-Menten kinetics. They describe that in sites characterized by lower availability of their substrate pool, like largely refractory systems, the response of enzymatic Michaelis-Menten constants to increasing temperatures become more relevant, allowing higher Q10 values. The natural wetlands within the Waccamaw watershed possess more complex and aromatic substrates, evidenced by an OM mobilization study in the region (Majidzadeh et al. 2017), and this suggests a similar rationale for their substantial sensitivity to warming. Moving forward, there remains a need to better characterize the relationship between aspects of molecular complexity, lability, and temperature sensitivity of organic matter degradation from varying source waters.

Continued urbanization and other anthropogenic activities will continue to cause DO impairment, and this is likely to be exacerbated in a warmer world. Under current climate predictions, natural ecosystems with higher refractory OM will likely be disproportionality impacted by warmer temperatures, leading to a greater risk of declining DO in these aquatic systems. Current IPCC best estimates suggest a 2.7 – 4.4°C average increase in global temperatures by 2100, for intermediate to very high greenhouse gas emissions (IPCC 2021). Assuming this magnitude of increase to average summer-time high temperatures of approximately 27.5 °C, our DO decay rates at wetland sites could increase by an average of 24.4 ± 8.06% to 41.4 ± 13.9%, dependent on emissions. This projected increase in DO decay is more than double the average calculated increases for both the Waccamaw River proper (11.6 ± 7.46% to 19.3 ± 12.8%) and ponds (10.2 ± 4.63% to 16.7 ± 7.84%), clearly demonstrating the interactions between warming and OM source in OM degradation rates.

It should be noted that coupled with higher temperatures such as those observed in the Waccamaw River, future predictions include higher pulses of extreme precipitation in the coastal zone (Degerman et al. 2013). These storm events will likely increase the flushing of refractory, temperature sensitive OM from wetlands into the river (Day et al. 2008), allowing for higher rates of oxygen demand and thus likely further DO impairment. The Waccamaw River is just one example of a carbon rich system suffering from DO impairment. Coastal blackwater rivers dominated by terrestrially derived, refractory OM are common to the Southeast and Gulf Coasts of the US, as well as other semi-tropical latitudes globally (Meyer et al. 1990), and are likely to suffer similar consequences of increasing temperatures.

Table 2.1 Ancillary measurements categorized of particulate and dissolved measurements.

	PARTICULATE					DISSOLVED			
Type	**Chl-α (μg L^{-1})**	**PC (μM)**	**PN (μM)**	**TPP (μM)**	**PC:PN**	**TDN (μM)**	**TDP (μM)**	**DOC (μM)**	**a_{355} (m^{-1})**
Wetland	12.4 ± 13.5	158.1 ± 87.0	11.59 ± 6.94	0.44 ± 0.13	14.5 ± 3.9	86.23 ± 24.77	0.52 ± 0.32	3790 ± 851	166.6 ± 38.5
Waccamaw River	11.0 ± 10.2	109.9 ± 34.5	9.87 ± 7.25	0.91 ± 0.24	13.4 ± 4.9	66.77 ± 9.67	1.90 ± 0.32	1091 ± 519	45.8 ± 21.8
Pond	27.7 ± 13.8	204.7 ± 100.9	18.55 ± 7.17	1.15 ± 0.55	10.7 ± 2.8	30.77 ± 9.90	0.58 ± 0.26	543 ± 152	13.7 ± 5.1

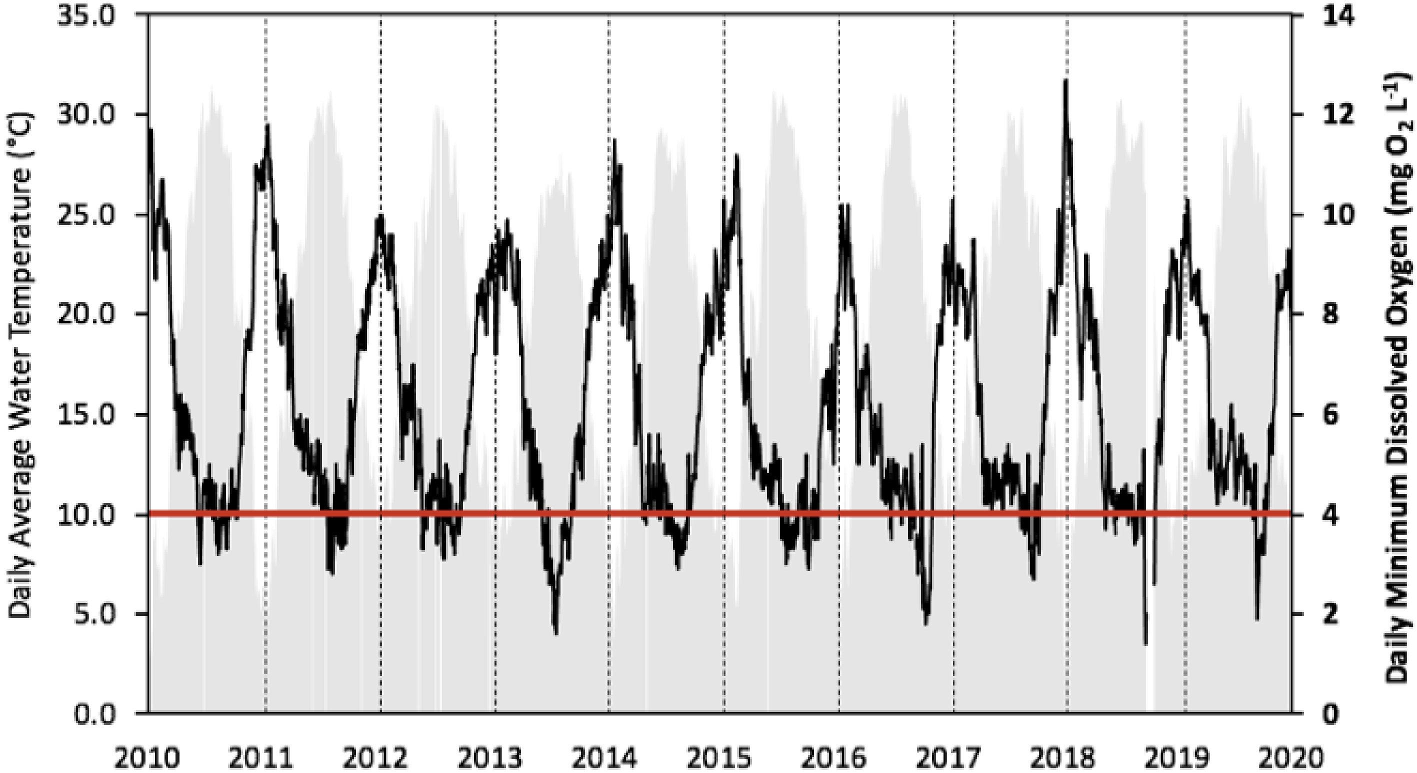

Figure 2.1 Continuous monitoring data from 2010-2020 including: Daily average water temperature (°C; filled gray region) and daily minimum dissolved oxygen (DO) concentrations (mg L^{-1}; solid black line); the red line indicates the threshold of DO impairment as defined by DO < 4 mg L^{-1} set by the South Carolina Department of Health and Environmental Control (SCDHEC, 2014a). Dashed black lines indicate the start of the annual cycle in January. Data gathered from Waccamaw River at Hagley Landing (USGS site number 2110815).

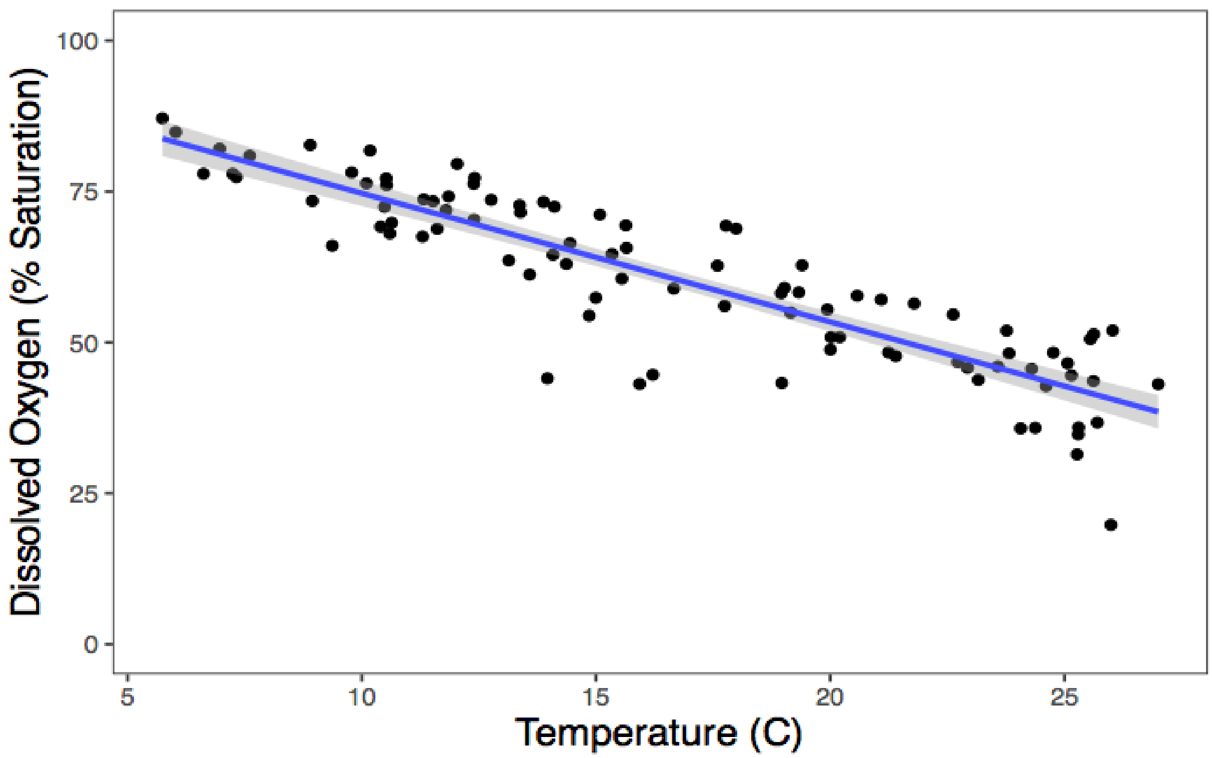

Figure 2.2 Linear regression of average monthly temperature (°C) and dissolved oxygen (% saturation) from 2010-2020 from data collected at USGS site 2110704 (Waccamaw River at Conway Marina at Conway, SC). Blue line represents a significant linear regression relationship ($p = 2.2 \times 10^{-16}$, $R^2 = 0.78$) and gray shaded region represents the 95% confidence interval.

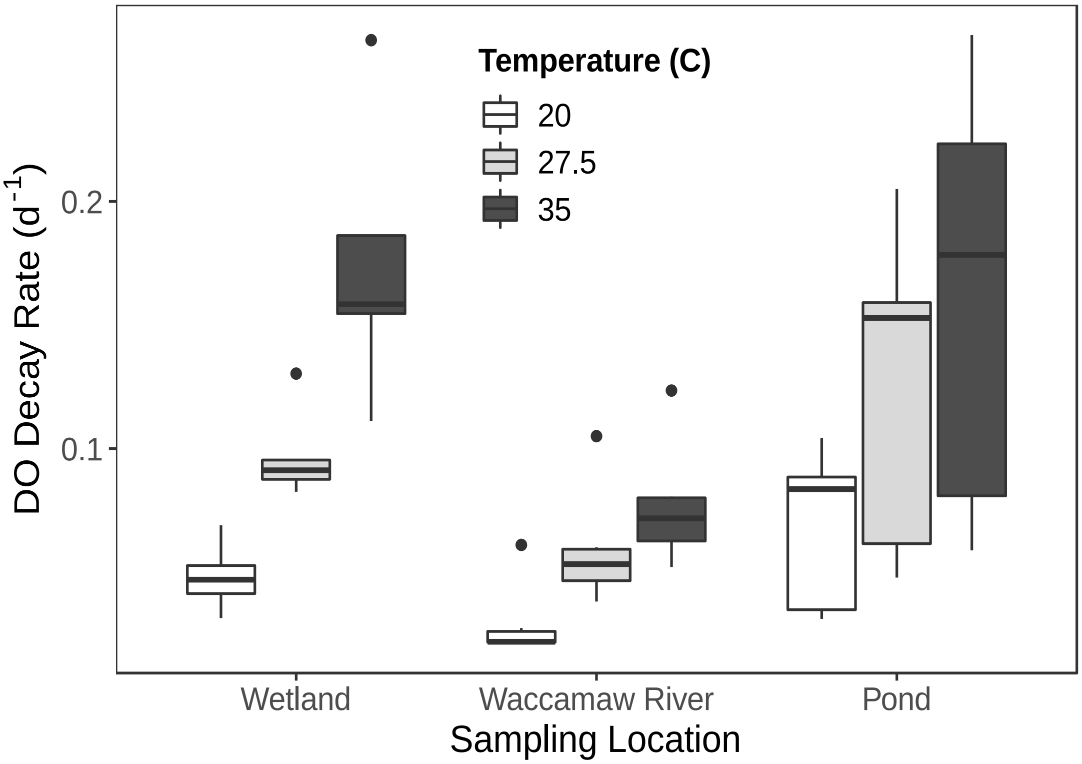

Figure 2.3 Boxplot displaying the decay rates of dissolved oxygen (DO) (d^{-1}) for each site type: forested wetlands, Waccamaw River, and stormwater detention ponds. Plots are grouped by incubation temperature: 20 (white), 27.5 (light gray), and 35°C (dark gray).

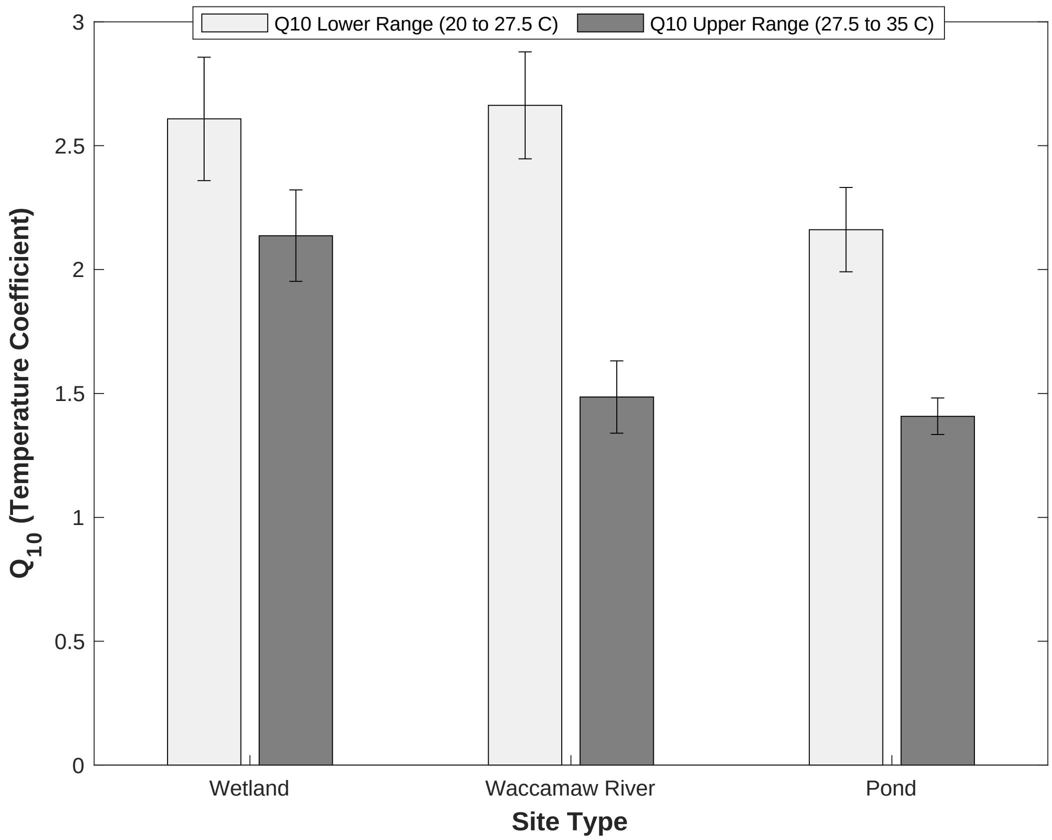

Figure 2.4 Bar graphs depicting the Q_{10} temperature coefficients for each site type (forested wetlands, Waccamaw River, and stormwater detention ponds) at two temperature ranges: Lower range (20 to 27.5 °C, light bars) and Upper range (27.5 to 35 °C, dark bars). Error bars represent standard error across group replication. ANOVA tests yielded no significant differences for the lower range Q_{10} values across sites ($p > 0.05$), however, for the upper range, wetlands yielded significantly higher values ($p < 0.05$).

CHAPTER 3

YEAR 2 – SENSITIVITY TO CHANGING NUTRIENTS

3.1 ABSTRACT

Inland waters are prone to episodic nutrient inputs on both spatial and temporal scales, and this loading may influence the degradation rate of aquatic organic matter (OM) by increasing microbial remineralization rates. Given the importance of OM degradation on global carbon budgets, it is critical that processes influencing OM degradation in a world predicted to be warmer and characterized by flashier nutrient rich precipitation. Utilizing samples collected from the Waccamaw River, South Carolina and various sources within its watershed (stormwater detention ponds, cypress wetland drainages, and upland forest drainages), we explored how changing nutrient, carbon, and temperatures impacted dissolved oxygen (DO) decay rates. Control treatments were combined with amendments in the form of carbon (+C) alone and nutrients (+NP) alone, added to samples prior to dark incubations within two experimental temperature regimes (ambient and ambient + 5°C). Our results indicate that the influence of nutrients on our samples were variable and led to significant ($p < 0.05$) increases in DO decay rates in approximately 42% of the treatments. In contrast, when nutrients were added in combination with warmer temperatures, significant increases in DO decay rates occurred in 83% of our samples. This suggests a synergistic interaction between both warming and nutrient loading to our sites. Thus, in a warmer world where flashier storm events cause episodic flushing of nutrients into receiving waters, both OM degradation and DO decay may be exacerbated.

3.2 INTRODUCTION

The degradation of organic matter (OM) within inland waters contributes a significant amount of CO_2 into the atmosphere (Drake et al. 2018), however, much remains unknown about the complex dynamics controlling the magnitude and rate of OM degradation. The world is also undergoing major changes, influenced by anthropogenic activity, such as climate change and extending urbanization, and thus, it is imperative to understand how these alterations may affect processes like OM decomposition. Jiang and O'Neill (2017) showed that the anthropogenic footprint on Earth is projected to continue, as extensive urbanization (50.4% - 2017) is expected to increase to 60 – 92% by the end of the century, depending on shared socioeconomic pathways. These pathways are a parameter within the Jiang and O'Neill (2017) study of describing potential societal trends, as they relate to development, and overall, they range between slow urbanization in all country groups, faster growth in more wealthy nations, and finally fast urbanization in all analyzed country groups. It is particularly important because in all scenarios urbanization has increased, and as land use changes with urbanization, there are associated changes in OM composition and an increase in the proportion of readily, bioavailable OM (Hosen et al. 2014), suggesting higher rates of OM degradation.

Expanding development is more prominent along shorelines and coastal watersheds, and this expansion of impermeable surface and subsequent runoff can increase the accumulation of limiting nutrients to estuarine environments (Freeman et al. 2019). Although coastal urbanization is more prominent than inland urbanization, inland waters are also prone to the effects of development. For example, harmful algal blooms (HABs) are expected to potentially increase as a result of the interaction between greater inorganic nutrient and organic contaminant loading (Brooks et al. 2015). This finding has been shown to exist within the region of our current study, the South Carolina coastal zone, and HAB prevalence can be traced back to greater production of manmade, stormwater detention ponds (Lewitus et al. 2008), related to greater development within the region. Gold et al. (2019) found that within coastal plain stream systems, similar to the systems within our current study, there is a direct, predictable link between high impervious surface coverage and an associated high export of inorganic nutrients, arguing for future decline in water quality in downstream waters.

A common threat to overall water quality and aquatic resources is widespread dissolved oxygen (DO) impairment (Saari et al. 2018; Bretiburg et al. 2018). Understanding how climate change and urbanization can influence DO is therefore crucial. Dissolved oxygen is consumed during OM degradation via aerobic respiration, and it has been found that shifts in the lability of aquatic OM, resulting from greater quantities of runoff through developed landscapes, can alter the DO dynamics of inland waters (Gold et al. 2020). The relative rate of OM decomposition is also linked to rising temperatures, as biologically mediated metabolic reactions should increase in rate with temperature (Lloyd and Taylor 1994). The exact relationship between microbial respiration and temperature varies, as suggested by Sierra (2012) and Tang et al. (2017), however, a number of soil science studies have suggested that recalcitrant OM is more sensitive to rising temperatures than that of its labile counterpart (Davidson and Janssens 2006; Vanhala et al. 2007; Conant et al. 2008). Investigating the interactive effects of nutrient loading via urbanization, increasing temperatures with ongoing climate change, and overall OM sensitivity to these changes will allow us to further understand the connectivity between OM degradation and DO dynamics moving forward, extending the preliminary investigation of temperature sensitivity as it relates to OM degradation (Chapter 2).

This study aims to further the understanding of nutrient and carbon loading to organic rich blackwater systems and determine synergistic effects of this loading as it occurs simultaneously with warming in the natural world. Systems classified as “blackwater” get their name from their tea-colored water rich in chromophoric dissolved organic matter (CDOM). Although they are commonly studied within the Southeast and Gulf coasts of the United States, these systems exist globally at semi-tropical latitudes (Meyer et al. 1990). Here, we focus on the watershed of the Waccamaw River, a coastal plain blackwater river in coastal South Carolina, U.S. (SCDHEC, 2014a). Although nutrient loading in other coastal regions is associated with eutrophication, blackwater systems can be largely light limited as a result of their color and CDOM levels (Phlips et al. 2000). Thus, nutrients may not necessarily promote large increases in algal blooms if light limitation is a primary driver of success within blackwater bodies (Lewis 1988; Carey et al. 2007). This does not always hold true, however, as work conducted in the Lower St. Johns River suggests that upstream autochthonous algal production, resulting from the loading of urban and labile organic nutrients, may be controlling DO deficits in the lower reaches of the system (Hendrickson et al. 2007). Mallin et al. (2004) has further shown that in blackwater river estuaries, nitrogen loading stimulated phytoplankton production, whereas phosphorus loading stimulated bacterial abundance. Biochemical oxygen demand

(BOD) was elevated in both instances, further exacerbating the already naturally low DO concentrations within blackwater systems. We focus this work on how nutrient and labile carbon loading influences OM degradation rates within the Waccamaw watershed via the alleviation of either readily available substrate limitation or nutrient limitation of microbial growth. We further build upon our previous work (Chapter 2) to explore how the combination of labile carbon and nutrients with rising temperatures impacts OM decay from various sources to the Waccamaw watershed.

3.3 METHODOLOGY

3.3.1 STUDY LOCATION AND SAMPLE COLLECTION

The Waccamaw River, located within the greater Pee Dee Basin, South Carolina, is designated as a "blackwater" river (SCDHEC 2007) characterized by DO impairment (DO < 4.0 mg L^{-1}). Large influxes of highly chromophoric dissolved OM (CDOM) from extensive wetlands (36.9% of watershed land use) are characteristic of this blackwater system, however, it is also undergoing urbanization. Increasing residential and peri-urban development makes up approximately 10.5% of the watershed land use, primarily within the Greater Myrtle Beach, SC area. Greater extent of urbanization within the region contributes substantial anthropogenic OM via stormwater detention ponds (Smith et al. 2018), which are increasingly suspected of elevating BOD in downstream waters (McCabe et al. 2020).

In order to characterize the dynamics of OM degradation rates in response to increasing temperatures and/or loading of nutrients and carbon, surface samples were collected from contrasting watershed sources during the summer season of 2021 (May 30

– July 30). Sample sites were chosen from the Waccamaw River proper, as well as a variety of systems across the Waccamaw watershed including: Stormwater detention ponds within residential communities, wetlands with upland forest drainage, and cypress wetland drainages. The DO impaired lower mainstem of the Waccamaw River was prioritized for sampling. Residential, stormwater detention pond samples were gathered from medium density developments within Georgetown and Horry counties, S.C. Sampled wetlands with upland forest drainage all consisted of wetlands located within Hobcaw Barony, a protected, undeveloped tract of land in the lower Waccamaw watershed (Tufford et al. 2003). Lastly, due to access difficulty, sampled creeks consisting of primarily cypress forest drainage were located outside of the Waccamaw watershed, within the greater Pee Dee Basin, however, these systems remain representative of the cypress drainages found within the Waccamaw watershed. During each sampling event, a Pentair Shurflow 8000 series diaphragm pump was utilized to pump surface water into a 20-liter carboy. DO incubations were conducted at two temperature regimes (ambient [ranged between ~23.5 to 28.5°C] and ambient + 5°C) for replicate samples (n=4) within three different treatments: ambient control, nutrient amendment (+NP), or labile carbon amendment (+C).

3.3.2 EXPERIMENTAL AMENDMENTS AND INCUBATIONS

In order to address the effects of changing conditions within these sites, primary efforts were focused on DO incubations of sample water, from triplicate sites, within each of the four studied groups. After sample collection and return to the laboratory, water from each site was parsed six ways into 2-liter polycarbonate bottles. These six bottles correspond to the three amendment treatments (control, +NP, and +C) at two incubation temperatures (ambient and ambient + 5°C). The bottles were allowed to equilibrate overnight, and the next morning, 10 mL amendments were added. Amendments were adjusted to obtain final concentrations of 100 uM carbon (glucose) serving as a labile carbon addition, 50 uM nitrogen (ammonium nitrate), and 5 uM phosphorus (disodium phosphate). For the control treatment, 10 mL of DI water were added to ensure consistency among treatments. Following the amendment additions, sample water from each of the six 2-liter bottles was divided into replicate (n=4), 300 mL borosilicate biochemical oxygen demand (BOD) bottles. DO concentrations (mg L^{-1}) were tracked approximately every 12 hours for 5 to 7-days utilizing a Wiltrox 1 chemiluminescent oxygen meter for mini sensors (Loligo Systems, Denmark) in the dark. This device allowed for repeated measurement of DO concentration without invasive opening of the bottle, via fiber optic technology. After incubations were complete, relative DO decay rates were calculated utilizing time-series curve fitting of DO measurements, per bottle.

3.3.3 ANCILLARY MEASUREMENTS

Upon initial sample collection, a subsample was collected for the characterization of ambient conditions among sites. Particulate and dissolved pools within these systems were separated with combusted 25 mm diameter, 0.7 um pore size, glass fiber filters (GF/F). Filters were analyzed for particulate carbon (PC), nitrogen (PN), total particulate phosphorus (TPP), and chlorophyll-α (Chl-α) concentrations. The filtrate was analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), nitrite-nitrate (NO_x), nitrite (NO_2), phosphate (PO_4), and ammonium

(NH_4) concentrations, in addition to DOM absorbance spectra. For each analysis, approximately 10% of samples were run in duplicates.

3.3.4 ANALYSES – PARTICULATE FRACTION

Both PC and PN were determined using a PerkinElmer Series II CHNS/O Analyzer 2400. TPP was measured using the ash/hydrolysis method (Aspila et al. 1976, Benitez-Nelson et al. 2007). A Turner Trilogy Laboratory Fluorometer was utilized to quantify Chl-α concentrations within samples, following U.S. EPA method 445.0 (Arar and Collins 1997).

3.3.5 ANALYSES – DISSOLVED FRACTION

Following Benner and Strom (1993), for the determination of DOC, samples were acidified to pH 2 with 10% hydrochloric acid (HCl) and then analyzed via high-temperature combustion (720°C) on a TOC-VCPN Shimadzu Analyzer. TDN, TDP, and NH_4 were analyzed on a SEAL Analytical AA3 nutrient auto analyzer following Standard Methods SM 4500-N C (Eaton et al. 2005). The determination of NO_x, NO_2, and PO_4 concentrations were conducted manually following colorimetric procedures, as described by Murphy and Riley (1962) and Jones (1984). These manually determined concentrations were then cross checked via the nutrient auto analyzer to ensure reliable results. Optical properties of the DOM were analyzed via absorbance spectra, measured from 250-550 nm with a Shimadzu UV-2450 UV-Vis dual beam spectrophotometer. To gain further insight into the optical properties of the DOM pool, absorbance spectra was converted to absorption coefficients at 355 nm (a_{355}) and standardized to DOC (a_{355} : DOC).

3.3.6 STATISTICAL AND DATA ANALYSIS

R (version 4.0.3) was used in order to complete the necessary statistical analyses. DO decay rates were calculated by taking the slope of the natural log transformed decay trends within each bottle. The average decay rate for each temperature and amendment combination was determined by quantifying the mean rate among the four replicates within each sampling location. In order to determine how temperature and nutrient/carbon amendments individually and in unison affected DO decay rates, a multiplicative two-way ANOVA was utilized for each site. Percent increase in DO decay rates were calculated for each temperature and amendment pair to help quantify the overall response of the system to these different treatments. In order to build upon previous work within the region, the effect of temperature increase alone on DO decay rates, was instead determined as Q_{10} temperature coefficients following the van't Hoff equation:

$$Q_{10} = \left(\frac{R2}{R1}\right)^{\frac{10}{(T2-T1)}}$$

Where R1 and R2 correspond to the DO decay rate over the incubation at a given temperature (T) treatment, T1 (lower T) and T2 (higher T). The value of Q_{10} can provide relevant information in regards to how a metabolic rate may respond to 10 degrees of warming, and evidence for site variability of temperature sensitivity may point towards OM source and lability within our studied sites (Chapter 2).

Ambient characteristics and responses in DO decay rates to changing conditions were compared using one-way ANOVA, after normality was tested. If needed, data were log transformed. In instances where ANOVA results yielded significant differences among groups ($\alpha = 0.05$), Tukey HSD post hoc comparisons were conducted.

In addition to our collected data, available data (USGS site 2110704) for the Waccamaw River at Conway Marine (Conway, SC) was explored to investigate patterns in DO trends over the past decade, 2010 up through 2019. In order to elucidate how nutrient loading could influence the oxygen trends within the river, data analysis included a focus on tidally filtered discharge and any potential relationship with DO % Saturation.

3.4 RESULTS

3.4.1 EXPERIMENTAL INCUBATIONS AND RESPONSE TO VARIED CONDITIONS

Our experimental incubations varied in their response as a function of both incubation conditions (temperature and amendment) and site type. Table 3.1 quantifies at which stations there were significantly higher ($p < 0.05$) DO decay rates relative to that at our control treatment within ambient temperatures. Overall, nutrient amendments yielded the lowest proportion of significant sites (~42%), followed by increasing temperature to ambient + 5°C (~58%). When the nutrient amendment was made to bottles in the warmer incubator and we induced the combined nutrient/temperature effect, the proportion of sites with significantly higher DO decay rates, relative to the ambient control, increased (~83%). In all studied sites, the amendments of carbon alone and in unison with warming yielded complete significance across sites (100%). Looking at Fig. 3.1, it is apparent that our four site types shared a rather consistent response distribution, measured as DO decay rate percent increase for the changing conditions relative to ambient control averages, however, some variability can be noted. Within the upland drainages, the addition of carbon, albeit significant in all three replicates ($p < 0.05$), yielded a smaller relative increase (51.8 ±

16.8%) in the DO decay rate relative to the other sites (134.0 ± 17.1% to 163 ± 42.8%). The effects of temperature and nutrients alone seemed to not promote large responses within the stormwater detention ponds, as temperature was significant at only one of the three triplicate sites (W5-0709, $p < 0.05$), and nutrients were never significant ($p > 0.05$). These trends are apparent in Fig. 3.1, as the average % increase in DO decay rates under warming conditions were smallest within stormwater detention ponds (24.0 ± 4.09%) and nutrients yielded the second smallest response within stormwater detention ponds (22.3 ± 18.0%). In all sites, the combined effect of temperature and nutrients (T+NP) yielded higher average responses across sites relative to either the influence of temperature or nutrients alone. The same apparent trend can be noted when comparing the combined amendment of temperature and carbon (T+C) to each component individually.

3.4.2 AMBIENT CONDITIONS – PARTICULATE FRACTION

Overall, data analyses of the particulate fraction have shown that our data exhibits some differences across sites when considering the ambient conditions at the time of sample collection, but overall within site replicates, variation yielded wide spreading data points (Fig 3.2). To begin, the PC among our sites did not differ significantly ($p > 0.05$), however, the largest average values were within stormwater detention ponds (199.52 ± 40.76 uM), which were more than double the average values within upland drainages (82.86 ± 79.88 uM). In addition to PC, our TPP data suggests no significant differences ($p > 0.05$) among the differing site types, although like PC, TPP values were once again lowest for the upland drainages (0.25 ± 0.19 uM), while stormwater ponds remained quite high (1.46 ± 0.64 uM). Similar to the previous two measurements, PN shared a similar trend, but instead, was significantly different across groups, where stormwater detention ponds were larger than both cypress wetlands ($p = 1.47 \times 10^{-2}$) and upland drainages ($p = 1.53 \times 10^{-3}$). The use of PC, PN, and TPP molar concentrations allowed for the determination of stoichiometric ratios, and these distributions are included in Fig. 3.2. C:N molar ratios display a significantly higher ratio ($p = 2.54 \times 10^{-2}$) within the urban drainages (18.84 ± 5.78) in comparison to the stormwater ponds (9.22 ± 0.39). In terms of the relative stoichiometric ratio between the molar concentrations of C:P, the only instance of significance ($p = 2.75 \times 10^{-2}$) was between the highest value within upland drainages (309.46 ± 88.85) and the lowest value within the Waccamaw River (105.48 ± 34.81). Albeit not statistically significant, the C:P ratios for cypress wetlands and stormwater detention ponds were both approximately half the average ratio for upland drainages. Looking at N:P ratios, no significant differences existed ($p > 0.05$), but both the upland drainages and stormwater ponds possessed values of 16.55 ± 1.12 and 16.83 ± 6.94 respectively, while the cypress drainages and Waccamaw river possess lower values of 8.94 ± 2.42 and 9.29 ± 3.97. Lastly, ambient concentrations of chlorophyll-a within stormwater detention ponds (59.97 ± 15.33 ug L^{-1}) were approximately 10 and 20-fold larger than the cypress wetlands (5.60 ± 1.10 ug L^{-1} $p = 2.12 \times 10^{-2}$) and upland forest drainages (3.09 ± 1.17 ug L^{-1}) respectively, whereas, the Waccamaw River was extremely variable (44.51 ± 50.01 ug L^{-1}).

3.4.3 AMBIENT CONDITIONS – DISSOLVED FRACTION

Like the particulate fraction, the dissolved fraction underwent a variety of data analyses, and our results suggest wide variability of ambient conditions within and between site types (Fig. 3.3). DOC showed a clear trend in that upland drainages (5266.22 ± 267.04 uM) were significantly higher than all other site types across the board ($p < 0.05$) and approximately 10-fold larger than stormwater detention ponds (527.82 ± 127.78 uM). TDN was largest within the upland drainages (99.59 ± 21.78 uM), which were significantly higher ($p < 0.05$) than both the Waccamaw River (55.82 ± 2.95 uM) and stormwater ponds (33.75 ± 3.11 uM). Cypress wetlands possessed a TDN value (68.72 ± 12.01 uM) between the upland and river sites, and it also was significantly higher than stormwater ponds ($p = 4.10 \times 10^{-2}$). TDP showed a different trend and concentrations were largest within the cypress drainages (2.81 ± 1.08 uM), which was significantly higher than the uplands (0.44 ± 0.10 um; $p = 4.79 \times 10^{-3}$) and stormwater ponds (0.53 ± 0.27 uM; $p = 6.74 \times 10^{-3}$). Inorganic nitrogen molecules were combined for the measure of Dissolved Inorganic Nitrogen (DIN), and our results show that the Waccamaw River (24.63 ± 7.09 uM) yielded significantly higher concentrations ($p < 0.05$) than both the upland drainages (5.96 ± 0.667 uM) and stormwater detention ponds (2.84 ± 1.11 uM). The independent trends between these inorganic forms (NO_x, NO_2, and NH_4) are variable and depicted in Fig. 3.3. PO_4 serves as our primary measure of DIP, and this trend mirrored that of the TDP pool, in that the cypress wetlands possessed values (2.03 ± 0.95 uM) significantly larger than both upland drainages (0.28 ± 0.14 uM; $p = 2.05 \times 10^{-2}$) and stormwater ponds (0.17 ± 0.15 uM; $p = 4.04 \pm 10^{-3}$ uM). Our final measurement normalized optical absorbance at 355 nm to

DOC concentrations (a355:DOC), and results indicate no significant relationships between these measures, although, average values for the upland drainages (0.0375 ± 0.0030) were the largest, whereas, stormwater ponds were the lowest (0.0241 ± 0.0210).

3.4.4 WACCAMAW RIVER – CONTINUOUS MONITORING PATTERNS

The continuous monitoring data from USGS site 2110704, the Waccamaw River at Conway Marina (Conway, SC), displayed a variable relationship between DO (% saturation) and tidally filtered discharge ($ft^3\ s^{-1}$) as a function of temperature (Fig. 3.4). To account for the potential skewness of our findings toward the most extreme, rare storm events, these analyses did not include the monthly averages during Hurricane Florence, Joaquin, and Matthew. It is apparent that this data shows a lack of a relationship in DO (% saturation) as discharge increases when temperatures are less than 20°C ($p > 0.05$). On the other hand, however, when temperatures are above 20°C linear regression analyses suggest a significant negative relationship ($p = 1.10 \times 10^{-7}$) between DO (% saturation) and tidally filtered discharge ($ft^3\ s^{-1}$).

3.5 DISCUSSION

3.5.1 VARIABLE SITE SENSITIVITY TO EXPERIMENTAL AMENDMENTS

The influence of nutrients was the weakest compared to our other experimental treatments, however, there was still a significant response in our DO decay rates 42% of the time. It is important to keep in mind that our incubations were performed in the dark, and thus we can interpret that a significant response likely alleviated limitation of microbial community growth. It has been shown in previous studies that nutrients, specifically phosphorus additions, can limit bacterial abundance within organic-rich blackwater systems (Mallin et al. 2004), however, our systems where a significant nutrient response was determined had higher DIP values relative to their within site replicates where no significant response was noted. Work conducted on the periphyton communities within a blackwater lake suggest a possible co-limitation of phosphorus and biologically available carbon substrate, especially under light deficiencies, when considering the heterotrophic components of the periphytic community (Sanches et al. 2018). Our results lack a clear relationship between ambient concentrations of nutrients and sites in which DO decay rates significantly increased, however, the literature is not clear on blackwater nutrient response patterns as different studies have noted different findings. Both Mallin et al. (2004) and Sanches et al. (2018) determined a response of phosphorus loading on heterotrophic growth, however, Guariento et al. (2011) found that heterotrophic periphytic organisms displayed no response to added nutrients under light-limiting conditions and under high-light conditions, nutrients had a negative effect due to autotrophic organisms outcompeting for nutrients. The above studies acknowledge that although the carbon pool within these blackwater systems is large, it is largely refractory and unavailable, and our work may support this claim because in 100% of our sites, the addition of a labile, more bioavailable carbon source (glucose) prompted significant increases in the rate of DO decay. It is important to note that much of the nutrient and carbon loading into the cypress and upland drainages is largely in the form of refractory DOM, thus the influence of glucose should prompt a response and is largely unsurprising. With that being said, however, viewing Fig. 3.1, it is apparent that although glucose significantly increased all DO decay rates, the relative percent increase in this rate was not uniform throughout sites, and it seemed stunted within the upland drainages, which are characterized as our most refractory, organic-rich system of the four.

The soil science field has displayed a number of studies that explain higher temperature sensitivities and relative shifts in OM degradation for sites where this large but refractory carbon pool is present (Davidson and Janssens 2006; Vanhala et al. 2007; Conant et al. 2008). Their suggestion is consistent in our data because the site types quantified by larger DOC values (cypress and upland drainages) yielded significant temperature responses. In addition to this, the significance of nutrients (42%) and temperature (58%) alone was exacerbated to 83% of our sites when both occurred simultaneously, suggesting a potential synergistic effect between warming and nutrients. Due to the consistent significance within our carbon amendment (100%), the effects of temperature and carbon in unison (100%) do not tell us much, however, viewing Table 3.1, it is apparent that the p-values for each site were largely smaller when accounting for temperature, thus further supporting the synergistic effects of loading and warming together.

3.5.2 AMBIENT CONDITION VARIABILITY

Overall, the above trends noted in the significance of our various experimental treatments is complicated and does not seem to relate specifically to any one ambient condition, however, this displays the shear variability within our sites and at a minimum tells us that nutrient loading can influence DO decay rates within this region. Although, they do not fully help us elaborate on the intricacies of nutrient influence, our ambient data upon sample collection does help provide us a perspective into how these sites may differ in their typical characterization. For example, the particulate fraction typically displayed larger values within the stormwater detention ponds, and this is expected, as much of their OM makeup is composed of autochthonous algal production, further supported by the high levels of chlorophyll-a within these sites. Oppositely, the upland forest drainages showed a small relevance of particulate nutrients and carbon compared to other systems, but instead a more dominant dissolved pool, particularly in the total dissolved pools and not the inorganic pools, suggesting a dominance of organic constituents. Both of these findings are consistent with previous studies, as stormwater pond OM is typically autochthonous algal production (Williams et al. 2013), whereas, upland forested wetland drainages are prone to high levels of organic nutrient loading (Pellerin et al. 2004). Our data further supports the claim that variable OM source and lability may be influencing the sensitivity of OM degradation to changes in both temperature and nutrients, as our particulate molar ratios of C:N suggest values more consistent with terrestrial vegetation (C:N of 20+) in upland drainages and lower values (C:N between 4 and 10) more consistent with algal production within stormwater ponds (Meyers 1994).

3.5.3 WACCAMAW RIVER CONTINOUS MONITORING

The USGS data provided insight on the conditions and trends experienced within the Waccamaw River. As suggested by our own data, there seemed to be a nutrient affect only 42% of the time, however, when warming was induced in unison with nutrient loading, we experienced about 83% significance among sites, and thus these two factors combine for a synergistic effect. Upon viewing Fig. 3.4, it is apparent that the same could be said for the continuous monitoring within the Waccamaw River, as the trend between tidally filtered discharge and DO saturation varied as a function of temperature. The use of tidally filtered discharge was chosen because it can be assumed that during large storm events, there will be episodic flushing and loading of nutrients into receiving waters (McClain et al. 2003). Thus, our analysis of the continuous monitoring data tried to infer how nutrient loading has influenced the % saturation of DO within the Waccamaw River. Overall, our findings show that when temperatures were high (>20°C), there is a significant negative relationship between discharge and DO saturation ($p = 1.10 \times 10^{-7}$), which suggests that when nutrients are loading into the system and warm temperatures are present, we can expect sites to increase in their DO decay rates, inferring higher rates of OM degradation. In summary, our findings between both a decade of continuous monitoring and our experimental manipulations suggest that nutrients alone cannot serve as a reliable predictor of OM degradation sensitivity, however, when paired with increases in temperature, it is likely that our system will increase in its degradation rate and overall DO consumption, further exacerbating DO impairment. These findings are quite relevant, as climate change predictions suggest both warming temperatures and flashier storm events (Degerman et al. 2013) and more work should be conducted to elaborate on the intricacies surrounding the influence of nutrient loading on the sensitivity of OM degradation within organic-rich systems like the blackwater regions within the southeastern US.

Table 3.1 Statistical significance of experimental amendments relative to ambient control.

STATION	TYPE	+ NP	+ C	TEMP	TEMP & NP	TEMP & C
R1-0530	Waccamaw River	0.593	2.50×10^{-6} *	0.697	3.97×10^{-4} *	1.99×10^{-7} *
R2-0530	Waccamaw River	0.855	3.91×10^{-6} *	0.962	0.198	2.77×10^{-8} *
R3-0730	Waccamaw River	2.32×10^{-2} *	1.35×10^{-3} *	0.135	9.81×10^{-4} *	3.16×10^{-6} *
W1-0608	Cypress Wetland	2.83×10^{-3} *	1.40×10^{-12} *	2.59×10^{-6} *	4.41×10^{-12} *	2.53×10^{-14} *
W2-0608	Cypress Wetland	0.874	3.04×10^{-14} *	2.12×10^{-5} *	5.25×10^{-7} *	2.53×10^{-14} *
W3-0623	Cypress Wetland	2.44×10^{-8} *	2.53×10^{-14} *	3.26×10^{-14} *	2.53×10^{-14} *	2.53×10^{-14} *
W4-0623	Upland Drainage	8.12×10^{-7} *	9.52×10^{-6} *	2.03×10^{-8} *	1.07×10^{-11} *	7.17×10^{-14} *
W5-0709	Upland Drainage	7.65×10^{-5} *	2.05×10^{-7} *	5.81×10^{-9} *	4.18×10^{-12} *	2.53×10^{-14} *
W6-0720	Upland Drainage	0.798	1.23×10^{-3} *	5.11×10^{-4} *	5.59×10^{-5} *	2.07×10^{-10} *
P2-0709	Stormwater Pond	0.995	6.99×10^{-6} *	0.952	0.399	8.68×10^{-10} *
P8-0720	Stormwater Pond	0.122	3.32×10^{-13} *	5.17×10^{-4} *	1.54×10^{-4} *	2.53×10^{-14} *
P4-0730	Stormwater Pond	0.123	5.90×10^{-8} *	0.491	2.69×10^{-6} *	8.55×10^{-13} *

* Denotes statistical significance relative to ambient control

	+ NP	+ C	TEMP	TEMP & NP	TEMP & C
Proportion of Sites with Significant Results	5/12	12/12	7/12	10/12	12/12
% of Total Sites	~42%	100%	~58%	~83%	100%

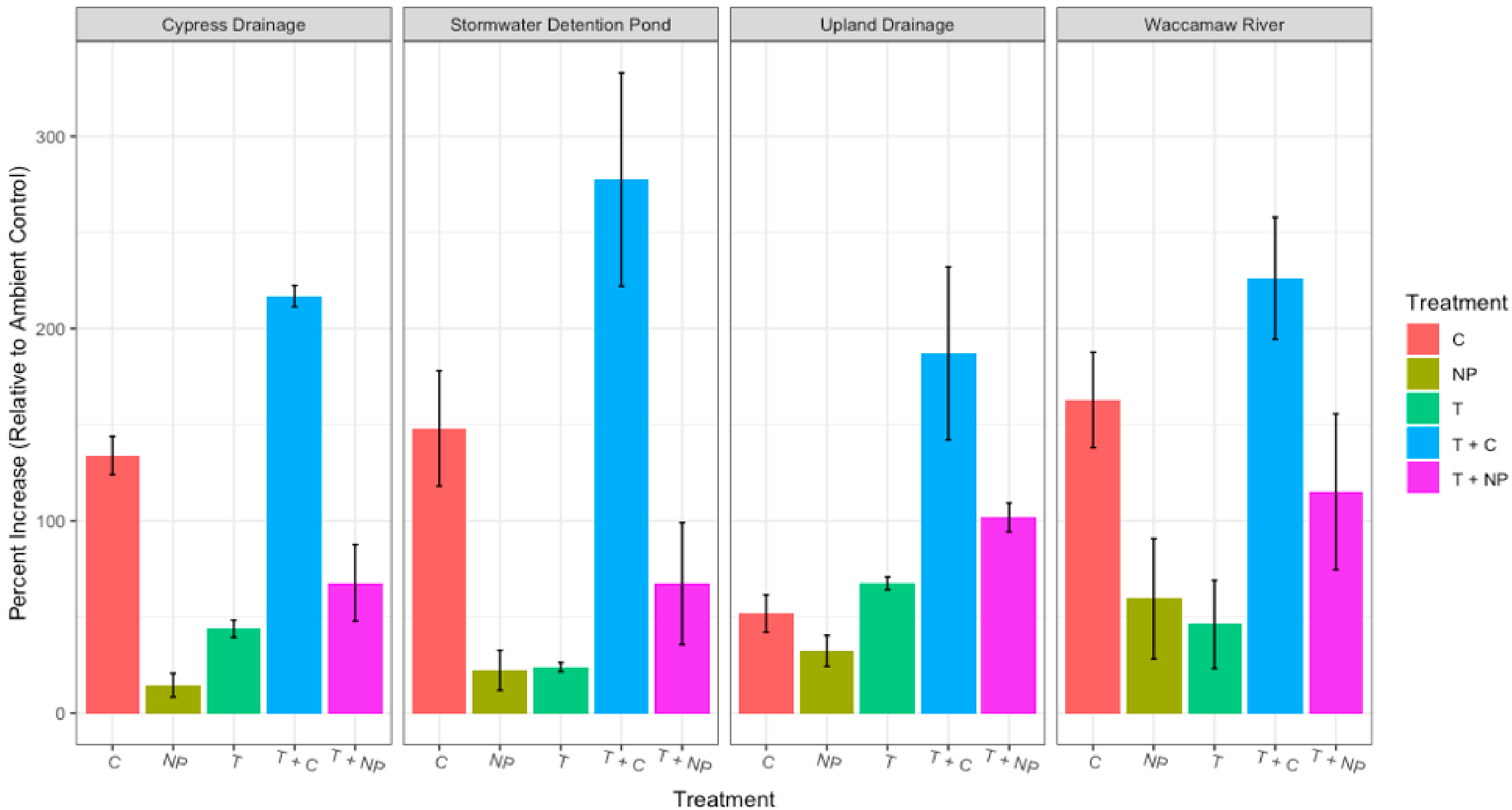

Figure 3.1 Percent Increase of DO decay rates relative to ambient control for each of the experimental treatments (Carbon – C, Nutrients – NP, Temperature – T, Temperature and Carbon – T+C, and Temperature and Nutrients, T+NP). Subplots are grouped by study site type: cypress drainage, stormwater detention pond, upland drainage, and the Waccamaw River proper. Error bars represent the standard error based on the triplicates within each site type.

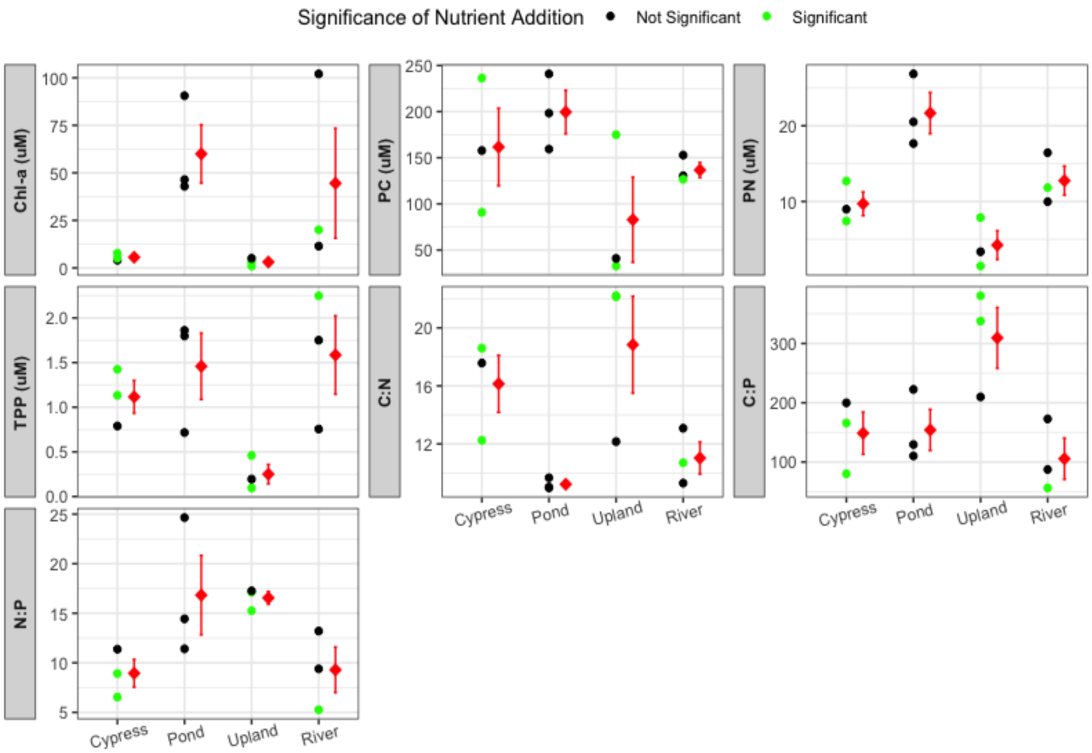

Figure 3.2 The particulate fraction categorized by site type. In each subplot, replicate dissolved oxygen (DO) decay rates are displayed, with symbol colored denoting significant differences higher ($p < 0.05$) relative to ambient controls (green = significant, black = not significant). Site means and standard errors are displayed in red to the right of the individual site points. The subplots display: chlorophyll-a (Chl-a), particulate carbon (PC), particulate nitrogen (PN), and total particulate phosphorus (TPP), particulate molar ration of carbon : nitrogen (PC:PN), carbon : phosphorus (PC:TPP), and nitrogen to phosphorus (PN:TPP).

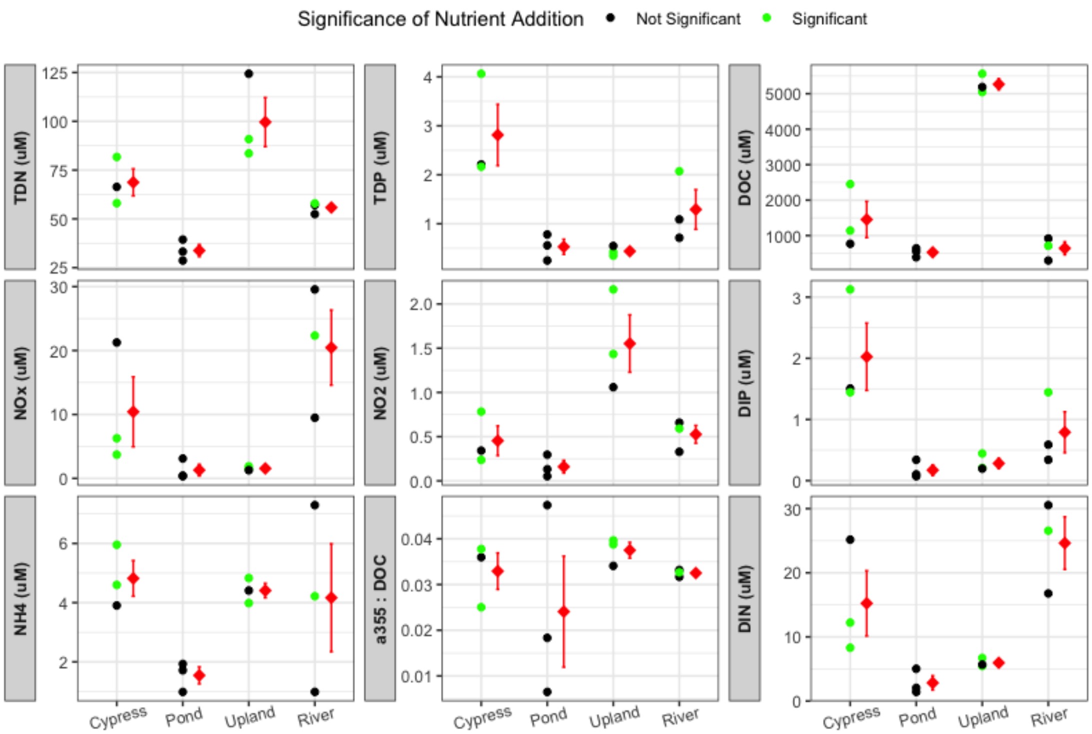

Figure 3.3 The dissolved fraction categorized by site type. In each subplot, replicate data points are displayed and colored by whether nutrient additions yielded significantly higher ($p < 0.05$) dissolved oxygen decay rates relative to the rates experienced in our ambient control treatments (green = significant, black = not significant). In addition to the individual data points, each site mean and standard error are displayed in red to the right of the individual site points, allowing a view of within site variability for some of these parameters. The subplots display: total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), dissolved organic carbon (DOC), nitrite + nitrate (NO_x), nitrite (NO_2), phosphate as dissolved inorganic phosphorus (DIP), ammonium (NH_4), absorption coefficient at 355 nm normalized to DOC (a355 : DOC), and dissolved inorganic nitrogen (DIN).

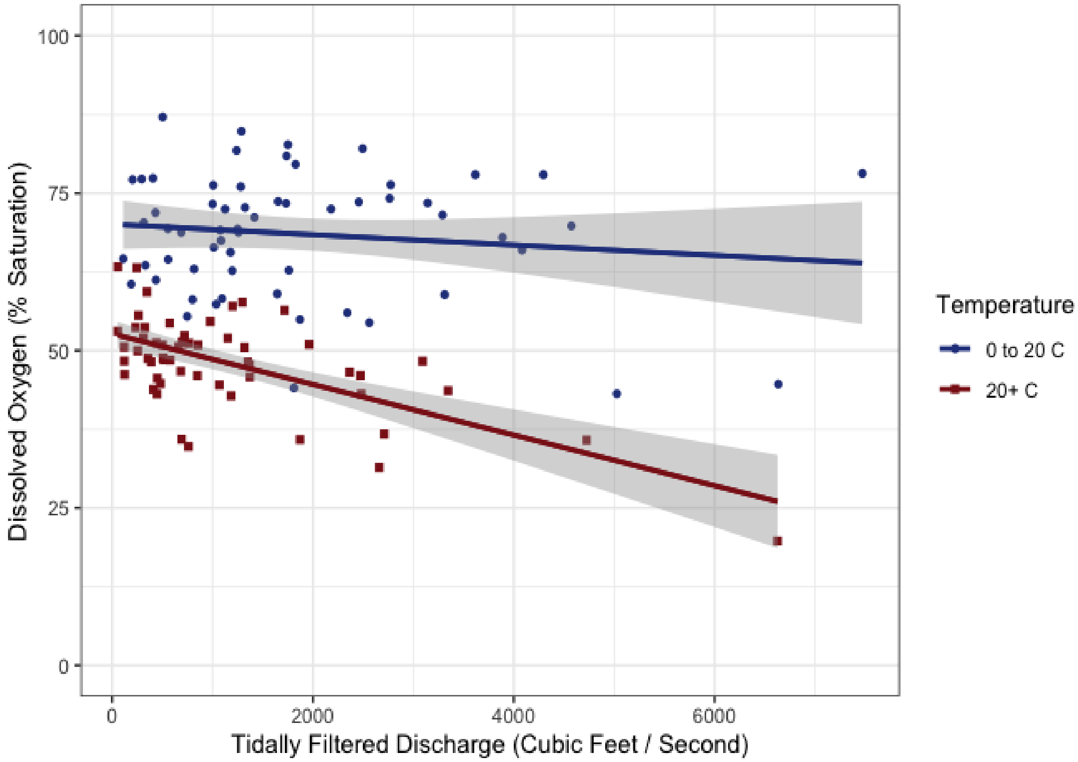

Figure 3.4 Dissolved oxygen (% saturation) versus tidally filtered discharge (ft^3 s^{-1}). Each data point represents monthly averages for the Waccamaw River at Conway Marina (Conway, SC), USGS site 2110704. Data is conditionally grouped by temperature and is either placed below 20°C (blue circles) or 20+°C (red squares). The shaded region represents the 95% confidence interval. When temperatures are below 20°C, no significant relationship is present. For data where temperatures exceed 20+°C, a significant negative relationship exists ($p = 1.10 \times 10^{-7}$). To account for the influence of rare, extreme storm events, this analysis removed monthly averages directly impacted by Hurricanes Florence, Joaquin, and Matthew.

CHAPTER 4

CONCLUSION

Our results indicate that both nutrient loading as well as rising temperatures may serve as a mechanism of elevated DO decay rates, likely through increases in the degradation of aquatic OM. This study is among the first to show a relationship between temperature sensitivity as it relates to OM source and lability within inland systems. Our study supports the suggestion of a temperature sensitivity relationship from the soil science community (Davidson and Janssens 2006; Vanhala et al. 2007; Conant et al. 2008), in which, refractory OM shows larger temperature sensitivities (measured as Q_{10} temperature coefficients) than labile OM. This finding strengthens the threat of climate change for blackwater systems around the globe, that are characterized as organic-rich, because the bulk pool of carbon is largely refractory and more sensitive to rising temperature, exposing a natural weakness within these systems.

Although ambient conditions did not fully correspond to when a site would display a significant nutrient response, it is still notable that for certain stations within the region (42%), nutrients were capable of inducing higher rates of DO decay, and further work should be focused on determining what site conditions may correspond to a nutrient response. Overall temperature had a larger independent effect on our study sites (53%), and thus it may be a stronger mechanism within the Waccamaw River watershed driving DO impairment. That being said, however, the world is not predicted to just get warmer or be solely prone to flashier storm events, but these trends are forecasted to correspond

(Degerman et al. 2013). Our experimental incubations and the continuous monitoring data for the Waccamaw River suggest a linked synergistic interaction between rising temperatures and nutrient loading, and thus, it is expected that with ongoing climate change, overall rates of OM degradation can be expected within this system, further worsening the characteristic DO deficits of this watershed.

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