Master's Theses and Capstones   Student Scholarship

Winter 2020

DYNAMIC DISSOLVED ORGANIC MATTER RESPONSE TO INCREASES OF ENERGY AVAILABILITY IN FORESTED HEADWATER STREAMS

Katherine Xiomara Perez-Rivera
University of New Hampshire, Durham

Follow this and additional works at: https://scholars.unh.edu/thesis

DYNAMIC DISSOLVED ORGANIC MATTER RESPONSE TO INCREASES OF ENERGY

AVAILABILITY IN FORESTED HEADWATER STREAMS

BY

KATHERINE X. PÉREZ RIVERA

B.S. Environmental Sciences, University of Puerto Rico, Río Piedras, 2018

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

in

Natural Resources

December, 2020

DYNAMIC DISSOLVED ORGANIC MATTER RESPONSE TO INCREASES OF ENERGY

AVAILABILITY IN FORESTED HEADWATER STREAMS

BY

KATHERINE X. PÉREZ RIVERA

This thesis was examined and approved in partial fulfillment of the requirements for the degree
of Master of Science in Natural Resources by:

Thesis Director, Dr. William H. McDowell, Professor
Natural Resources and the Environment

Dr. Adam S. Wymore, Research Assistant Professor
Natural Resources and the Environment

Dr. Jessica Ernakovich, Assistant Professor
Natural Resources and the Environment

On August 5th, 2020

Approval signatures are on file with the University of New Hampshire Graduate School.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

DYNAMIC DISSOLVED ORGANIC MATTER RESPONSE TO INCREASES OF ENERGY

AVAILABILITY IN FORESTED HEADWATER STREAMS

By

Katherine X. Pérez Rivera

University of New Hampshire

Forested headwater streams receive inputs of dissolved organic matter (DOM) that can be transformed by biota as they are transported downstream. Biotic uptake of ambient DOM is difficult to assess through direct experimentation, as the fate of a specific organic compound added to a stream is unlikely to mirror that of the ambient DOM pool. Here we examined the dynamics of DOM by using a hybrid approach that combines uptake metrics of a simple organic compound with a direct assessment of the effects of adding this presumably labile organic carbon source (acetate) on the ambient DOM pool. We proposed that adding acetate could result in three different types of responses in the ambient DOM pool: inert, production and priming. Our results provide evidence that each of these scenarios can occur in small streams, but among all our study sites the overall response of ambient dissolved organic (DOC) to added acetate was production of additional DOC, while the dissolved organic nitrogen (DON) pool was unresponsive to addition of acetate (inert response).

CHAPTER 1: INTRODUCTION

Dissolved organic matter (DOM) plays a critical role in both aquatic and terrestrial

ecosystems (Van Stan and Stubbins 2018), serving as the major link between the two. DOM is

the largest pool of organic matter present in aquatic systems (Mulholland 2003; Álvarez-Cobelas

et al. 2012; Kaplan and Cory 2016) and is responsible for regulating biogeochemical reactions

which influence the concentration and export of other solutes (Fisher and Likens 1972; Vannote

et al. 1980; Prairie 2008; Catalán et al. 2018; Seybold and McGlynn 2018). Our knowledge

regarding in-stream processing of DOM is limited, especially for understanding the ecological

and biogeochemical controls on the highly diverse pool of ambient DOM. Most research efforts

that have addressed DOM dynamics in streams have done so by studying the fate of added

organic solutes focusing on single labile low molecular weight compounds (Brookshire et al.

2005; Rodríguez-Cardona et al. *In Press)*, ^{13}C labeled DOM (Kaplan et al. 2008), or from a

single source such as leaf leachate (Bernhardt and McDowell 2008; Wymore et al. 2018). While

the addition of a specific compound or a group of compounds from a specific source provides

valuable information on their uptake, compound-specific removal from streams proves to be a

poor proxy for the dynamics of ambient DOC in streams and rivers (e.g., Mineau et al. 2016).

Alternatively, other studies have examined how ambient DOM concentrations,

stoichiometry, or composition predict rates of nitrate uptake (e.g., Rodríguez-Cardona et al.

2016, Wymore et al. 2016, Rodríguez-Cardona et al. 2020). Inferences regarding the effects of

DOM concentrations and composition on other biogeochemical cycles must be treated with

caution, however, since measures of DOM in this context are essentially a post-hoc correlative

assessment of those substrates that either did not participate in, or are a product of, the reaction

in question (Wymore et al. 2019). As such, there is little experimental work that directly addresses the ambient DOM pool as a whole and how it responds to changes in the availability of readily available forms of energy or nutrients (Wymore et al. 2015; Catalán et al. 2018). In particular, the mechanism regulating the priming of the aquatic DOM pool remains controversial and unresolved (Catalán et al. 2015). Priming has been shown to increase the decay of DOC in rivers, however, the drivers of this response remain unknown (Hotchkiss et al. 2014).

In this study we develop a novel hybrid approach to understanding DOM dynamics that combines measurements of the disappearance of a specific organic solute (acetate) coupled with an assessment of the solute's impacts on the ambient DOM pool. This is similar to previous studies of acetate uptake (Johnson and Tank 2009; Johnson et al. 2009; Seybold and McGlynn 2018) with a crucial difference: we measure the acetate uptake directly, rather than assume its uptake can be measured by changes in overall DOC concentrations (e.g. Johnson et al 2009, Johnson and Tank 2009). Simultaneously, we also track changes in concentration of DOC and dissolved organic nitrogen (DON), which represent two different ways to measure the DOM pool. The indirect manipulation of the highly diverse DOM pool through the addition of compound specific solutes can provide unique insights into the biogeochemical controls on DOM (Lutz et al. 2012; Wymore et al. 2015; Rodríguez-Cardona et al. *In Press*) And while similar to the approach of Lutz et al. (2012) where both acetate and DOC concentrations were assessed, our experimental design creates a wide and dynamic range of acetate concentrations and assesses changes in both the added solute as well as the ambient pool of DOC and DON throughout the experimental manipulation.

Our fundamental question is: ***How does the ambient pool of dissolved organic matter (DOM) respond to increased availability of labile C in streams of widely differing DOM***

concentrations? With this overarching question we test three specific hypotheses about ambient

DOM based on past research: the ambient DOM pool is refractory and unaffected by an

additional energy source (inert response); concentrations of DOC decrease in response to

additional labile carbon (priming response, Catalán et al. 2015); or concentrations of DOC

increase in response to added labile carbon (production response, similar to what has been

proposed for the sources of most soil organic matter where DOC is microbially derived;

Kallenbach et al. 2016). These three categories of response mirror the categories proposed by

Wymore et al. (2015) to describe the response of DON to additional inorganic nitrogen. To test

our hypotheses, we conducted short-term whole-stream enrichments with acetate and examined

both acetate uptake dynamics and the response of ambient DOM to the additional energy source.

CHAPTER 2: METHODS

Study sites

We selected four forested headwater streams across New England (USA) based on a wide range of DOC and DON concentrations (2.43 – 43.0 mg C L-1 and 0.08 – 0.67 mg L-1, respectively; Table 1). This gradient in DOM (DOC and DON) allows us to determine how ambient and background concentrations affect the response of DOM to changes in the availability of labile C. We also selected sites based on concentrations of dissolved inorganic nitrogen (DIN: NO3- + NH4+) (Table 1). Low concentrations of DIN are required to calculate concentrations of DON with precision, as it cannot be measured directly and must be calculated as the difference between total dissolved nitrogen (TDN) and DIN (Equation 1).

Equation 1:

$$[\boldsymbol{DON}] = TDN - (NO_3 + NH_4)$$

In-situ manipulation of labile DOC

Short-term pulse additions of acetate were conducted at each study site (16 total additions) from May to October 2019. Acetate (CH3COO-) was added as a labile form of DOM, given that it is a compound that is easily assimilated by stream microorganisms and found naturally at very low concentrations in freshwater ecosystems (Johnson and Tank 2009; Johnson et al. 2009), facilitating its manipulation (Johnson et al. 2009; Mineau et al. 2016; Catalán et al. 2018). Prior to each addition, discharge (Q) was measured either a day before or during the same day of the manipulation. Discharge was determined using a dilution gauging approach (Kilpatrick and Cobb 1985) where sodium chloride (NaCl) was mixed with stream water and added to the experimental reach and changes in conductivity were logged every 5 seconds using

a HOBO conductivity data logger (Onset, Bourne, MA). The measured Q was used to determine the dry mass of acetate and NaCl needed to elevate by approximately 2X the background concentration of the respective DOC and Cl in each study site.

Acetate was added along with NaCl, using short-term pulse additions that allow assessment of the fate of added and unmanipulated solutes across a gradient of manipulated solute concentrations (Tank et al. 2008; Covino et al. 2010; Wymore et al. 2015). The general criteria used to select experimental reaches consisted of avoiding areas with tributaries and large pools. As the pulse of added solutes was transported downstream, it was tracked through changes in conductivity using a field meter (YSI ProDSS, Yellow Spring, OH). Once the pulse of solutes arrived at a downstream sampling station, samples were collected throughout the breakthrough-curve (BTC). The number of samples collected during each addition varied between 25 and 32 samples. Prior to each addition background samples were collected in duplicates (2 upstream and 2 downstream). Sampling through the BTC allows us to assess the responses of the ambient DOM pool to various levels of the added acetate as well as calculate acetate uptake (Tank et al. 2008; Covino et al. 2010; Wymore et al. 2015).

Chemical analyses

Samples collected during field manipulations were filtered through pre-combusted glass fiber filters (0.7µm; Whatman GF/F) into 60 mL acid washed HDPE bottles and amber vials (for DOM optical properties). Samples were placed in a cooler with ice until returned to the lab and then frozen or refrigerated until analysis. Samples were analyzed for concentrations of NO3-, NH4+, total dissolved nitrogen (TDN), dissolved organic carbon (DOC), specific ultraviolet absorbance (SUVA254), and major cations and anions analyses were conducted in the Water

Quality Analysis Laboratory at the University of New Hampshire. Samples were analyzed for TDN and DOC using high temperature catalytic oxidation (Shimadzu TOC-V with a TNM-1 nitrogen analyzer), for NO3-, nitrite if present, acetate, and major anions and cations using ion chromatography (Anions/Cations Dionex ICS-1000 with an AS-DV autosampler). NH4+ was determined using automated colorimetry with a WestCo Scientific SmartChem 200 discrete analyzer.

Ambient DOC

For all the samples collected throughout the BTC, ambient DOC was computed as the difference between the DOC concentration and the acetate concentration (Equation 2), where both DOC and acetate concentrations are in units of mg C per liter (mg C L-1). Molar ratios of ambient DOC and DON were computed to determine how the stoichiometry of DOM and the energy-nutrient balance is changing in response to the acetate manipulation.

Equation 2:

$$[\textbf{\textit{Ambient DOC}}] = [DOC] - [Acetate]$$

DOM optical properties

Background samples collected prior to each addition were analyzed for DOM optical properties. Ultraviolet (UV) absorbance was measured using a spectrophotometer (Thermo ScientificTM GENESYSTM 150 UV-Vis). Specific ultraviolet absorbance at 254nm (SUVA254) was calculated by dividing the UV absorbance at 254nm by the concentration of DOC (Equation 3). SUVA is used as an index of the aromaticity of DOM (McKnight et al. 2001; Weishaar et al. 2003). Humification index (HIX), a fluorescence property that allows one to determine degree of humification (Ohno 2002), was calculated by dividing the area under the emission spectra 435-480nm and the sum of the peak area 330-345nm and 435-490nm at an

excitation wavelength of 254 nm. Fluorescence index (FI) was used to identify DOM origin (i.e.,

allochthonous or autochthonous) and was determined as the ratio between 470 and 520 nm

emission intensity using an excitation wavelength of 370nm (McKnight et al. 2001; Cory and

McKnight 2005). To determine a spectral slope (S), absorption spectra was log transformed for

the ranges of 275-295 and 350-400nm and fit non-linearly to an exponential function (Helms et

al. 2008). From S the ratio of slopes at ranges of 275-295 and 350-400nm was determined as the

slope ratio (SR), a parameter that provides information on DOM aromaticity and molecular

weight (Helms et al. 2008).

Equation 3:

$$SUVA_{254} = \frac{UV\ Absorbance}{[DOC]}$$

DOM spiraling metrics

      To determine uptake metrics for the added solutes, the breakthrough curve integration

method was used (Tank et al. 2008). The uptake length (Sw), or average distance traveled by a

solute (acetate), was determined by the negative inverse of the longitudinal loss rate (kL in m-1)

which is computed as the ratio of the natural log background-corrected Acetate:Cl for each

sample and the distance of the reach length (Equation 4).

Equation 4:

$$S_w = \frac{-1}{k_L}$$

Uptake velocity (Vf), which is defined as a mass transfer coefficient, was determined using

equation 5, where Q is discharge, w represents stream width and Sw is the uptake length which

was previously calculated using equation 4. Vf is normalized for stream physical properties such

as stream depth and velocity and is therefore often used for cross-site comparisons (Stream

Solute Workshop 1990; Peterson et al. 2001; Plont et al. 2020).

Equation 5:

$$V_f = \frac{\frac{Q}{w}}{S_w}$$

Statistical analyses

Simple linear regressions (SLR) were used to determine the response of DOM (as

Ambient DOC or DON), molar DOC:DON ratios to the added labile C (acetate). Variation in

uptake metrics were also compare to background concentrations of DOM via SLR. ANOVA was

used to determine whether differences in uptake across sites were statistically significant and

experiments were considered replicates. A Principal Components Analysis (PCA) was used to

examine which variables contributed the most to the variation in responses among additions. Our

PCA evaluated ambient DOM concentrations, the optical properties of ambient DOM, stream

characteristics (e.g., DO, pH, temperature, specific conductance and reach length) and uptake

kinetics of acetate. The amount of variation explained by a component was considered

significant when it was > 0.7 (Martí et al. 2009). Variables (e.g., nutrients concentrations, stream

characteristics, etc.) have loaded scores which describe how they relate to the components or

PCA axes (Wymore et al. 2017). These scores from axes 1 and 2 (PC1 and PC2) were used in

SLR along with the slopes that resulted from the relationships between the response variables

and the manipulated acetate. The level of significance was set at $p < 0.05$ and all statistical

analyses were performed in R studio (RStudio, Inc., Boston, MA 2019) except for PCA and

among sites comparisons which were conducted in JMP (JMP®, Version 15, SAS Institute Inc.,

Cary, NC, 1989-2019).

CHAPTER 3: RESULTS

Variability in DOM response to added acetate

In-situ manipulations of acetate resulted in variable responses for DOM across study sites. The response of ambient DOM to added acetate resulted in all three response scenarios (inert, priming and production). Overall, in almost half of the manipulations no response to acetate addition was observed in ambient DOC (Figure 1). This "inert" response to added acetate was observed for ambient DOC in 44% (n = 7) of the manipulations conducted and occurred more often in summer than in fall. In 25 % (n = 4) of the manipulations conducted among sites a priming effect was observed, with concentrations of ambient DOC declining during the addition of acetate (Figure 2). In 31% (n = 5) of our acetate additions, ambient DOC concentrations increased, supporting the "production" hypothesis (Figure 3).

The response of DON to acetate addition was strikingly different from the response in ambient DOC. In all but two of the manipulations no response in DON concentration was observed during the acetate addition. In both instances a "production" response of DON to added acetate was observed in the same site, Rum Brook (RMB; Figure 4). A summary of all individual responses can be found in Table 2. Individual responses of ambient DOC and DON to the added acetate can be found on APPENDIX B.

In addition to examining the response of DOC and DON to acetate concentrations, the response in DOM can also be related to the amount of acetate that has been removed from solution, based on the differences between observed acetate concentrations and the concentration expected to occur based on the inert tracer (Cl) concentration. This has the advantage of providing an overall assessment of the response to the biotic activity in response to acetate

addition, across experiments with widely differing levels of acetate addition. Overall, we see an increase in ambient DOC concentrations in response to the amount of acetate removed from solution (Figure 5), whereas ambient DON concentrations were unchanged, and thus could be categorized as the "inert" response pattern (Figure 6). A similar approach was used for examining individual additions and our results show greater removal of acetate at Crawford Brook (CRB), whereas less acetate removal was found at Trout Pond Brook (TPB) (Table 3).

DOM stoichiometry

To further evaluate the energy and nutrient balance in our study sites throughout the different additions conducted we looked at the relationship between ambient DOC and DON molar ratios. Identical to our individual assessment of DOM responses to the manipulated acetate our results showed evidence for the three hypothesized scenarios (inert, production, and priming) for DOM molar ratios. Around 38% (n=6) of the additions showed an inert response for DOM molar ratios. Similarly, around 38% (n=6) of the additions showed a production response, while only 25% (n=4) of the manipulations show some sort of evidence for priming (Table 3). For all sites, the response pattern of DOM molar ratios to the individual additions of acetate was similar to the response of ambient DOC previously presented with the exception of one summer addition in DCF, one fall addition in TPB and one fall addition in RMB. Individual molar ratios response to the manipulation of acetate can be found on APPENDIX C.

Potential influences of variable responses

Principal component analyses (PCA) showed than more than half of the variability in our results could be explained by axes 1 and 2 (PC1 and PC2) for all 4 models ran which included variables such as physicochemical properties, DOM background concentrations and composition, and uptake metrics (APPENDIX D). Physicochemical properties explained about

70% of the variability by PC1 and PC2 and the identified top predictor variable for this model was dissolved oxygen (DO) concentrations (APPENDIX D). For DOM background concentrations and composition, our model explained 67% of the variability by axes 1 and 2, where top predictor variables were background DOC concentrations, Abs254, DON, and DOC: DON molar ratios (APPENDIX D). When uptake metrics were included in the model of physicochemical properties the explanation of variation decreased to 55%, while DO remain being the number 1 predictor variable. However, our DOM background concentrations and composition model with the incorporation of uptake metrics explained the same percent of variability (67%) by axes 1 and 2, with the difference in the percent of contribution from each axis (PC1 = 45.6%, PC2= 21.6%). Similar to the previous DOM model without uptake metrics, top predictors variables remained the same (APPENDIX D).

Uptake kinetics

Measurable acetate uptake kinetics from the overall BTC response could only be obtained for 50% (n = 8) of the additions conducted (Table 4). Uptake lengths (Sw) ranged between 16 and 914 m (Table 4). The greatest uptake lengths were observed at sites with shorter experimental reaches (i.e., CRB; Table 4). Uptake velocity (Vf) ranged between 1.34- and 48.63- mm min -1, with the greatest uptake at DCF (Figure 7). Additions that were conducted later in the summer and fall tended to produce no measurable acetate uptake kinetics. Our analysis of variance (ANOVA) showed that uptake among all sites was significantly statistically different. In addition to determining uptake velocity, we examined whether the values were related to discharge (Q), ambient DOM concentrations and molar DOC:DON ratios. We found no relationship between Q, ambient DOM concentrations or molar DOC:DON ratios and the amount of acetate that was being take up during the in-situ manipulations.

11

CHAPTER 4: DISCUSSION

Effects of labile C availability on DOM

Our study showed variable responses of DOM to increases in energy availability. This variable response of DOM to acetate enrichments has been previously reported. Lutz et al. (2012) found that even after saturating streams with different amounts of acetate (2-16x above background level), DOC and DON still did not show significant changes in concentration (Lutz et al. 2012). In their study Lutz et al. (2012) suggested that DOC's variable response to acetate enrichment could be due in part to the analytical uncertainty involved with subtracting acetate concentrations in order to estimate the concentration of non-acetate DOC (what we refer to here as ambient DOC) (Lutz et al. 2012).

The patterns of change in the ambient DOM concentration suggest that the addition of acetate has different effects on how the DOM pool responds, and such responses can be attributed to the availability of DOC and DON as well as seasonality. Acetate additions that resulted in an increase in ambient DOC concentrations were most common during the late spring and summer, suggesting that seasonality could play a role in how the ambient DOM pool responds to the addition of labile C. The effect of seasonality on DOM dynamics has been previously addressed (Johnson et al. 2009; Wymore et al. 2015; Seybold and McGlynn 2018) and the results reported differed across studies. Ambient concentrations of DOM tend to fluctuate temporally, given the inputs aquatic ecosystems receive as their surrounding landscape changes (i.e., snowmelt, litterfall). Solute concentrations in streams reflect processes that have already taken place (i.e., uptake, retention; Seybold and McGlynn 2018). For most of our study sites (CRB, DCF and TPB) background DOM availability increased throughout the sampling

period, particularly as the transition from summer to fall began. This change in the concentration of both DOC and DON from late spring to fall seems to have played a role in the energy-nutrient balance within the DOM pool, affecting the response of these solutes to the enrichment of acetate. As the concentration of a solute increases, it is likely that the demand for labile DOM is being satisfied by ambient sources, resulting in the biota not taking up the added acetate.

Priming of DOC during a manipulation of labile C is a response of the ecosystem that can often result from increased rates of mineralization of organic matter such as leaf litter in the stream bed. Rates of priming are constrained by the capacity of microorganisms to break down complex forms of organic matter (Catalán et al. 2015). It is also likely that the addition of labile C can stimulate mineralization of ambient DOC under certain conditions, given that the bioavailability of ambient organic carbon can vary dramatically among different sources (Kuzyakov et al. 2000; Hotchkiss et al. 2014).

Direct test of on-going hypotheses

Our study serves as a direct test of several hypotheses regarding in-stream processing of DOM. Through our study we were able to test the energetic role of DON in streams (Wymore et al. 2015) as well as the effects of carbon and nutrient availability on the uptake of acetate (Seybold and McGlynn 2018). DON's ecological duality (energy source vs. nutrient source) in headwater streams was assessed by determining the response of DON to the added acetate. Most individual in-situ manipulations of acetate did not result in a response of DON to the added solute. This lack of response does not support our initial hypothesis, given that adding labile C was expected to stimulate nitrogen uptake causing DON concentrations to decrease. However, the few DON responses (which were DON increases) were only found at RMB, our site with the greatest background DIN concentrations (Table 1: RMB). DON's direct response to the addition

of inorganic nitrogen has been previously addressed and this type of enrichment has demonstrated a duality, where DON switches between serving as an energy or a nutrient source to the ecosystem throughout seasons (Wymore et al. 2015). When comparing our results directly with what has been reported by Wymore et al (2015), we see that in our experiments DON's positive response to the added acetate in RMB takes place in June and September and the direction of this response does not change. The additions of NO_3^- conducted by Wymore et al. (2015) caused DON's response in RMB to shift (from negative to positive) between July and August (Fig. 3G-H in Wymore et al. 2015). The fact that DON's was unresponsive to the added acetate did not change in our study and that most of our additions elicited no response in DON concentrations suggests that alteration of DON concentrations requires simultaneous addition of both labile C and N into the system. This new proposed hypothesis suggests that DON's response is dependent on inorganic nitrogen availability, given that adding labile C alone was not sufficient to alter the ambient pool of DON.

Through our study we were also able to compare our results with what has been reported by Seybold and McGlynn (2018), where they found that seasonal changes can lead to different uptake kinetics and export of solutes. They reported that decreases in the availability of DOC and NO_3^- (from May to August) resulted in a limitation of uptake (Seybold and McGlynn 2018). Our results are not consistent with what has been reported by Seybold and McGlynn (2018). Generally, as ambient concentrations increased uptake of acetate was less likely to occur. This is true specifically for sites on the higher end for our gradient of background DOC and DON concentrations (CRB, DCF and RMB) and sampling rounds conducted late in the fall (October; CRB4 and TPB4), where concentrations tend to be greater due to litterfall.

Acetate uptake in a global context

Uptake velocity (Vf) is the most broadly useful parameter for comparing nutrient and carbon uptake kinetics among sites because it corrects for discharge and normalizes for the concentration of solutes (e.g., Mulholland et al. 2009; Catalán et al. 2018). Uptake velocity across our study sites showed that demand for labile C is limited and can be variable for each studied system. Generally, for CRB and TPB uptake was more likely to take place when concentrations of ambient DOM were lower; as ambient concentrations of DOM increased, uptake of acetate decreased to zero or non-detectable. In DCF a different pattern was observed, where uptake velocity increased between the first two experiments along with ambient DOM concentrations. However, when ambient DOM concentrations were the highest there was no uptake registered. For RMB, uptake took place during the peak of ambient DOM concentrations suggesting that there are other drivers that influence the uptake of added solutes. When uptake velocity was evaluated against different potential explanatory variables, it was found that uptake velocity was related to fluorescence index, which suggests that DOM's source influences uptake kinetics (Rodríguez-Cardona et al. 2016; Wymore et al. 2016). Our results for acetate uptake were comparable to those obtained by Catalán et al. (2018) for acetate uptake across a geographical gradient along European ecoregions. The values reported for acetate Vf in Catalán et al. (2018) ranged between 0.31 mm min-1 to 7.9 mm min-1. The results of our study overlap for the most part with what was reported by Catalán et al. in 2018, except for the high acetate Vf values found at DCF, which were greater than 7.9 mm min-1, with the highest uptake value being 48.6 mm min-1 (Table 4). In addition to Catalán et al (2018), Johnson and Tank (2009) investigated the effects of diurnal cycles on the uptake of DIN, DOC and DON as NH4+, acetate and glycine, respectively. Values for uptake velocity of acetate ranged between 0 and 25.8-mm

min $^{-1}$. In their study acetate Vf was found to be higher during the day for only half of the experiments conducted which reflected no diurnal patterns in their findings (Johnson and Tank 2009). The range in the values reported for acetate uptake in this study is greater than the ones reported by Catalán et al (2018), however, their results overlap and are consistent with ours. Our results are also the first to assess uptake of acetate measured directly, rather than as a change in total DOC.

DOM composition

Recent studies highlight the role of DOM composition in understanding in-stream processing and drivers of uptake kinetics (e.g., Rodríguez-Cardona et al. 2016; Wymore et al. 2016; Catalán et al. 2018). DOM is a complex mixture of compounds and in order to assess any aspect of its functionality and reactivity its chemistry needs to be characterized (Mineau et al. 2016; Catalán et al. 2018). The evaluation of optical properties of DOM serves as a characterization of its sources and origins which provides insight on the different process that influence its reactivity. Of the DOM optical parameters evaluated in this study, acetate uptake (Vf) was found to be strongly and significantly correlated to fluorescence index (FI) (Figure 8). Values of FI lower than 1.2 correspond to terrestrial sources, while values greater than 1.8 imply an autotrophic origin (Fellman et al. 2010). The range in FI values for our samples suggests that DOM in our study streams is mainly derived from terrestrial sources (Fellman et al. 2010). However, the positive relationship between FI and acetate uptake suggests that uptake increases as the source of DOM shifts from terrestrial to microbial (Figure 8). Our study sites can be considered non-C limited (given their high C:N ratios) which coincides with the low demand (limited uptake) of labile C (acetate). DOM in these streams is tightly connected to the landscape which means that further studies should incorporate the connectivity between terrestrial and

aquatic ecosystems and how changes in the landscape influence in-stream processes along with the effect it has on DOM composition including its sources, transformation, fate, and transport. Incorporating DOM composition is key to teasing apart the different drivers of DOM reactivity particularly in freshwater ecosystems.

Implications for DOM dynamics in stream ecosystems

DOM dynamics in streams are tightly connected to changes in the landscape (Vannote et al. 1980; Newbold et al. 1982). As atmospheric deposition, temperature and hydrology changes, inputs of terrestrial DOM in streams and river networks can be affected, altering levels of stream water DOM (Dawson et al. 2008) as well as its quality and reactivity (Kothawala et al. 2014; Kaplan and Cory 2016). DOM's reactivity is dependent on the chemical composition of its fractions which influence its fate (Cory and Kaplan 2012; Casas-Ruiz et al. 2017). Our study shows that DOC is often responsive to the manipulation of acetate, however, this response is variable and can switch across seasons and different background concentrations. These dynamic responses of DOM did not always follow acetate uptake kinetics. Out of the 8 additions that resulted in non-detectable uptake of acetate, only 4 of them were associated with an inert response in DOC concentrations. The other half of these additions with non-detectable uptake resulted in a priming response. Given that DOC appears to be sometimes responsive to the manipulation of acetate and that such responses differ primarily based on ambient DOM availability, it can be suggested that our study sites could have been saturated especially during times where background DOM availability was greater. As for DON, the dominance of inert responses to increases in energy availability in our study suggest that DON's energetic role within these systems is minimal and therefore the ecological duality previously proposed by Wymore et al. (2015) was not supported during our acetate manipulations.

17

CHAPTER 5: CONCLUSION

Our study shows the influence that the availability of additional labile carbon (C) has on the ambient DOM pool. We proposed that the addition of an external energy source (acetate) could result in three different responses in the ambient DOM pool: inert, production and priming, and provided evidence for each of these scenarios. C availability has no direct effect on DON concentration, suggesting that for DON to respond some component of the N pool needs to be stimulated.

The relationship between acetate uptake and fluorescence index (FI) highlights the influence the landscape has on inputs of organic matter in aquatic ecosystems. Our results showed that the DOM in our study sites is mainly derived from terrestrial sources, supporting linkages between aquatic and terrestrial systems. As uptake increases FI increases suggesting that DOM's source could be shifting from terrestrial to microbial or that the terrestrial DOC is labile and being consumed rapidly at the same time, leaving only the aquatic signature behind. However, this relationship needs to be further explored given that the strength for this result was strictly due to a single data point with high leverage. To develop a more comprehensive understanding of DOM in freshwater ecosystems, future studies should combine uptake metrics as well as DOM composition to explore critical explanatory variables that will enhance our understanding in deciphering the role, drivers, and controls of DOM processing in streams.

LIST OF REFERENCES

Alvarez-Cobelas M, Angeler DG, Sánchez-Carrillo S, Almendros G (2012) A worldwide view of organic carbon export from catchments. Biogeochemistry 107:275–293. https://doi.org/10.1007/sl0533-010-9553-z

Bernhardt ES, McDowell WH (2008) Twenty years apart: Comparisons of DOM uptake during leaf leachate releases to Hubbard Brook valley streams in 1979 versus 2000. J Geophys Res Biogeosciences 113:1–8. https://doi.org/10.1029/2007JG000618

Brookshire ENJ, Valett HM, Thomas SA, Webster JR (2005) Coupled Cycling of Dissolved Organic Nitrogen and Carbon. 86:2487–2496

Casas-Ruiz JP, Catalán N, Gómez-Gener L, et al (2017) A tale of pipes and reactors: Controls on the in-stream dynamics of dissolved organic matter in rivers. Limnol Oceanogr 62:S85–S94. https://doi.org/10.1002/lno.10471

Catalán N, Casas-Ruiz JP, Arce MI, et al (2018) Behind the Scenes: Mechanisms Regulating Climatic Patterns of Dissolved Organic Carbon Uptake in Headwater Streams. Global Biogeochem Cycles 32:1528–1541. https://doi.org/10.1029/2018GB005919

Catalán N, Kellerman AM, Peter H, et al (2015) Absence of a priming effect on dissolved organic carbon degradation in lake water. Limnol Oceanogr 60:159–168. https://doi.org/10.1002/lno.10016

Cory RM., McKnight DM (2005) Fluorescence Spectroscopy Reveals Ubiquitous Presence of Oxidized and Reduced Quinones in Dissolved Organic Matter. Environ Sci Technol 39:8142–8149. https://doi.org/10.1021/es0506962

Cory RM, Kaplan LA (2012) Biological lability of streamwater fluorescent dissolved organic matter. Limnol Oceanogr 57:1347–1360. https://doi.org/10.4319/lo.2012.57.5.1347

Covino TP, McGlynn BL, McNamara RA (2010) Tracer Additions for Spiraling Curve Characterization (TASCC)- Quantifying stream nutrient uptake kinetics from ambient to saturation. Limnol Oceanogr 484–498. https://doi.org/10.1109/tasc.2010.2040612

Dawson JJC, Soulsby C, Tetzlav D, et al (2008) Influence of hydrology and seasonality on DOC exports from three contrasting upland catchments. Biogeochemistry 90:93–113. https://doi.org/10.1007/s10533-008-9234-3

Fellman JB, Hood E, Spencer RGM (2010) Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems : A review. 55:2452–2462. https://doi.org/10.4319/lo.2010.55.6.2452

Fisher SG, Likens GE (1972) Stream Ecosystem : Organic Energy Budget. Bioscience 22:33–35
Helms JR, Stubbins A, Ritchie JD, et al (2008) Absorption spectral slopes and slope ratios as

indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol Oceanogr 53:955–969. https://doi.org/10.4319/lo.2008.53.3.0955

Hotchkiss ER, Hall RO, Baker MA, et al (2014) Modeling priming effects on microbial consumption of dissolved organic carbon in rivers. J Geophys Res Biogeosciences 119:982–995. https://doi.org/10.1002/2013JG002599

Johnson LT, Tank JL (2009) Diurnal variations in dissolved organic matter and ammonium uptake in six open-canopy streams. J North Am Benthol Soc 28:694–708. https://doi.org/10.1899/08-107.1

Johnson LT, Tank JL, Arango CP (2009) The effect of land use on dissolved organic carbon and nitrogen uptake in streams. Freshw Biol 54:2335–2350. https://doi.org/10.1111/j.1365-2427.2009.02261.x

Kallenbach CM, Frey SD, Grandy AS (2016) Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat Commun 7:. https://doi.org/10.1038/ncomms13630

Kaplan LA, Cory RM (2016) Dissolved Organic Matter in Stream Ecosystems: Forms, Functions, and Fluxes of Watershed Tea. In: Streams Ecosystems in a Changing World. pp 241–320

Kaplan LA, Wiegner TN, Newbold JD, et al (2008) Untangling the complex issue of dissolved organic carbon uptake: a stable isotope approach. Freshw Biol 53:855–864. https://doi.org/10.1111/j.1365-2427.2007.01941.x

Kilpatrick FA, Cobb ED (1985) Techniques of water-resources investigations of the United States Geological Survey:Measurement of discharge. In: Application of Hydraulics

Kothawala DN, Stedmon CA, Müller RA, et al (2014) Controls of dissolved organic matter quality: evidence from a large-scale boreal lake survey. Glob Chang Biol 20:1101–1114. https://doi.org/10.1111/gcb.12488

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32:1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5

Lutz BD, Bernhardt ES, Roberts BJ, et al (2012) Distinguishing dynamics of dissolved organic matter components in a forested stream using kinetic enrichments. Limnol Oceanogr 57:76–89. https://doi.org/10.4319/lo.2012.57.1.0076

Martí E, Fonolla P, von Schiller D, et al (2009) Variation in stream C , N and P uptake along an altitudinal gradient : A space- for-time analogue to assess potential impacts of climate change. Hydrol Res 40.2–3:123–137. https://doi.org/10.2166/nh.2009.090

McKnight DM, Boyer EW, Westerhoff PK, et al (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr 46:38–48. https://doi.org/10.4319/lo.2001.46.1.0038

Mineau MM, Wollheim WM, Buffman I, et al (2016) Dissolved organic carbon uptake in streams: A review and assesment of reach-scale measurements. J Geophys Res Biogeosciences 121:2019–2029. https://doi.org/10.1002/2015JG003204.Received

Mulholland PJ. (2003) Large-scale patterns in dissolved organic carbon concentration, flux, and sources. In: Findla SEG., Sinsabaugh RL (eds) Aquatic Ecosystems Interactivity of Dissolved Organic Matter. Academic Press, New York, pp 139–159

Newbold JD, Mulholland PJ, Elwood JW, O'Neill R V. (1982) Organic Carbon Spiralling in Stream Ecosystems. Oikos 38:266. https://doi.org/10.2307/3544663

Ohno T (2002) Fluorescence Inner-Filtering Correction for Determining the Humification Index of Dissolved Organic Matter. Environ Sci Technol 36:742–746. https://doi.org/10.1021/es0155276

Peterson BJ., Wolheim WM., Mulholland PJ., et al (2001) Control of Nitrogen Export from Watersheds by Headwater Streams. Science (80-) 292:86–89

Plont S, O'Donnell BM, Gallagher MT, Hotchkiss ER (2020) Linking carbon and nitrogen spiraling in streams. Freshw Sci 39:126–136. https://doi.org/10.1086/707810

Prairie YT (2008) Carbocentric limnology : Looking back , looking forward. Can J Fish Aquat Sci 65:543–548. https://doi.org/10.1139/F08-011

Rodríguez-Cardona B, Wymore AS, McDowell WH (2016) DOC:NO$_3^-$ ratios and NO$_3^-$ uptake in forested headwater streams. J Geophys Res Biogeosciences 121:205–217. https://doi.org/10.1002/2015JG003146

Rodríguez-Cardona BM, Coble AA, Wymore AS, et al Wildfires lead to decreased carbon and increased nitrogen concentrations in upland arctic streams. https://doi.org/10.1038/s41598-020-65520-0

Rodríguez-Cardona BM, Wymore AS, McDowell WH. *In Press.* Nitrate uptake enhanced by availability of dissolved organic matter in tropical montane streams. Freshw Sci

Seybold E, McGlynn B (2018) Hydrologic and biogeochemical drivers of dissolved organic carbon and nitrate uptake in a headwater stream network. Biogeochemistry 138:23–48. https://doi.org/10.1007/s10533-018-0426-1

Stream Solute Workshop (1990) Concepts and Methods for Assessing Solute Dynamics in Stream Ecosystems. J North Am Benthol Soc 9:95–119. https://doi.org/10.2307/1467445

Tank JL, Rosi-Marshall EJ, Baker MA, Hall RO (2008a) Are Rivers Just Big Streams? A Pulse Method to Quantify Nitrogen Demand in a Large River. Ecology 89:2935–2945. https://doi.org/10.1890/07-1315.1

Van Stan JT, Stubbins A (2018) Tree-DOM: Dissolved organic matter in throughfall and stemflow. Limnol Oceanogr Lett 3:199–214. https://doi.org/10.1002/lol2.10059

Vannote RL, Minshall GW, Cummins KW, et al (1980) The River Continuum Concept. Can J Fish Aquat Sci 37:130–137

Weishaar JL, Aiken GR, Bergamaschi BA, et al (2003) Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ Sci Technol 37:4702–4708. https://doi.org/10.1021/es030360x

Wymore AS, Brereton RL, Ibarra DE, McDowell WH (2017) Critical zone structure controls concentration-discharge relationships and solute generation in forested tropical montane watersheds. Water Resour Res 53:6279–6295. https://doi.org/10.1002/2016WR020016.Received

Wymore AS, Coble AA, Rodríguez-Cardona B, McDowell WH (2016) Nitrate uptake across biomes and the influence of elemental stoichiometry: A new look at LINX II. Global Biogeochem Cycles 30:1183–1191. https://doi.org/10.1002/2016GB005468

Wymore AS, Leon MC, Shanley JB, McDowell WH (2019) Hysteretic Response of Solutes and Turbidity at the Event Scale Across Forested Tropical Montane Watersheds. Front Earth Sci 7:. https://doi.org/10.3389/feart.2019.00126

Wymore AS, Rodríguez-Cardona B, McDowell WH (2015) Direct response of dissolved organic nitrogen to nitrate availability in headwater streams. Biogeochemistry 126:1–10. https://doi.org/10.1007/s10533-015-0153-9

Wymore AS, Salpas E, Casaburi G, et al (2018) Effects of plant species on stream bacterial communities via leachate from leaf litter. Hydrobiologia 807:131–144. https://doi.org/10.1007/s10750-017-3386-x

TABLES

Table 1. Range of background concentrations of inorganic and organic solutes during field manipulations.

	Study Site			
	CRB	**DCF**	**TPB**	**RMB**
DOC (mg C L^{-1})	15.99 - 42.96	5.56 - 8.34	2.43 - 3.56	3.35 - 9.14
NO$_3^-$ (mg N L^{-1})	0.001 - 0.004	0.03 - 0.14	0.01 - 0.02	0.07 - 0.15
TDN (mg N L^{-1})	0.31 - 0.68	0.29 - 0.39	0.10 - 0.19	0.31 - 0.50
NH$_4$ (ug N L^{-1})	3.38 - 7.49	2.27 - 27.28	4.14 - 14.13	27.38 - 39.28
DON (mg N L^{-1})	0.30 - 0.67	0.23 - 0.30	0.08 - 0.15	0.12 - 0.35
Acetate (mg C L^{-1})	0 – 0.04	0 - 0.11	0 - 0.04	0

Table 2. Summary of DOM's response to the manipulated acetate for every addition conducted at each site. All r^2 values reported have a statistical significance of $p < 0.05$.

| | | | Response and r^2 | | |
| | | | **Ambient DOC vs. Acetate** | **DON vs. Acetate** | **AmbDOC:DON vs. Acetate** |
Addition	**Date**	**Season**			
CRB1	5/6/2019	Spring	production, 0.27	inert	production, 0.04
CRB 2	5/7/2019	Spring	production, 0.33	inert	production, 0.11
CRB 3	7/5/219	Summer	production, 0.15	inert	production, 0.14
CRB 4	10/11/2019	Fall	priming, 0.29	inert	priming, 0.42
DCF1	5/24/2019	Spring	inert	inert	inert
DCF2	6/27/2019	Summer	inert	inert	production, 0.27
DCF3	7/25/2019	Summer	inert	inert	inert
DCF4	9/6/2019	Fall	priming, 0.57	inert	priming, 0.17
TPB 1	5/31/2019	Spring	priming, 0.38	inert	priming, 0.15
TPB 2	7/19/2019	Summer	inert	inert	inert
TPB 3	8/16/2019	Summer	inert	inert	inert
TPB 4	10/30/2019	Fall	priming, 0.35	inert	inert
RMB 1	6/19/2019	Summer	production, 0.83	production, 0.22	production, 0.32
RMB 2	7/3/2019	Summer	production, 0.77	inert	production, 0.59
RMB 3	8/23/2019	Summer	inert	inert	inert
RMB 4	9/20/2019	Fall	inert	production, 0.11	priming, 0.15

Table 3. Average values for changes (Δ) in concentration of samples collected during the breakthrough curve (BTC) for all the additions conducted. Acetate expected was determined as the difference between the Acet:Cl in BTC samples and the Acet:Cl in the stock solution that was added during each manipulation. Acetate uptake (mg C/L) was computed as the difference between the expected acetate and the Δ Acetate (mg C/L).

Addition	Date	ΔAcetate (mg C/L)	ΔCl (mg/L)	ΔAcetate expected	Acetate uptake (mg C/L)	Δ Ambient DOC (mg C/L)	Δ DON (mg/L)	Δ AmbDOC:DON (molar)
CRB1	5/6/2019	13.22	12.47	26.03	12.81	1.48	0.03	0.03
CRB2	5/7/2019	11.50	9.88	20.77	9.27	1.41	0.02	2.10
CRB3	7/5/219	10.45	11.44	24.09	13.64	36.47	0.59	72.55
CRB4	10/11/2019	7.10	8.02	16.10	9.00	40.99	0.66	73.05
DCF1	5/24/2019	1.89	15.60	4.30	2.40	-1.51	-0.09	53.10
DCF2	6/27/2019	1.66	28.43	7.85	6.19	10.29	0.18	94.73
DCF3	7/25/2019	1.12	12.31	3.41	2.29	8.64	0.29	47.29
DCF4	9/6/2019	1.01	15.49	3.95	2.94	5.26	0.24	25.84
TPB1	5/31/2019	2.00	7.02	3.93	1.93	-0.86	-0.01	-6.42
TPB2	7/19/2019	0.61	3.20	1.78	1.16	2.95	0.17	20.48
TPB3	8/16/2019	0.22	4.02	2.25	2.03	2.87	0.08	47.78
TPB4	10/30/2019	1.35	5.43	3.11	1.76	3.41	0.14	33.55
RMB1	6/19/2019	1.44	133.31	5.88	4.44	8.98	0.35	41.85
RMB2	7/3/2019	4.19	87.01	18.16	13.98	11.12	0.37	35.63
RMB3	8/23/2019	0.49	66.79	3.33	2.84	6.98	0.28	29.13
RMB4	9/20/2019	1.07	80.69	4.83	3.77	3.26	0.13	29.28

Table 4. Stream characteristics, uptake kinetics and DOM optical properties for every addition at every site. Where length is in (m), Q in (L s^{-1}) , BTC S$_w$ in (m) and BTC V$_f$ in (mm min^{-1}).

Addition	Length	Q	BTC S$_w$	BTC V$_f$	FI	Abs254	HIX	SlopeRatio	SUVA
CRB1	24	13.47	**108.17**	**3.76**	1.15	0.96	0.99	0.73	6
CRB2	24	8.18	**194.49**	**1.34**	1.13	0.97	1	0.73	5.73
CRB3	24	3.41	**914.88**	**0.16**	1.15	1.9	0.97	0.7	5.45
CRB4	24	1.63	-	-	1.2	2.11	0.99	0.71	4.94
DCF1	33	95.26	**75.64**	**25.96**	1.28	0.33	0.97	0.76	5.99
DCF2	33	38.61	**16.36**	**48.63**	2.2	0.41	0.96	0.71	5.43
DCF3	33	89.82	-	-	1.33	0.42	0.95	0.76	4.94
DCF4	33	6.72	-	-	1.36	0.28	0.94	0.73	5.04
TPB1	152.7	101.3	-	-	1.33	0.19	0.96	0.75	6.85
TPB2	152.7	28	-	-	1.36	0.42	0.93	0.76	15.33
TPB3	152.7	21.58	**69.18**	**4.31**	1.36	0.41	0.93	0.75	16.92
TPB4	152.7	91.05	-	-	-	-	-	-	-
RMB1	64.1	53.22	**129.75**	**6.08**	1.31	0.49	0.95	0.74	6.34
RMB2	64.1	33.07	**247.51**	**1.95**	1.51	0.53	0.41	0.73	5.84
RMB3	64.1	11.19	-	-	1.33	0.36	0.96	0.77	5.23
RMB4	64.1	26.96	-	-	1.38	0.16	0.93	0.77	4.87
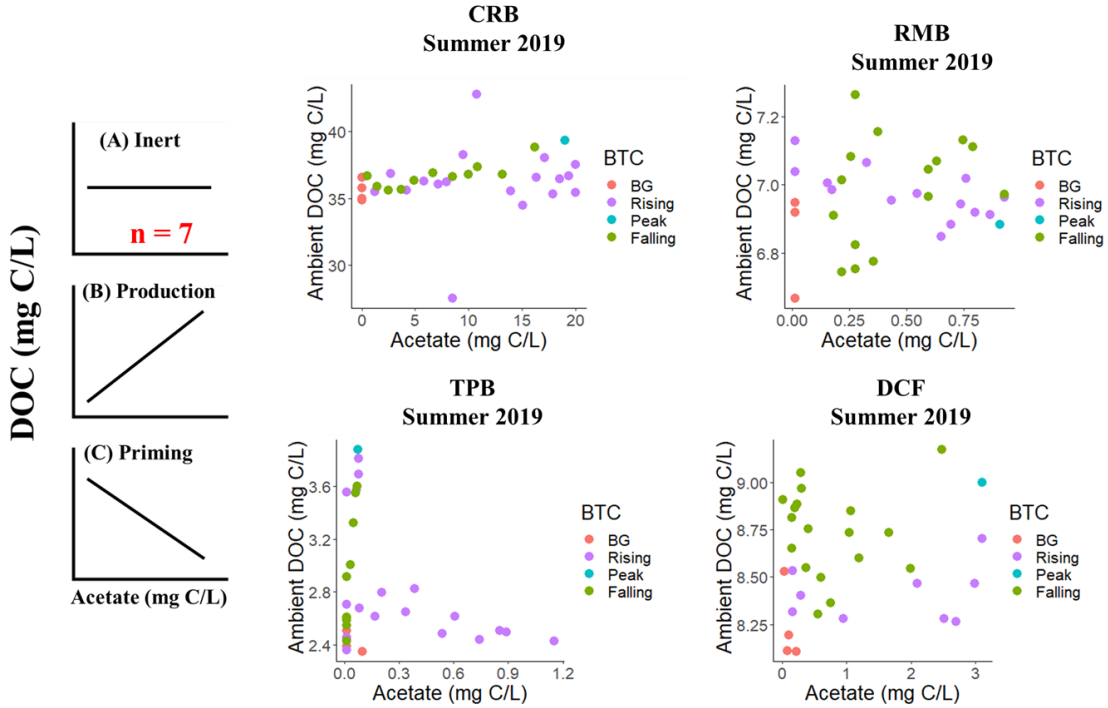
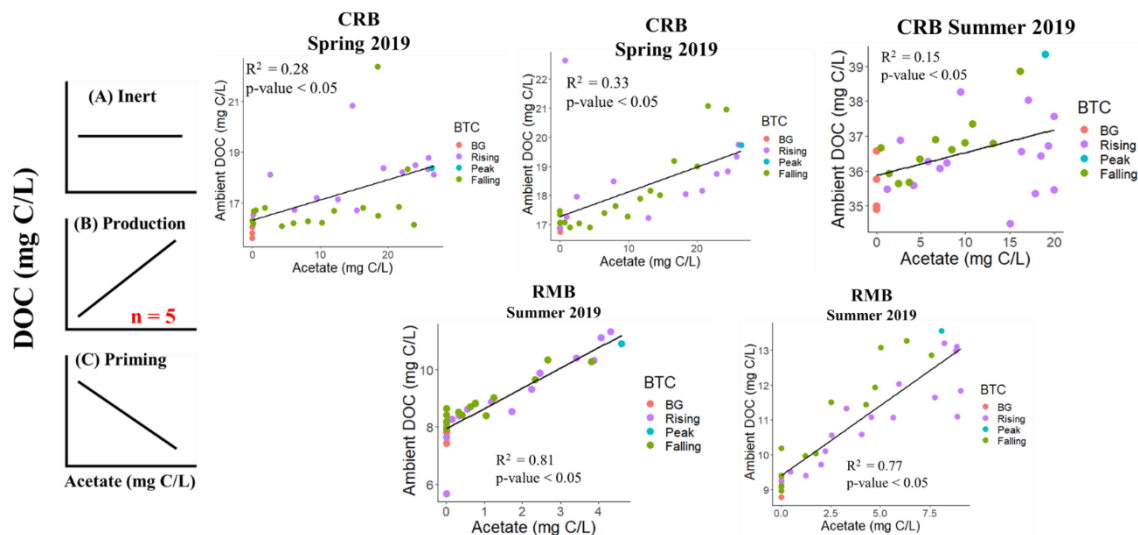**Figure 1.** Concentrations of ambient DOC and acetate in stream water during manipulations in which ambient DOC concentrations did not respond to acetate additions (inert response; 7 of 16 total manipulations). Each plot describes results from a single addition with samples taken along the breakthrough curve (BTC). Data points are color coded by the point at which samples are taken along the BTC. Pink = background before addition, purple = signal of arrival of solution (rising), blue= bulk of solution added has arrived (peak), and green = stream is returning to background conditions (falling).

27
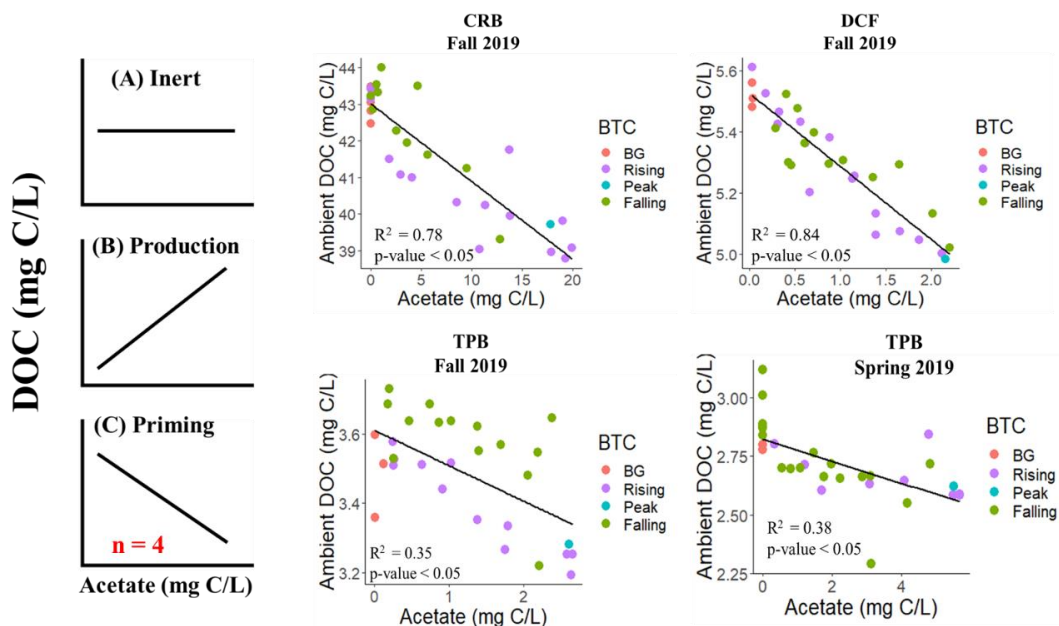
Figure 2. Concentrations of ambient DOC and acetate in stream water during manipulations in which ambient DOC responded to acetate additions with a priming response (4 of 16 total manipulations). Each plot describes results from a single addition with samples taken along the breakthrough curve. Data points are color coded by the point at which samples are taken along the breakthrough curve. Pink = background before addition, purple = signal of arrival of solution (rising), blue= bulk of solution added has arrived (peak), and green = stream is returning to background conditions (falling).

Figure 3. Concentrations of ambient DOC and acetate in stream water during manipulations in which ambient DOC responded to acetate additions with a production response (5 of 16 total manipulations). Each plot describes results from a single addition with samples taken along the breakthrough curve. Data points are color coded by the point at which samples are taken along the breakthrough curve. Pink = background before addition, purple = signal of arrival of solution (rising), blue= bulk of solution added has arrived (peak), and green = stream is returning to background conditions (falling).

29
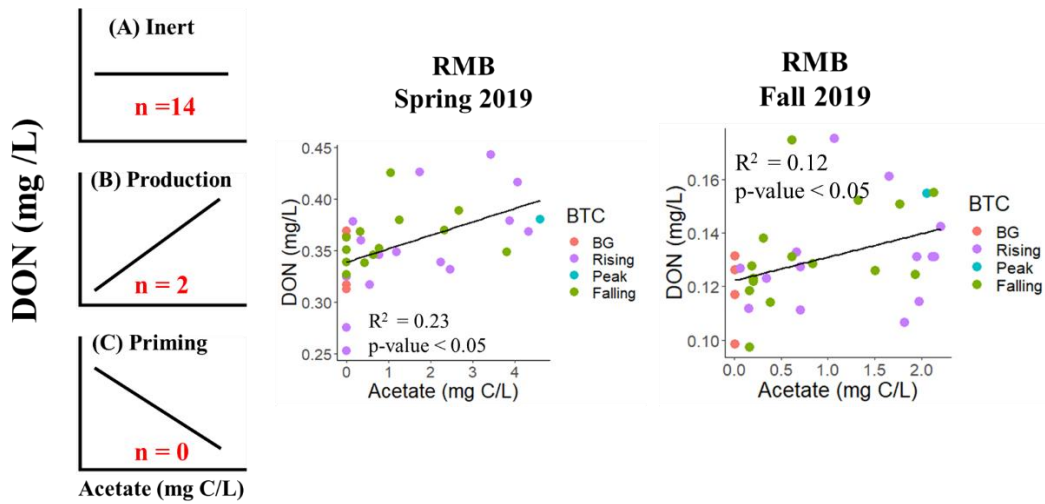
Figure 4. Summary of responses found for ambient DON to the addition of acetate for all 16 manipulations conducted among the four sites. A total of 14 additions showed an inert response, 2 of the additions showed a production response and no additions showed any signal of priming. Each plot describes results from a single addition with samples taken along the breakthrough curve. Data points are color coded by the point at which samples are taken along the breakthrough curve. Pink = background before addition, purple = signal of arrival of solution (rising), blue= bulk of solution added has arrived (peak), and green = stream is returning to background conditions (falling).
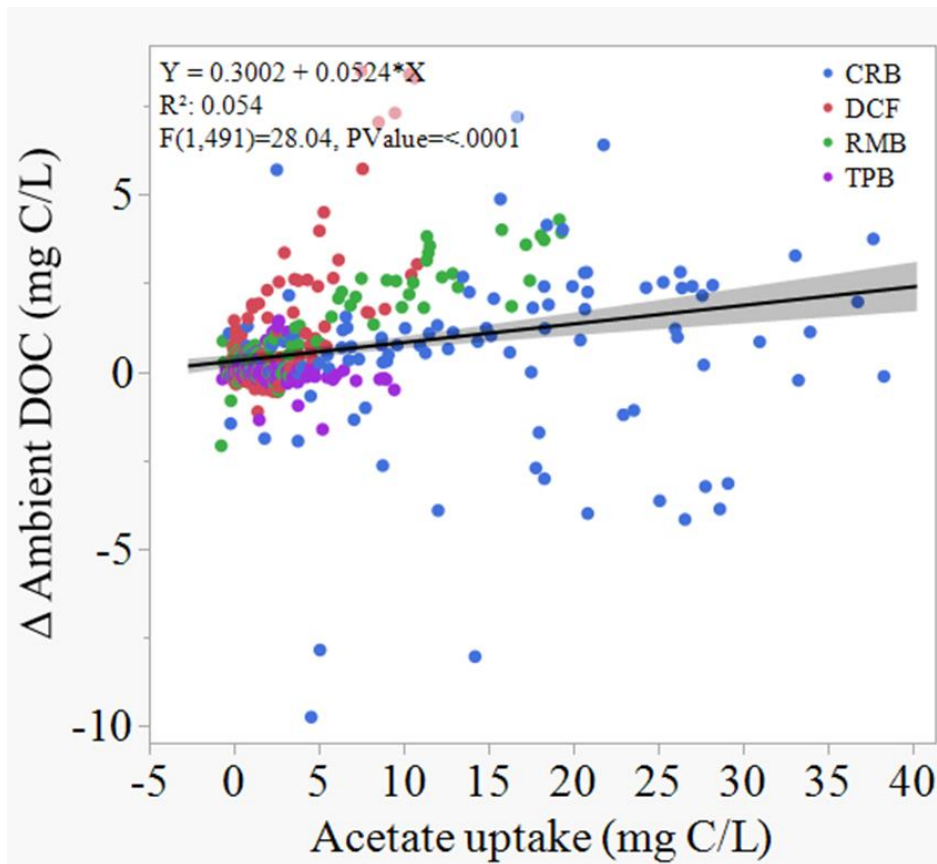
Figure 5. Change in ambient DOC concentration (mg C L^{-1}) as a function of acetate uptake (mg C L^{-1}) for all 16 additions conducted among all study sites.
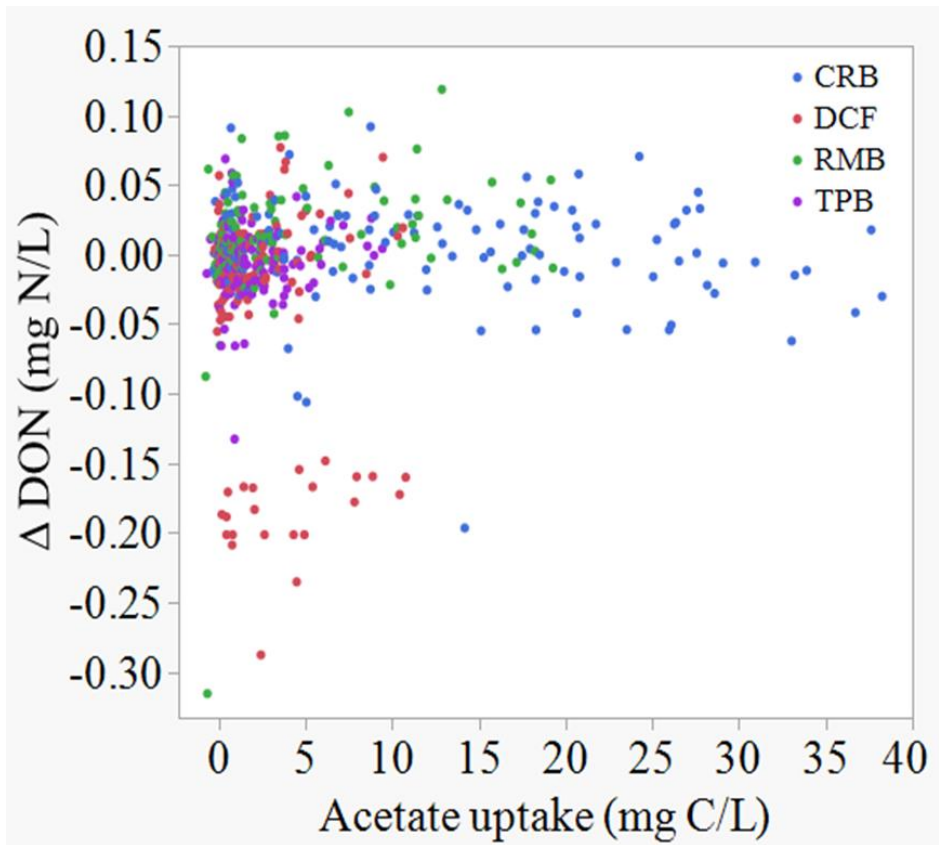
Figure 6. Change in DON concentration (mg N L^{-1}) as a function of acetate uptake (mg C L^{-1}) for all 16 additions conducted among all study sites.
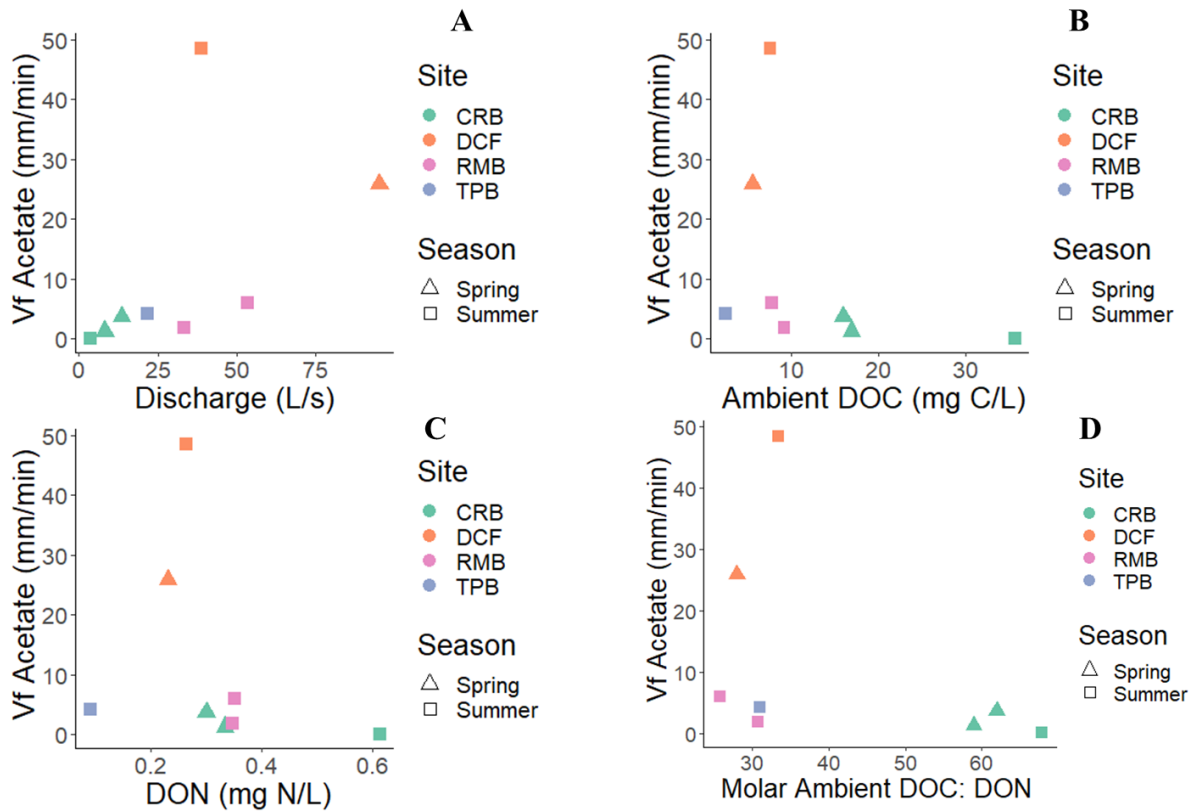
Figure 7. Uptake velocity (V_f) of acetate for all sites grouped by season: spring (triangles), summer (squares) related to (A) discharge, (B) ambient DOC concentration, (C) DON concentration and (D) Ambient DOC: DON ratios. Data points showed, correspond to only the 8 acetate additions that registered uptake.
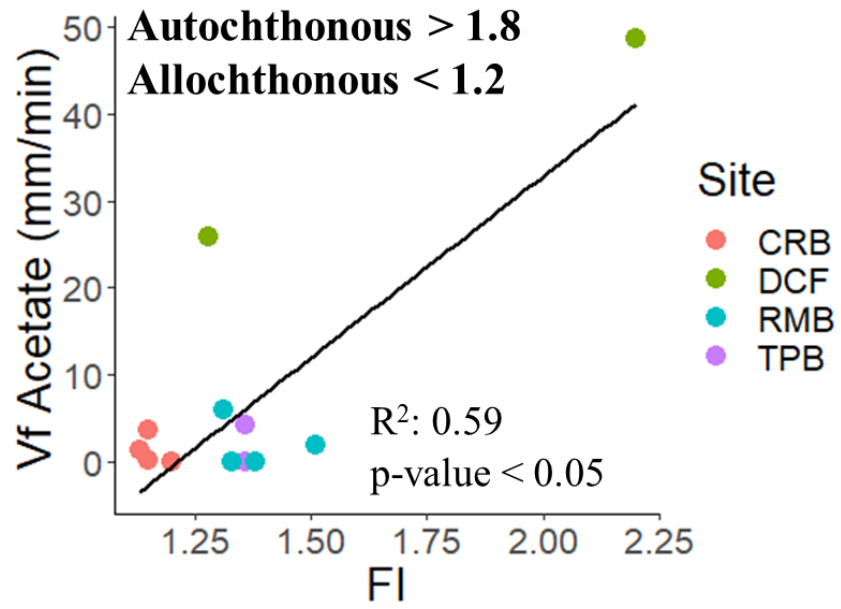
Figure 8. Relationship between uptake velocity (V_f) of acetate among all sites and fluorescence index (FI).

APPENDICES

APPENDIX A

Stream physicochemical properties and amount of acetate and salt added in each addition.

Addition	Mean Width (m)	Q (L/s)	SPC (uS/cm)	Temp (ºC)	DO (mg/L)	pH	NaCl (g)	Acetate (g)
CRB1	1.99	13.47	26.2	4.4	10.51	4.48	133.14	414.45
CRB2	1.89	8.18	26.5	4.5	10.31	4.07	119.45	374.46
CRB3	1.46	3.41	33.6	13.4	6.69	4.43	54.24	170.29
CRB4	1.30	1.63	39.9	10	6.43	4.2	33.43	100.04
DCF1	2.91	95.26	48.7	15.8	9.11	6	972.5	399.5
DCF2	2.91	38.61	55.2	19.7	8.07	6.25	838	345
DCF3	3.13	89.82	44.6	19.1	8.59	6.05	327.87	135.53
DCF4	2.73	6.72	62.8	15.7	9.1	6.66	131.9	50.1
TPB1	4.01	101.30	17	14.6	9.86	5.35	1315.5	1099
TPB2	4.36	28.00	19.3	18.7	8.57	5.99	140	116
TPB3	4.35	21.58	19.5	17.1	9.02	6.08	215.5	180
TPB4	4.28	91.05	20.7	9.9	11.06	5.99	468.67	400.99
RMB1	4.05	53.22	231.6	18.3	7.97	6.8	5930	390
RMB2	4.11	33.07	228.5	21.1	7.54	6.95	2000	622.5
RMB3	3.70	11.19	255.9	21.1	7.85	6.95	501	37.3
RMB4	3.62	26.96	266.7	13.7	9.25	6.97	1036	92.5

Individual responses

CRB 1 (5/6/2019)
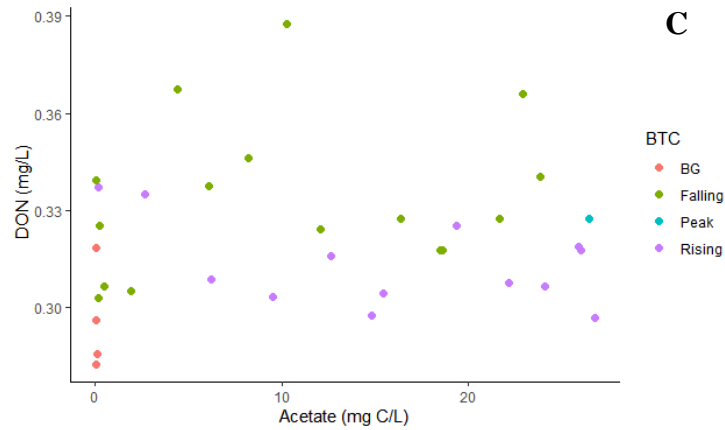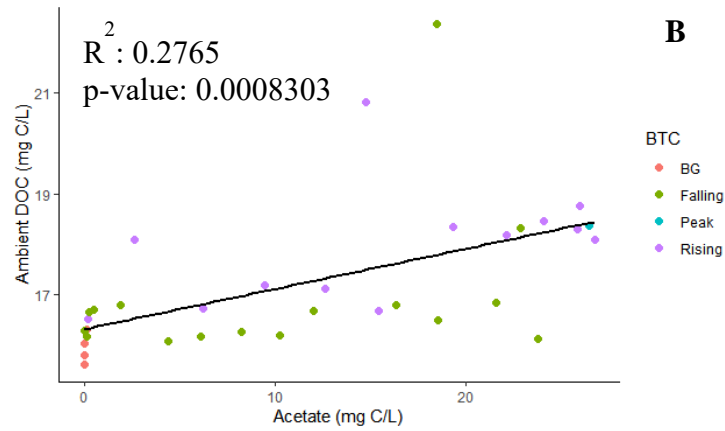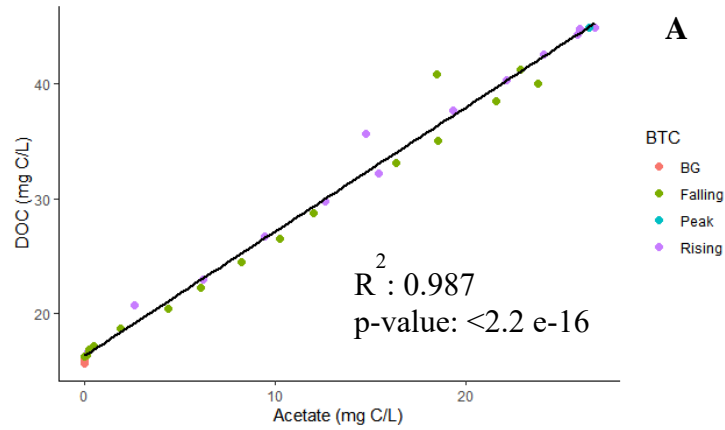
Relationship between manipulated acetate (mg C L^{-1}) in CRB (5/6/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved

organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
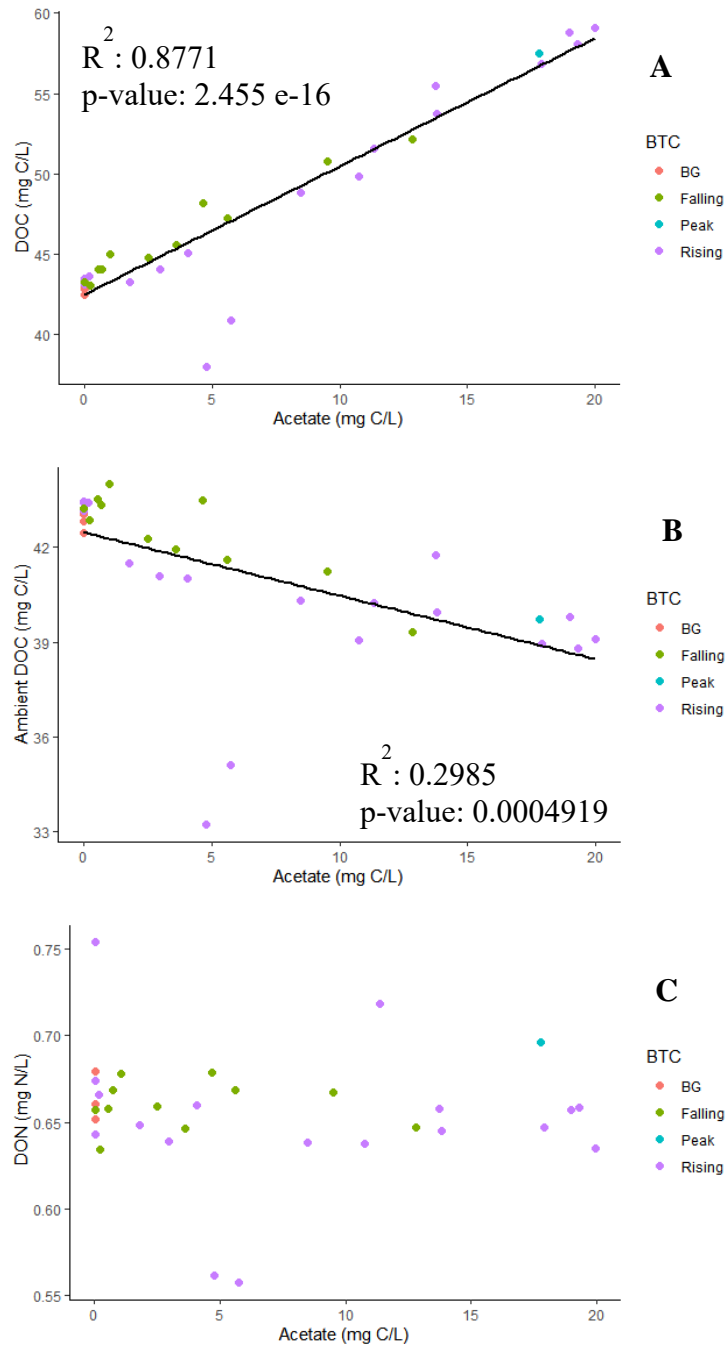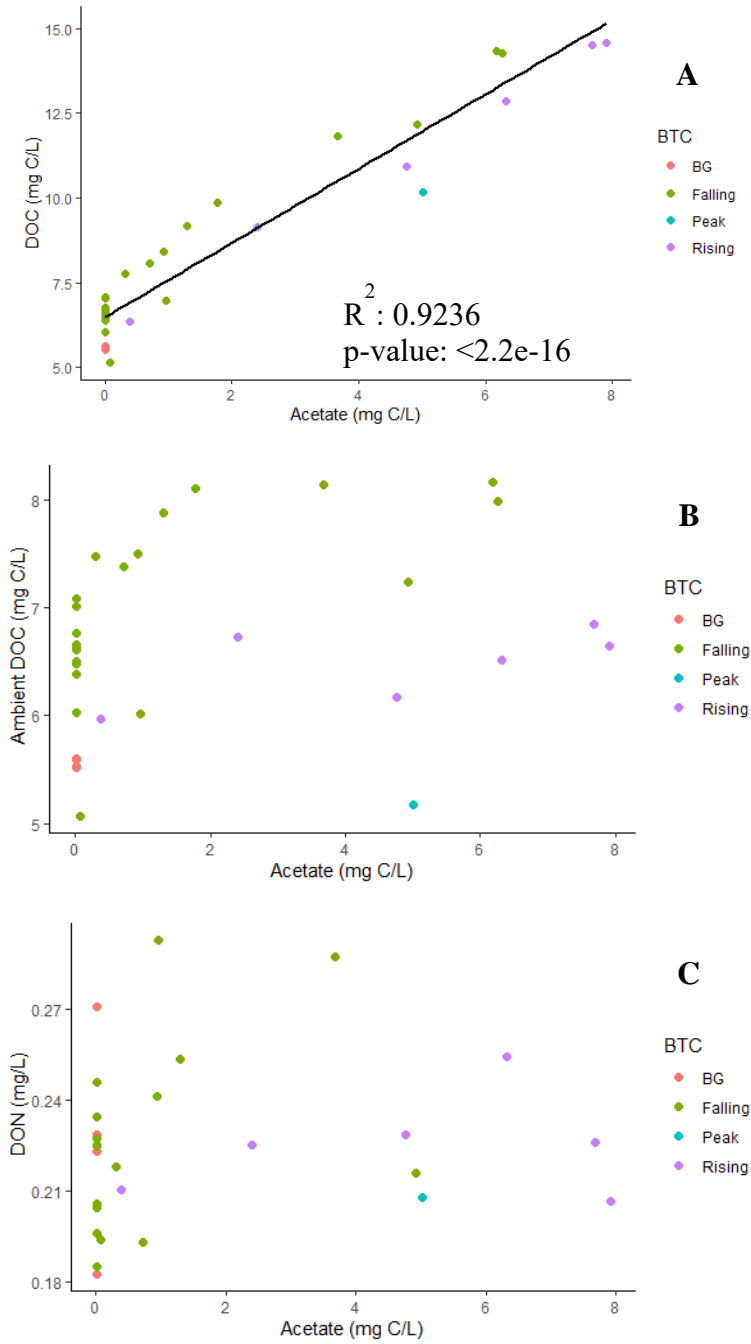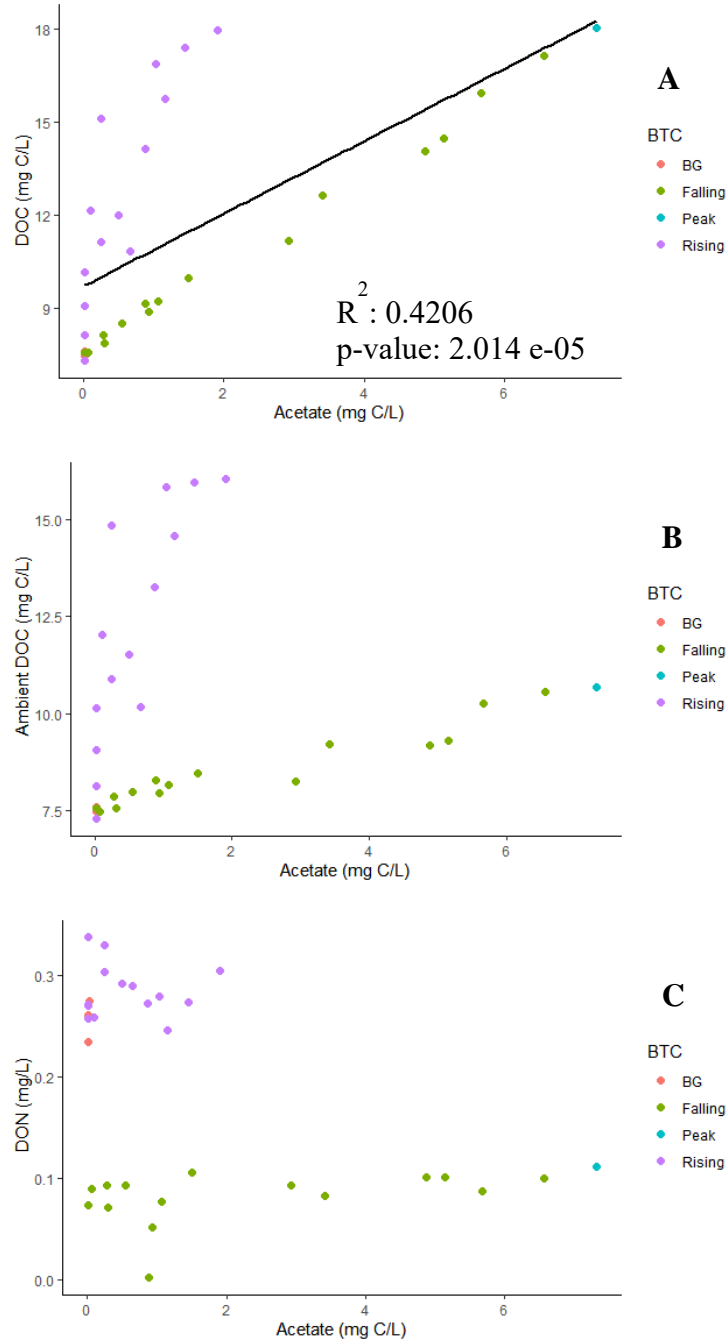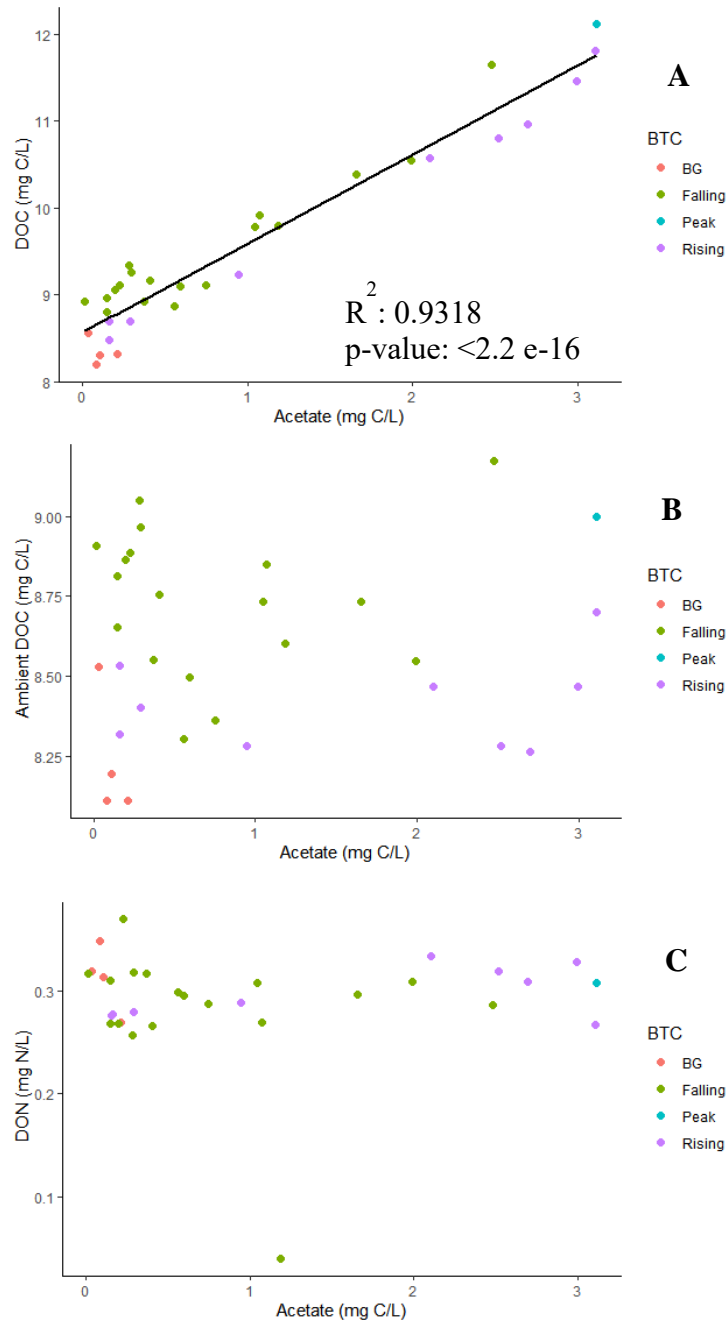CRB 2 (5/7/2019)
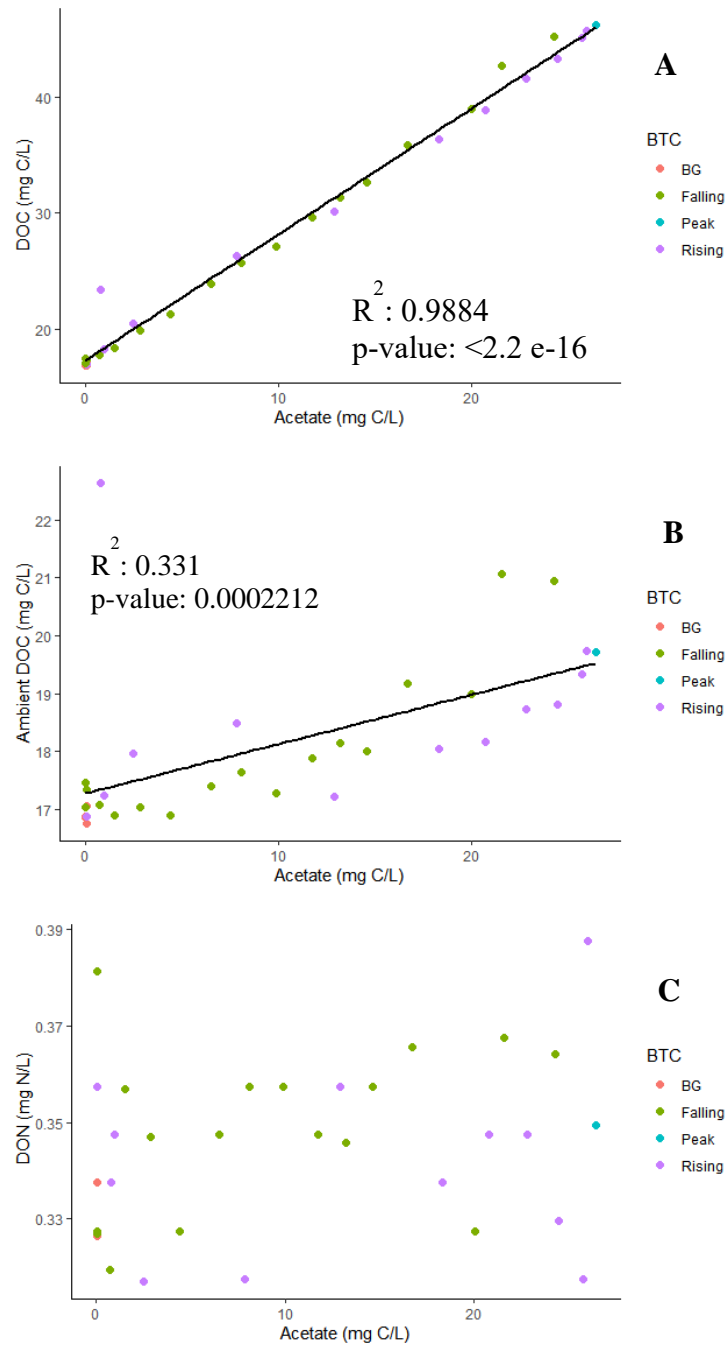
Relationship between manipulated acetate (mg C L^{-1}) in CRB (5/7/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).

CRB 3 (7/5/2019)
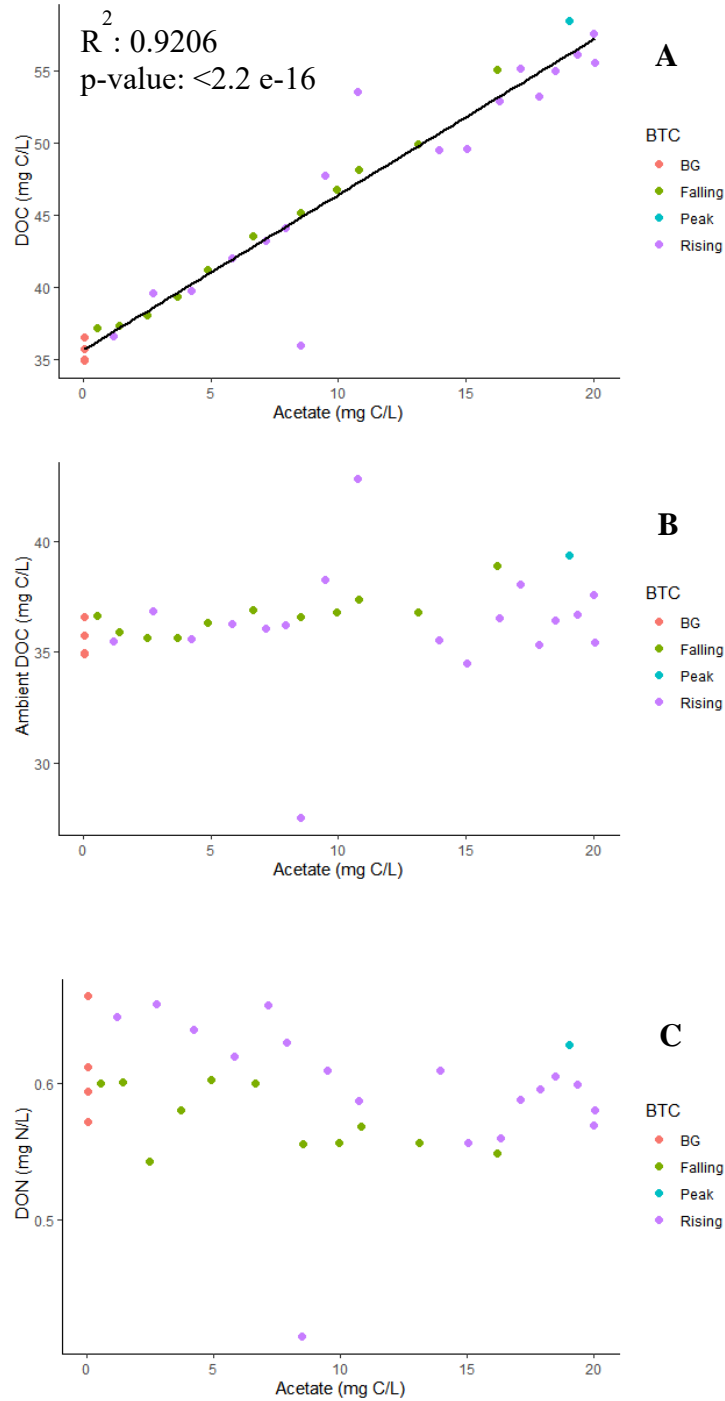
Relationship between manipulated acetate (mg C L^{-1}) in CRB (7/5/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
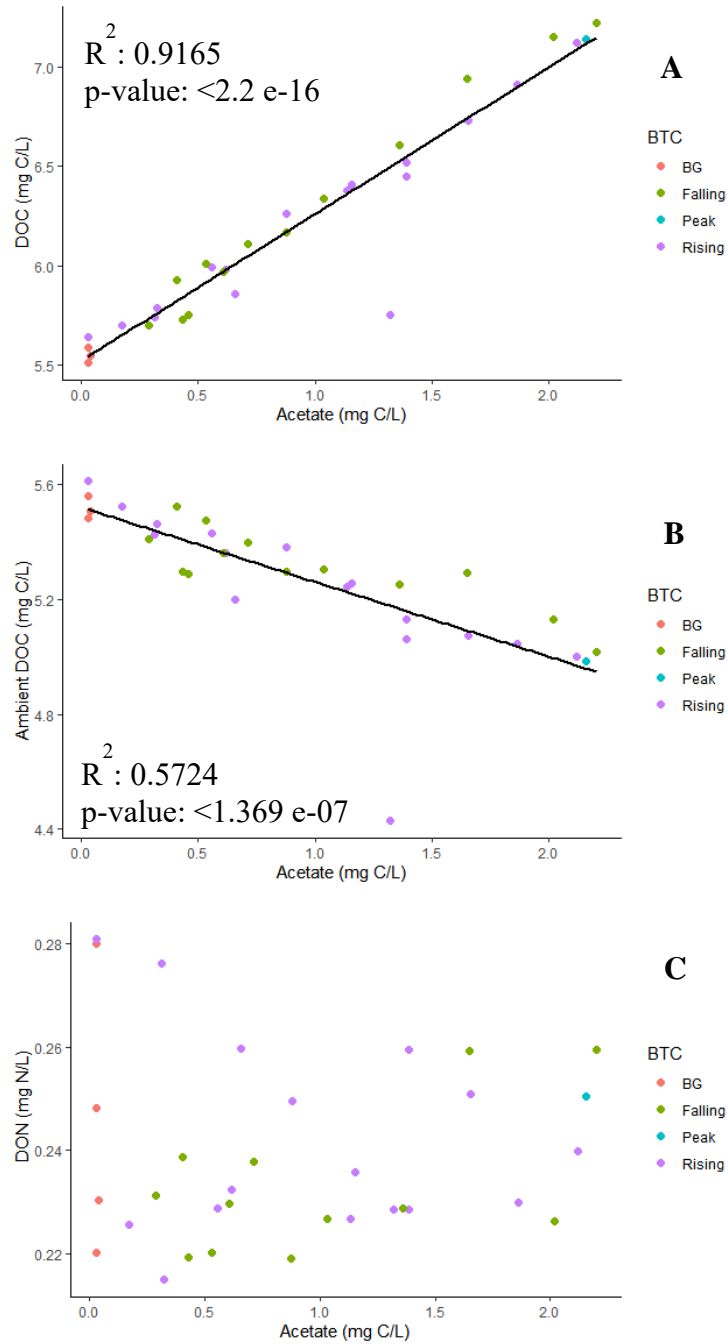
CRB 4 (10/11/2019)

Relationship between manipulated acetate (mg C L^{-1}) in CRB (10/11/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
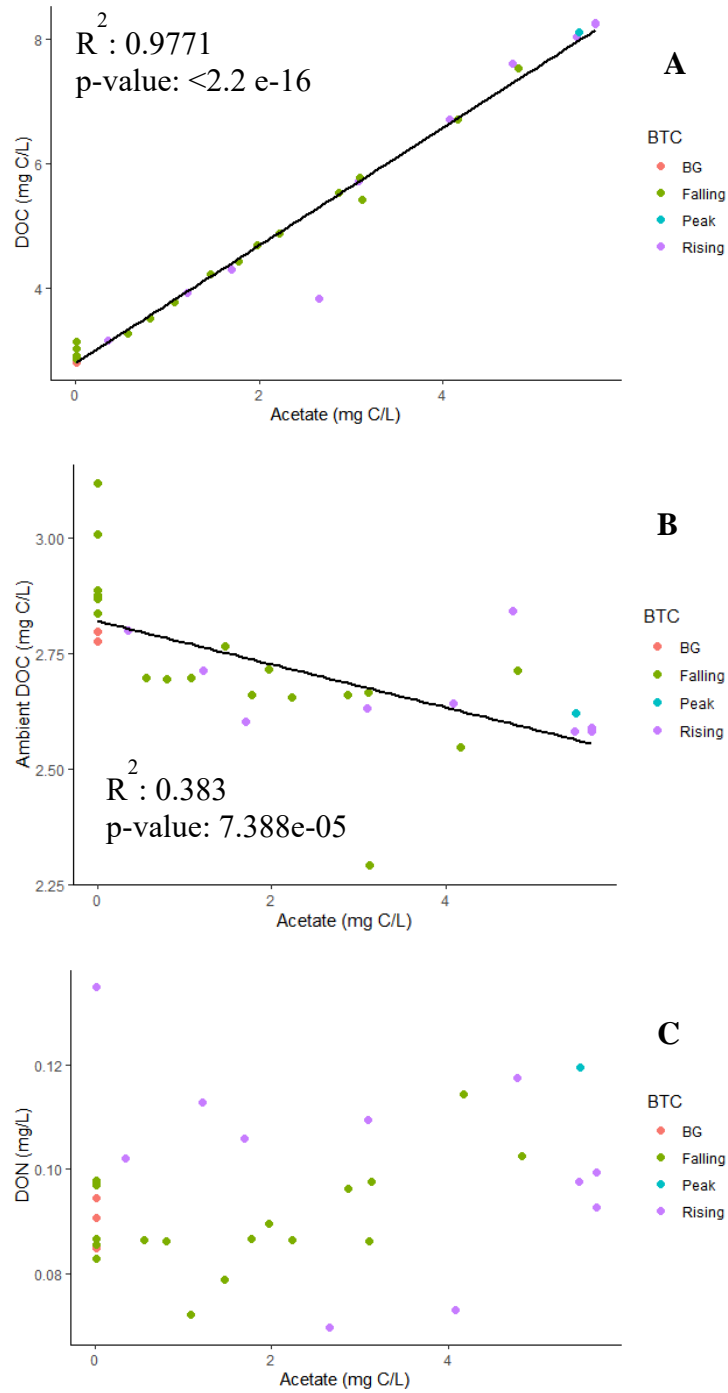
DCF 1 (5/24/2019)

Relationship between manipulated acetate (mg C L^{-1}) in DCF (5/24/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
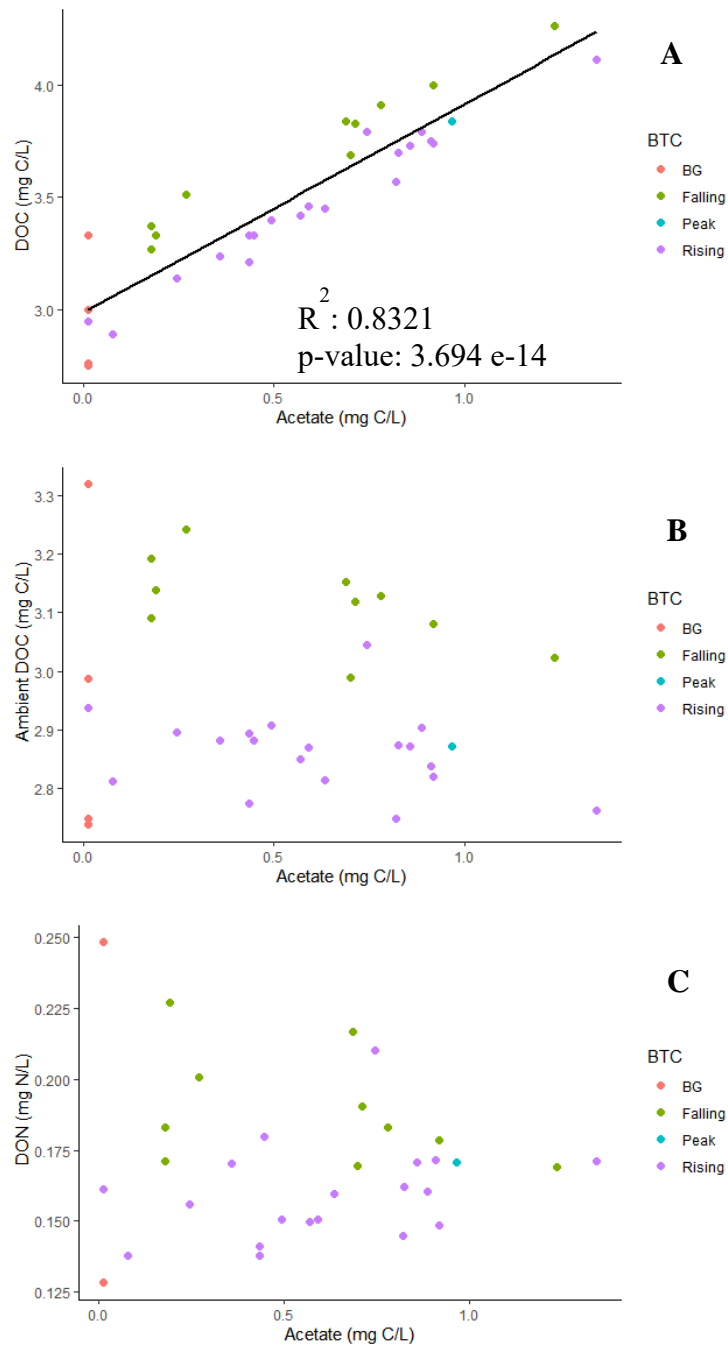
DCF 2 (6/27/2019)

Relationship between manipulated acetate (mg C L^{-1}) in DCF (6/27/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
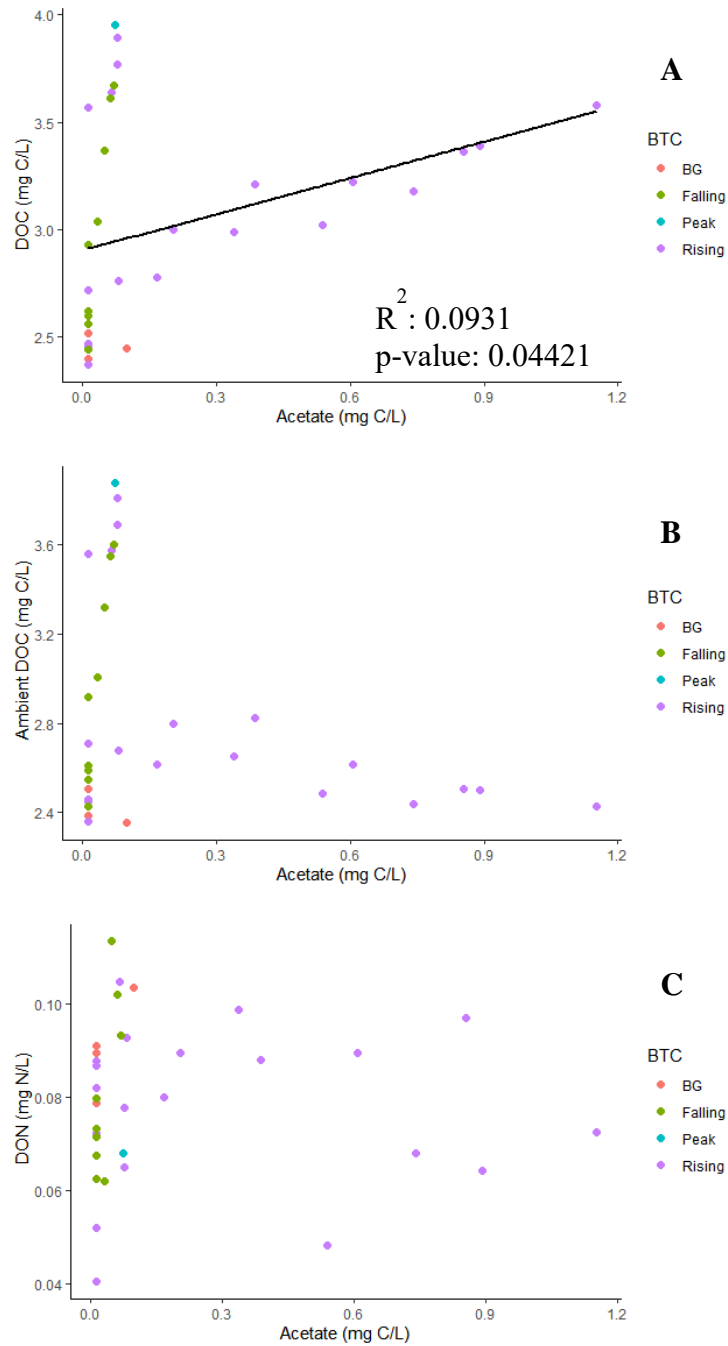
DCF 3 (7/25/2019)

R^2: 0.9318
p-value: <2.2 e-16

Relationship between manipulated acetate (mg C L^{-1}) in DCF (7/25/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
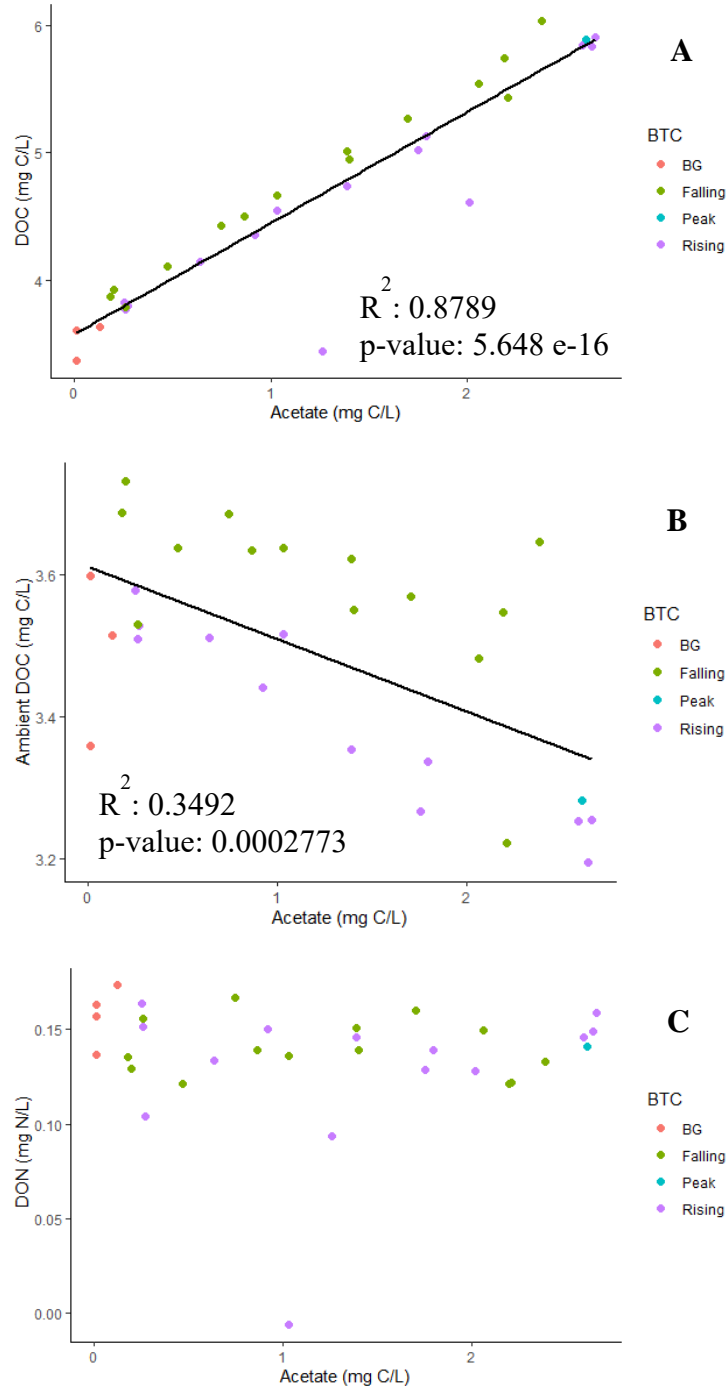
DCF 4 (9/6/2019)

Relationship between manipulated acetate (mg C L^{-1}) in DCF (9/6/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).

Relationship between manipulated acetate (mg C L^{-1}) in TPB (5/31/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
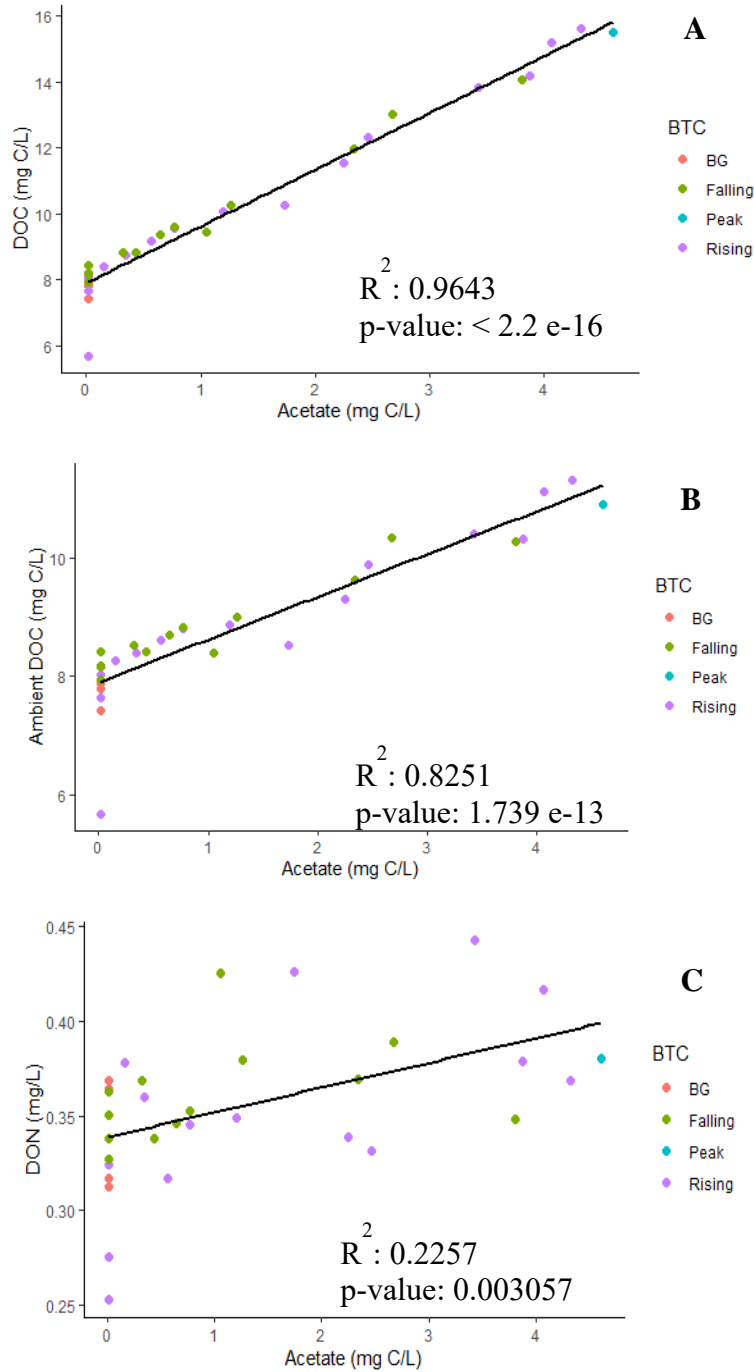
Relationship between manipulated acetate (mg C L^{-1}) in TPB (7/19/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
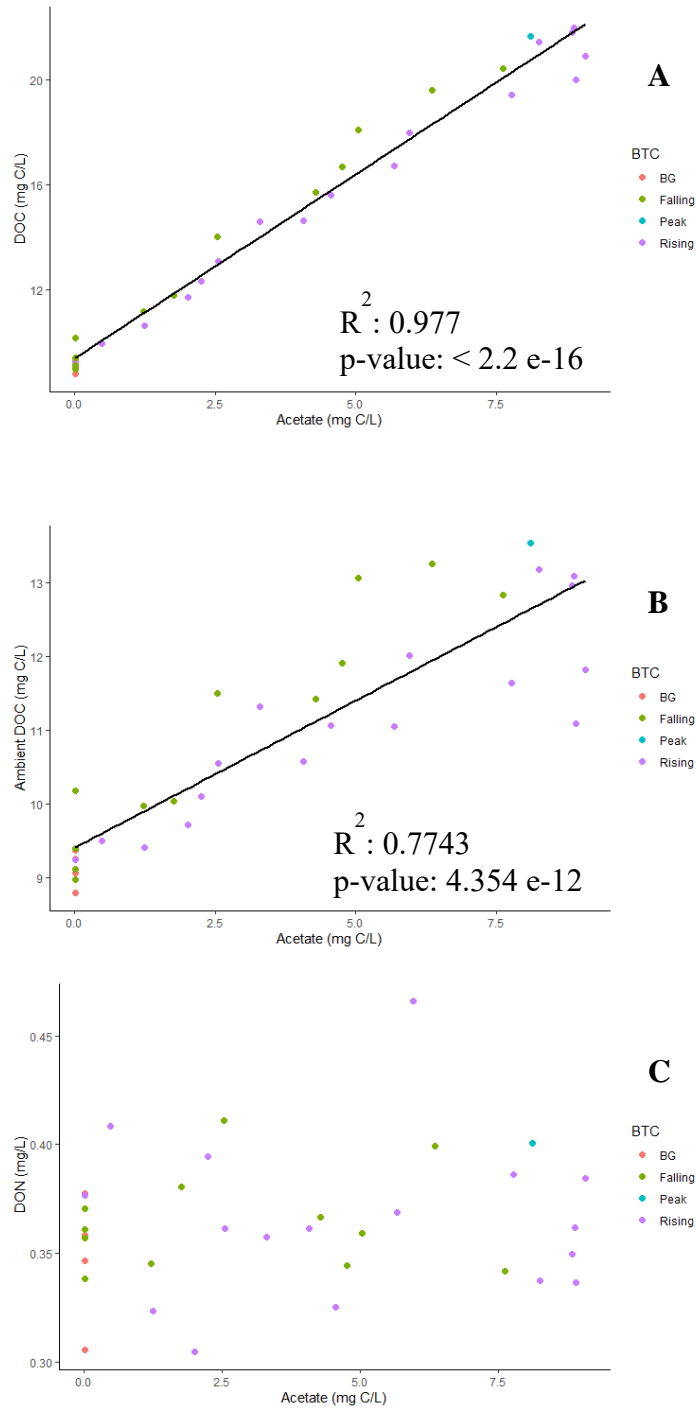
TPB 3 (8/16/2019)

Relationship between manipulated acetate (mg C L^{-1}) in TPB (8/16/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
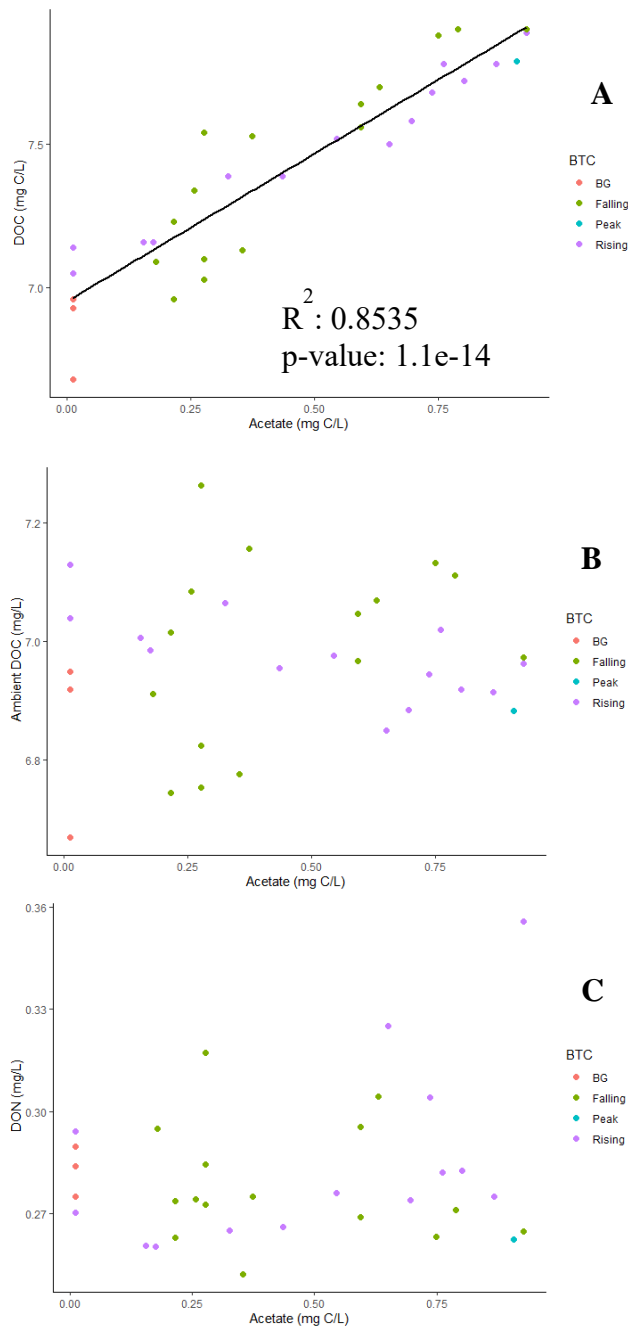
TPB 4 (10/30/2019)

Relationship between manipulated acetate (mg C L^{-1}) in TPB (10/30/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).

RMB 1 (6/19/2019)

Relationship between manipulated acetate (mg C L^{-1}) in RMB (6/19/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
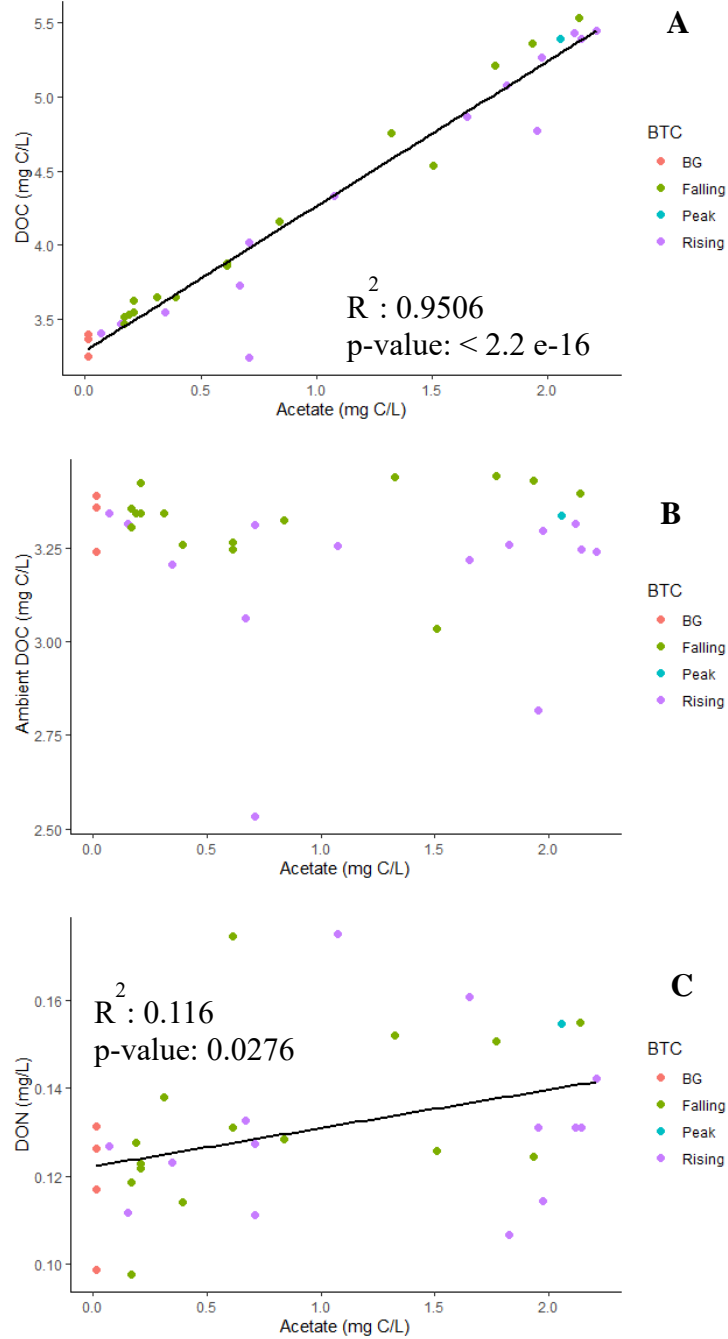
Relationship between manipulated acetate (mg C L^{-1}) in RMB (7/3/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).

Relationship between manipulated acetate (mg C L^{-1}) in RMB (8/23/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).

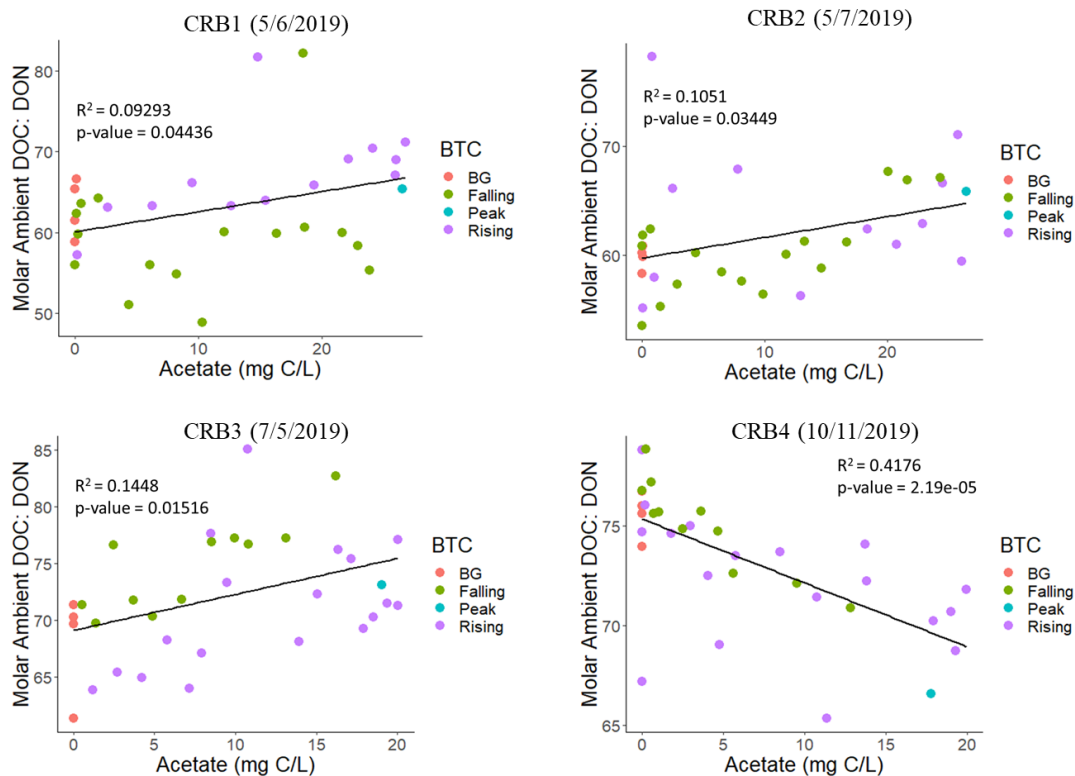Relationship between manipulated acetate (mg C L^{-1}) in RMB (9/20/2019) and (**A**) dissolved organic carbon (DOC), (**B**) ambient dissolved organic carbon (AmbDOC) and (**C**) dissolved
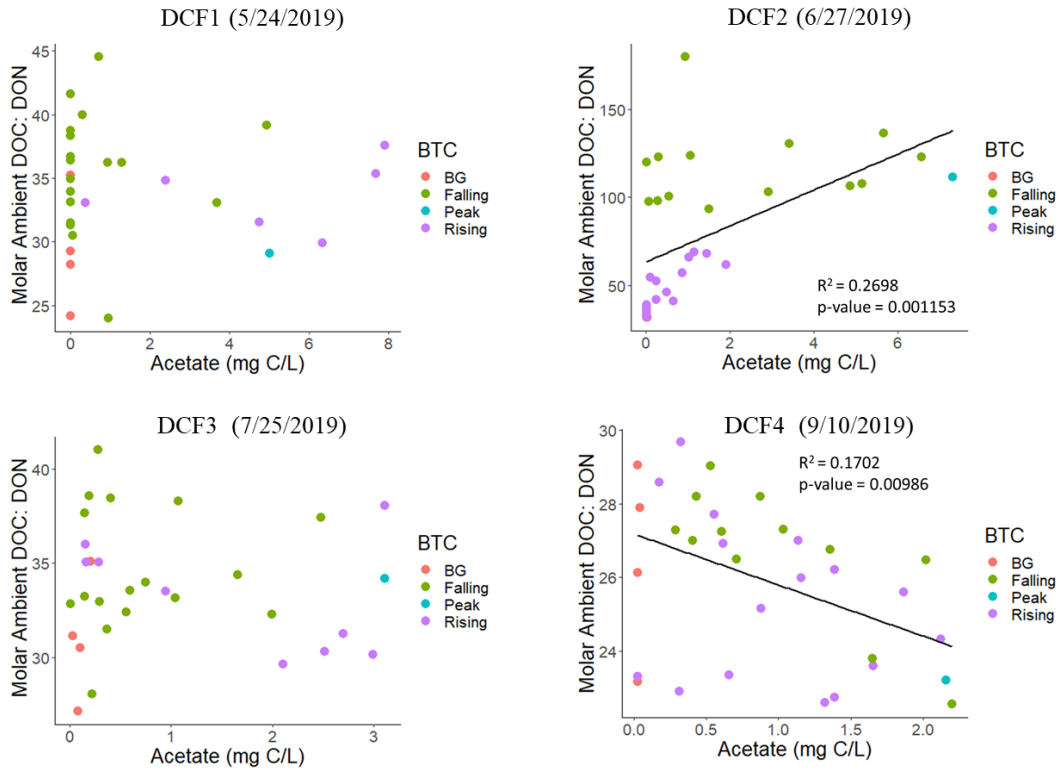
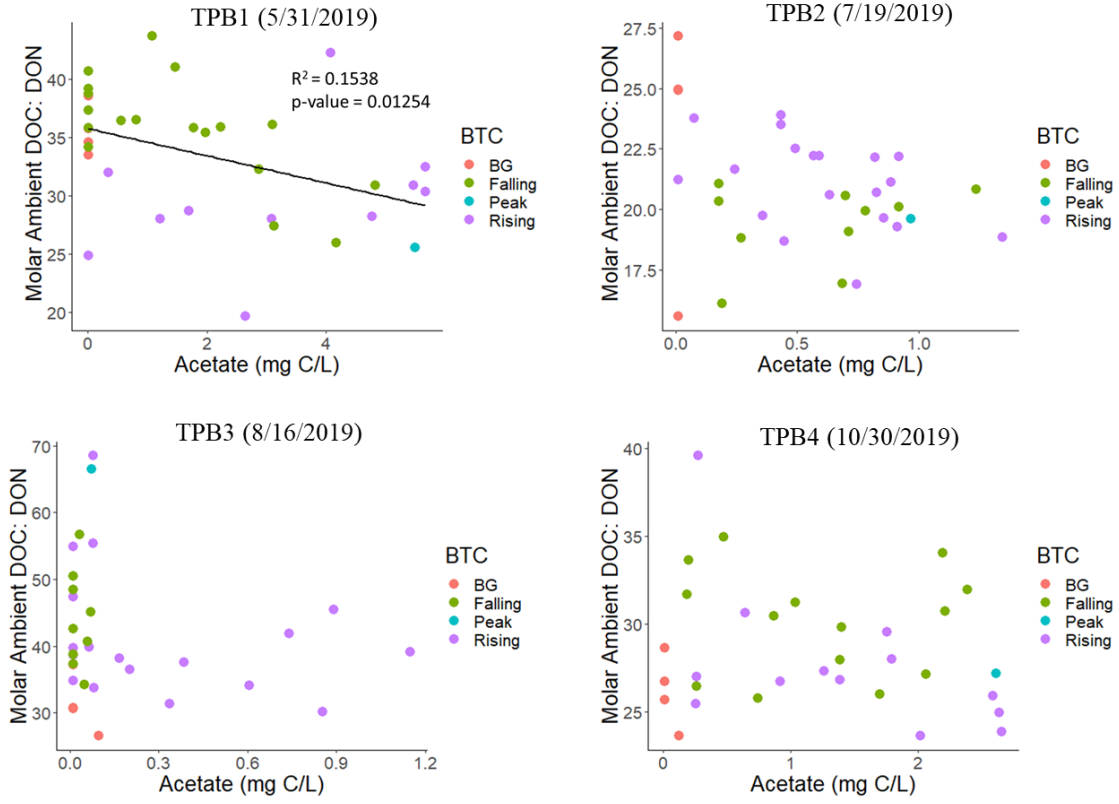organic nitrogen (DON). Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
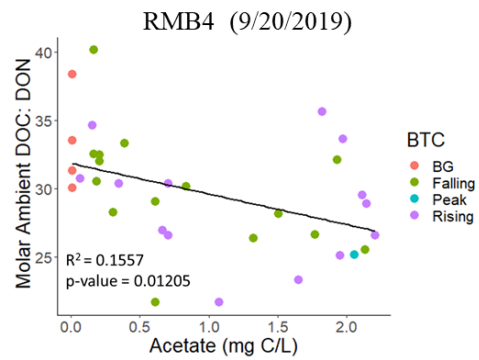
Individual responses for molar DOM ratios

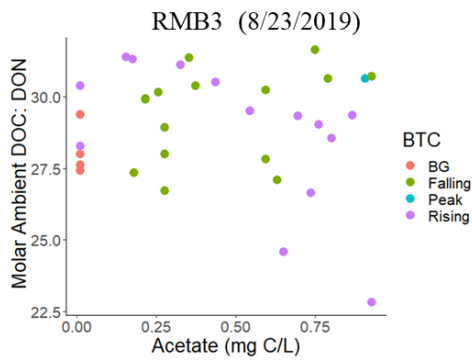Relationship between manipulated acetate (mg C L^{-1}) and molar ambient DOC: DON ratios in CRB for all additions conducted. Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).

Relationship between manipulated acetate (mg C L^{-1}) and molar ambient DOC: DON ratios in DCF for all additions conducted. Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
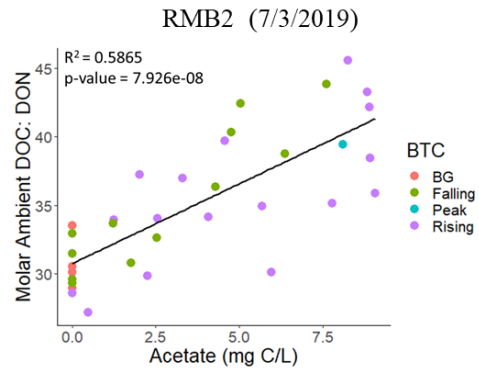
Relationship between manipulated acetate (mg C L^{-1}) and molar ambient DOC: DON ratios in TPB for all additions conducted. Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
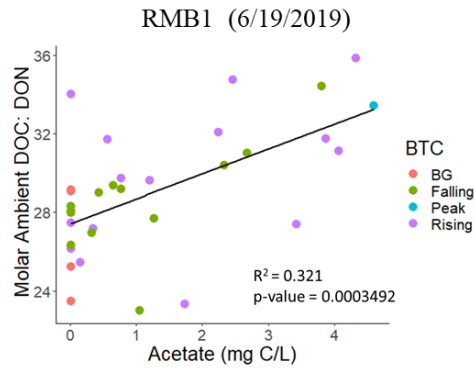
Relationship between manipulated acetate (mg C L^{-1}) and molar ambient DOC: DON ratios in RMB for all additions conducted. Figure represents all samples collected before the addition (BG) and during the BTC (rising, peak and falling).
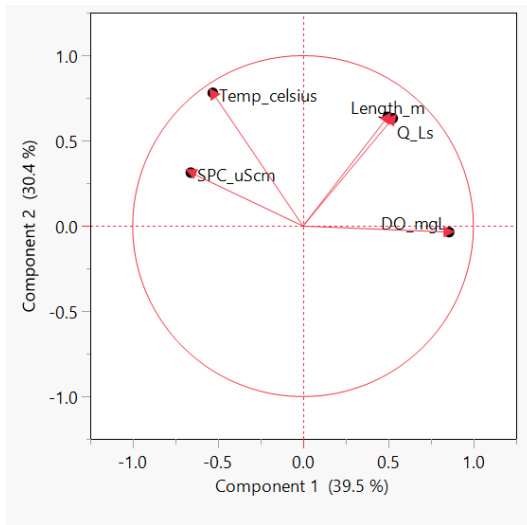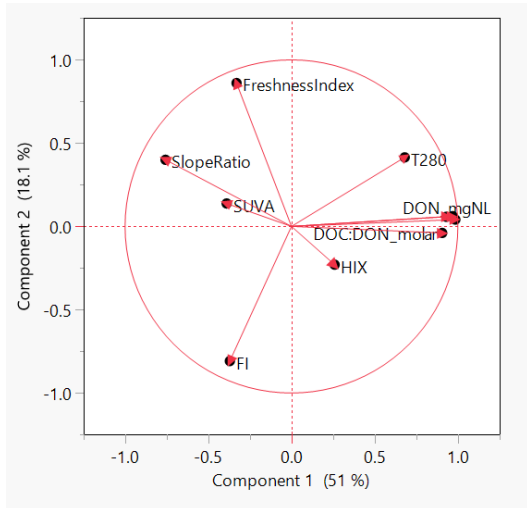
Principal Component analyses (PCA's)

Formatted Loading Matrix		
	Prin1	Prin2
DO_mgL	0.857362	-0.035219
Temp_celsius	-0.529686	0.781427
Length_m	0.496523	0.640162
Q_Ls	0.526401	0.632205
SPC_uScm	-0.658812	0.313506

Principal component analysis for physicochemical properties for all 16 additions conducted.

Formatted Loading Matrix		
	Prin1	Prin2
DOC_mgC.L	0.984657	0.040983
Abs254	0.968922	0.061811
DON_mgNL	0.930349	0.057899
DOC:DON_molar	0.905699	-0.038928
T280	0.680536	0.414720
FreshnessIndex	-0.330307	0.860624
SlopeRatio	-0.757081	0.400068
HIX	0.259362	-0.229668
SUVA	-0.389645	0.137782
FI	-0.370502	-0.807882

Principal component analysis for background dissolved organic matter concentrations and composition for all additions. One addition was excluded from the model given that not composition data was available for that day.

Formatted Loading Matrix

	Prin1	Prin2
DO_mgL	0.845211	-0.263786
Q_Ls	0.648279	0.432901
Length_m	0.634222	0.309704
Temp_celsius	-0.276010	0.896685
SPC_uScm	-0.455540	0.502703
BTC Vf (mm min-1)	-0.052935	0.335329
BTC Sw (m)	-0.566592	-0.423831
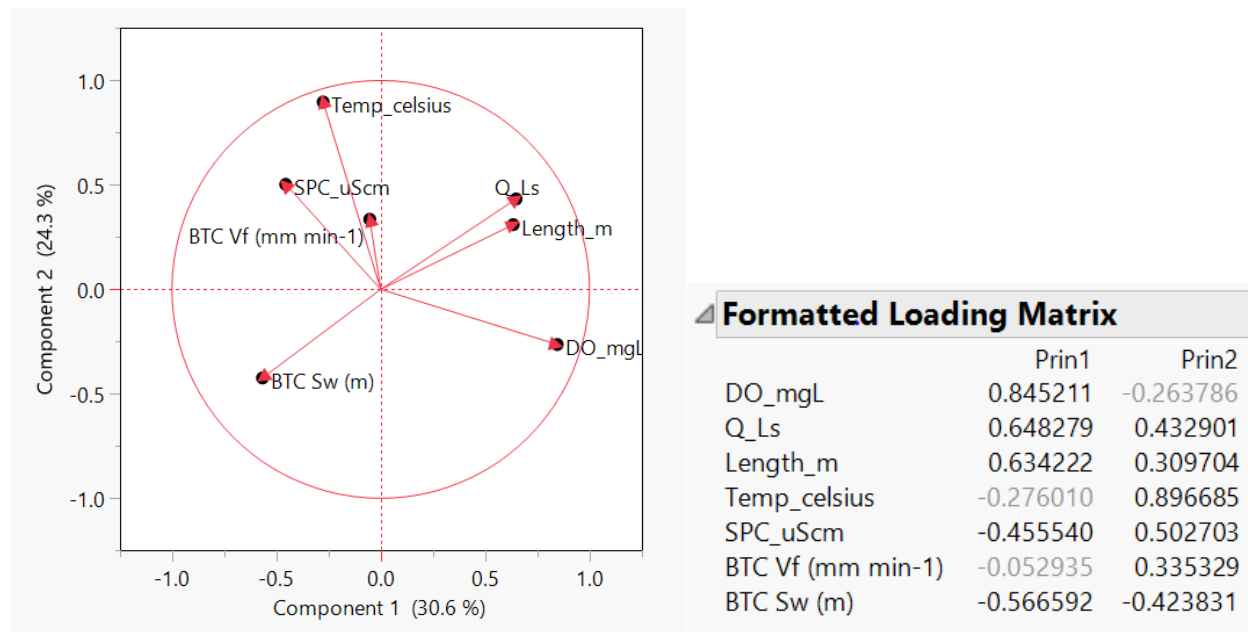
Principal component analysis for physicochemical properties and acetate uptake kinetics for all 16 additions conducted.
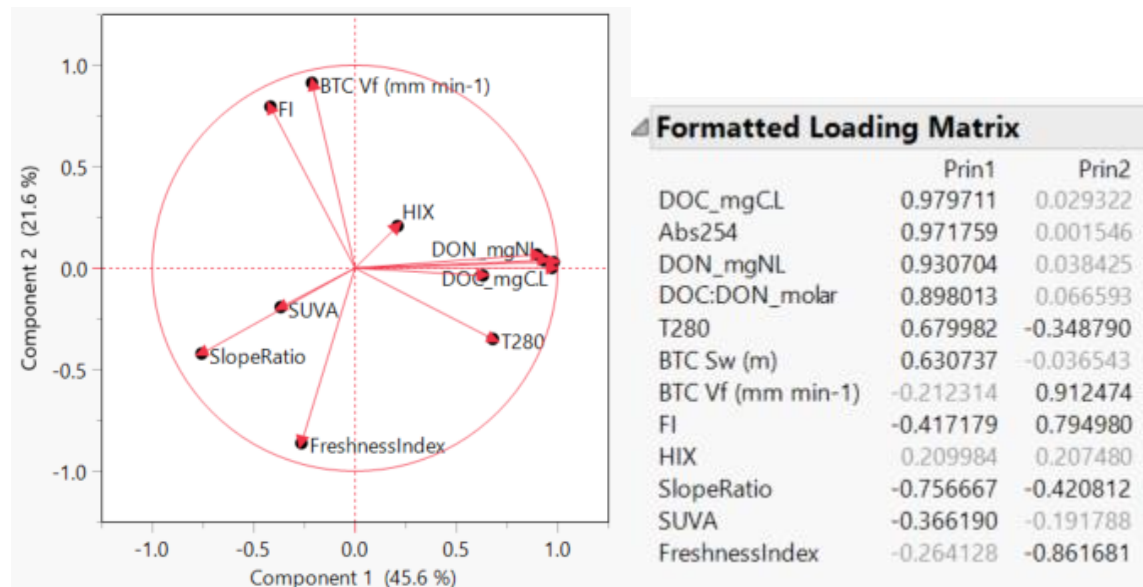
Formatted Loading Matrix

	Prin1	Prin2
DOC_mgCL	0.979711	0.029322
Abs254	0.971759	0.001546
DON_mgNL	0.930704	0.038425
DOC:DON_molar	0.898013	0.066593
T280	0.679982	-0.348790
BTC Sw (m)	0.630737	-0.036543
BTC Vf (mm min-1)	-0.212314	0.912474
FI	-0.417179	0.794980
HIX	0.209984	0.207480
SlopeRatio	-0.756667	-0.420812
SUVA	-0.366190	-0.191788
FreshnessIndex	-0.264128	-0.861681

Principal component analysis for background dissolved organic matter concentrations and composition, and acetate uptake kinetics for all additions. One addition was excluded from the model given that not composition data was available for that day.

58