PULSE OF THE STREAM:
EVALUATION OF HIGH-FREQUENCY DISSOLVED ORGANIC CARBON AND NITRATE CONCENTRATIONS IN STREAM WATER FOR DIEL AND AUTUMN PERIODS

by

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A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Water Science and Policy

Summer 2016

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ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Dr. Shreeram Inamdar, for his guidance and patience over the past two years. He allowed me to grow independently while pushing me to step outside my comfort zone and truly expand my ability to understand data within a greater environmental context. Thank you to Dr. Thomas Parr and Dr. Angelia Seyfferth for serving on my thesis advisory committee. Through your guidance I have learned many perspectives of and approaches to scientific thinking, and I appreciate all of the feedback you provided me.

I would like to thank: Fair Hill Natural Resource Management Area for allowing us to study water chemistry in their streams; the DEOS staff for providing meteorological data for analysis and their technical expertise setting up the electronics and telemetry for our instrumentation in the second order stream; and the NSF for providing the funding that made my research possible, grant number IIA 1330238. Thank you to Jillian Matz for assisting me with my diel study during her summer internship and to all the interns who helped sample every hour for three days during a heat wave.

I extend a special thanks to my fellow students Janice Hudson, Erin Johnson, Chelsea Krieg, Doug Rowland, Dan Warner, Brianna McEvoy, and Brié Levesque for creating a space of intellectual exchange on and off campus and for their amazing support through the ups and downs that are a part of graduate student life. Last, but certainly not least, thank you, Mom, Dad, Anna, and Phil for your love and support throughout the years.
# TABLE OF CONTENTS

LIST OF TABLES ....................................................................................................................... vii
LIST OF FIGURES ..................................................................................................................... ix
ABSTRACT ................................................................................................................................. xxvi

Chapter

1 INTRODUCTION ...................................................................................................................... 1

2 LITERATURE REVIEW ........................................................................................................... 6
   2.1 Importance of Sub-hourly, in situ Measurements ............................................................... 6
   2.2 Abiotic Processes ............................................................................................................... 8
      2.2.1 Temperature and light .............................................................................................. 8
      2.2.2 Dissolved gases ....................................................................................................... 9
      2.2.3 Hydrology ............................................................................................................... 10
      2.2.4 Effects of abiotic factors on solutes ....................................................................... 10
   2.3 Biologic Processes .......................................................................................................... 11
      2.3.1 Effects of biotic factors on solutes ....................................................................... 12
   2.4 Comparing abiotic vs. biotic influence of DOM cycling ............................................... 13
   2.5 Leaf chemistry and decomposition .............................................................................. 14
      2.5.1 Tracking decomposition ....................................................................................... 15
   2.6 Microbial processing and nutrients in streams ............................................................. 16
   2.7 Leaf fall and stream chemistry .................................................................................... 18

3 METHODS ............................................................................................................................. 21
   3.1 Site Description ............................................................................................................ 21
   3.2 Field Methods .............................................................................................................. 21
      3.2.1 In-stream sensors ................................................................................................. 22
3.2.1.1 Sensor calibration ......................................................... 23
3.2.2 Water sampling ............................................................ 25
3.2.3 Chemical analyses ......................................................... 25

3.3 Exploration of diel patterns of stream water DOC and NO$_3^-$-N ........ 26
3.4 Exploration of DOC and NO$_3^-$-N Dynamics during Autumn Leaf Fall .. 28

3.4.1 Stream water incubations .............................................. 28

4 RESULTS .............................................................................. 37
4.1 Diel Variations in Stream Water DOC and NO$_3^-$-N .................. 37
4.1.1 Seasonal diel signals throughout the watershed .................... 37
4.1.2 Differences in 2015 and 2016 winters and the strength of diel patterns ............................................................................................................. 42

4.2 Incubation ............................................................................. 42
4.2.1 Nitrogen consumption ..................................................... 43
4.2.2 Carbon concentration and composition .............................. 45

4.3 Coupled Changes in Stream Water NO$_3^-$-N and DOC during Autumn... 48
4.3.1 Tracking patterns with grab samples ................................... 48
4.3.2 High-frequency measurements capture autumn NO$_3^-$ depression ................................................................. 48

5 DISCUSSION: COUPLED RESPONSES OF AND CONTROLS ON STREAM WATER CARBON AND NITROGEN ............................................. 104
5.1 Diel Patterns in Stream Water Carbon and Nitrogen ................. 104
5.2 Incubations Indicate Carbon Limitation ................................. 112
5.3 Changes in Carbon Input to Stream Waters Stimulate Heterotrophic Activity during Autumn ................................................................. 115
5.4 Conceptual Models of Carbon and Nitrogen Dynamics in a Forested Piedmont Headwater System ......................................................... 120
5.4.1 Temporal controls of solute patterns .................................... 121
5.4.2 Spatial controls of solute patterns ....................................... 122

6 CONCLUSIONS ....................................................................... 127
REFERENCES ............................................................................ 131
Appendix

A  ADDITIONAL DIEL GRAPHS ........................................................... 138
B  ADDITIONAL AUTUMN GRAPHS .................................................. 183
C  INCUBATION TIME SERIES ........................................................... 192
LIST OF TABLES

Table 3.1 R² and adjusted R² values for the four models used to calibrate the sensor data used throughout this thesis, which show no evidence of the issue of multicollinearity among variables sometimes evident when using multiple regression analyses................................................................. 32

Table 3.2 Laboratory incubation treatment abbreviations and descriptions............. 36

Table 4.1 Summary of the ST12 monthly averages of the daily values and timing of maximum and minimum DOC and NO₃-N, the amplitude of the change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.......................................................... 52

Table 4.2 Summary of the ST12 monthly averages of the daily values and timing of the maximum DO, maximum and minimum temperature, amplitude of temperature change over the course of a day, and how long the temperature change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month......................................................................................... 53

Table 4.3 Summary of the ST12 monthly averages of the daily values and timing of the maximum and minimum Q and FDOM, amplitude of change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.......................................................... 54

Table 4.4 Summary of the monthly averages of the daily values and timing of the maximum and minimum ground water depth at two wells, amplitude of change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.................................................. 54
Table 4.5 Summary of the ST3 monthly averages of the daily values and timing of maximum and minimum DOC and NO$_3$-N, the amplitude of the change over the course of a day, and how long the change took during 2015 and spring 2016. Values used to determine the maximum and minimum daily concentrations of DOC and NO$_3$ were moving averages calculated for every four hours, or eight measurements, of concentration data. Red values indicate data or patterns were present for fewer than 10 days during that month. ........................................55

Table 4.6 Summary of the ST3 monthly averages of the daily values and timing of the maximum and minimum Q, amplitude of Q change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month. ........................................56

Table 4.7 Carbon to nitrogen ratios for each treatment of the lab incubation whose purpose was to add carbon and/or nitrogen to the system..................78

Table 4.8 The bioavailability of each carbon treatment solution and the overall BDOC. BDOC$_{DOM}$ is the bioavailability of the treatment solution and %BDOC is the overall BDOC of the treatment when added to stream water. ........................................................................................79

Table 5.1 Table of previous findings of diel patterns of DOC. Site description, summary of patterns, and controlling mechanisms behind the patterns are included. The text for the process controlling the patterns is color coded bases on which process was dominant: blue indicates hydrology dominated, green indicates biotic processes dominated, and purple indicates a mix of controls were found. ........................................123

Table 5.2 Table of previous findings of diel patterns of NO$_3$-N. Site description, summary of patterns, and controlling mechanisms behind the patterns are included. The text for the process controlling the patterns is color coded bases on which process was dominant: blue indicates hydrology dominated, green indicates biotic processes dominated, and purple indicates a mix of controls were found. ........................................124
LIST OF FIGURES

Figure 3.1 Map of our watershed with sampling sites marked. ST3 is the outlet of the 12 ha catchment and ST12 is the outlet of the 79 ha catchment where the spectrolysers are deployed. LW2 and LW5 are groundwater wells................................................................. 32

Figure 3.2 PLSR calculated DOC values regressed on lab DOC for ST3 2015 data... 33

Figure 3.3 PLSR calculated NO$_3^-$-N values regressed on lab NO$_3^-$-N for ST3 2015 data. .................................................................................................................. 33

Figure 3.4 PLSR calculated DOC values regressed on lab DOC for ST12 2015 data. 34

Figure 3.5 PLSR calculated NO$_3^-$-N values regressed on lab NO$_3^-$-N for ST12 2015 data. .................................................................................................................. 34

Figure 3.6 DO, temperature, NO$_3^-$-N, DOC, FDOM, and Q all exhibited diel patterns at ST12 in April 2015. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. ............................................ 35

Figure 3.7 Comparison of diel patterns of groundwater and stream discharge. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph...................... 36

Figure 4.1 Warmer temperatures during winter 2016 vs. winter 2015 increased the magnitude of diel patterns in stream solutes during spring 2016......... 51

Figure 4.2 Diel patterns at ST12 were observed in NO$_3^-$, DO, temperature, and DOC in March 2015. FDOM appeared to also exhibit a diel pattern, but not enough data was present to definitively conclude if a pattern was present. Diel Q cycling is weak during the early spring. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.. 57
Figure 4.3 DO, temperature, NO$_3^-$, DOC, FDOM, and Q all exhibited patterns at ST12 in April 2015. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.................................58

Figure 4.4 DO, temperature, NO$_3^-$, DOC, FDOM, and Q all exhibited patterns at ST12 in May 2015 but the amplitudes of the patterns were less than April. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. ................................................................. 59

Figure 4.5 Groundwater data in both wells indicate a drought occurred throughout later summer and early autumn 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.......................................................... 60

Figure 4.6 The strength of NO$_3^-$ and DOC patterns increased during October 2015 compared to summer months. DO and temperature amplitudes were similar to the preceding months. Discharge measurements were inaccurate during this time and inaccurate data was reported as a minimum value. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A...................................................... 61

Figure 4.7 After a February 2016 storm, NO$_3^-$ diel patterns recovered more rapidly than Q. Diel DOC patterns were not observed during this month. The grey box indicates the storm period and immediate return to diel NO$_3^-$ -N patterns as baseflow resumes. Solid lines are midnight. Dotted lines are noon. ............................................................. 62

Figure 4.8 A March storm had little effect on the diel patterns of NO$_3^-$-N during spring 2016. The grey box indicates the storm period and highlights that the NO$_3^-$-N pattern goes through a brief dilution at the peak of the storm but recovers the diel pattern in less than 24 hrs. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. ......................... 63
Figure 4.9 Stronger and warmer temperature signals led to increased amplitude in NO$_3$-N and DOC patterns during March 2016. Solid lines are midnight. Dotted lines are noon. Black arrow highlights the temperature increase. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. .......................... 64

Figure 4.10 ST12 diel conductance pattern in phase with NO$_3$-N, water temperature, discharge, and groundwater during May 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .......................... 65

Figure 4.11 ST12 diel conductance pattern in phase with NO$_3$-N, water temperature, discharge, and groundwater during December 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .......................... 65

Figure 4.12 ST12 diel conductance pattern in phase with NO$_3$-N, water temperature, discharge, and groundwater during March 2016. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .......................... 66

Figure 4.13 ST12 diel conductance pattern out of phase with NO$_3$-N, water temperature, and groundwater during September 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .......................... 66

Figure 4.14 ST12 diel conductance pattern out of phase with NO$_3$-N, water temperature, and groundwater during November 2015 until the 11/19/15 storm which brought conductance back into phase with the other parameters. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .......................... 67

Figure 4.15 Daily solar radiation values plotted with ST12 March 2015 time series of NO$_3$-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .......................... 67

Figure 4.16 Daily solar radiation values plotted with ST12 May 2015 time series of NO$_3$-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .......................... 68
Figure 4.17 Daily solar radiation values plotted with ST12 February 2016 time series of NO₃⁻-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph............................................. 68

Figure 4.18 Daily solar radiation values plotted with ST12 April 2016 time series of NO₃⁻-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph......................................................... 69

Figure 4.19 Daily solar radiation values plotted with ST12 April 2015 time series of NO₃⁻-N, DOC, discharge, and groundwater. Weaker diel patterns of DOC occur on low solar radiation days (grey box) compared to high solar radiation days (green box). Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph......................................................... 69

Figure 4.20 Daily solar radiation values plotted with ST12 March 2016 time series of NO₃⁻-N, DOC, discharge, and groundwater. Weaker diel patterns of DOC occur on low solar radiation days (grey box) compared to high solar radiation days (green box). Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph......................................................... 69

Figure 4.21 High variability in scan data mutes March 2015 NO₃⁻-N and DOC patterns at ST3. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A......................................................... 70

Figure 4.22 Diel NO₃⁻-N and DOC patterns emerge strongly at ST3 in April 2015. Discharge begins to show clear diel patterns. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A......................................................... 71

Figure 4.23 Diel patterns are still evident for NO₃⁻-N and DOC at ST3 in May 2015 but daily magnitudes are less than those in April. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A......................................................... 71
Figure 4.24 Weak diel patterns in DOC and NO$_3^-$-N return in October after being muted during summer drought. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. .......................... 72

Figure 4.25 Comparison of diel patterns of groundwater and stream discharge. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. ........................................ 72

Figure 4.26 Diel patterns of DOC, NO$_3^-$-N, and groundwater level are non-existent at ST3 during March 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .............................................................. 73

Figure 4.27 Diel patterns of DOC, NO$_3^-$-N, and groundwater level begin to emerge at ST3 during April 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. ........................................................................................................ 73

Figure 4.28 The amplitude of diel patterns in groundwater level increased at ST3 during May 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .............................................................. 74

Figure 4.29 Diel patterns of DOC, NO$_3^-$-N, and groundwater levels at ST12 during May 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .............................. 74

Figure 4.30 Diel patterns of DOC, NO$_3^-$-N, and groundwater levels at ST12 during August 2015. For groundwater levels, red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. All other red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph. .................................................................................. 75
Figure 4.31 Diel patterns of DOC, NO$_3$-N, and groundwater levels at ST12 during October 2015. For groundwater levels, red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. All other red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.32 Diel patterns in NO$_3$-N and DOC show an increase in strength during March 2016 at ST3 during the same time water temperature was shown to increase the strength of diel patterns at ST12, indicated by the blue arrow. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.33 Since diel pattern of NO$_3$-N and DOC at ST3 is not as strong as ST12, the winter 2016 storms increase the time it takes for diel variations in NO$_3$-N to resume at ST3 compared to at ST12. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.34 Overall percent change in total nitrogen from $t_0$ to $t_{25}$ days for all incubation treatments.

Figure 4.35 ANOVA on rates of consumption or production of TN across all incubation treatments. Positive rates indicate consumption.

Figure 4.36 Overall percent change in NO$_3$-N from $t_0$ to $t_{25}$ days for all incubation treatments. Green dots for LEAF and GLU represent the value of the one replicate bottle which had data for those treatments.

Figure 4.37 ANOVA on rates of consumption NO$_3$-N across all incubation treatments. Positive rates indicate consumption. Green dots LEAF and GLU represent the value of the one replicate bottle which had data for those treatments.

Figure 4.38 Overall percent change in DOC from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.39 ANOVA on rates of consumption of DOC across all incubation treatments. Positive rates indicate consumption................................. 84

Figure 4.40 Overall percent change in protein-like fluorescence from t₀ to t₂₅ days for all incubation treatments............................................................... 85

Figure 4.41 ANOVA on rates of consumption of protein-like fluorescence across all incubation treatments. Positive rates indicate consumption.............. 86

Figure 4.42 Overall percent change in humic-like fluorescence from t₀ to t₂₅ days for all incubation treatments............................................................... 87

Figure 4.43 ANOVA on rates of consumption of humic-like fluorescence across all incubation treatments. Positive rates indicate consumption.............. 88

Figure 4.44 Overall percent change in fulvic-like fluorescence from t₀ to t₂₅ days for all incubation treatments............................................................... 89

Figure 4.45 ANOVA on rates of consumption of fulvic-like fluorescence across all incubation treatments. Positive rates indicate consumption.............. 90

Figure 4.46 Overall percent change in SUVA₂₅₄ from t₀ to t₂₅ days for all incubation treatments............................................................... 91

Figure 4.47 ANOVA on rates of change in SUVA₂₅₄ across all incubation treatments. Positive rates indicate decrease in SUVA₂₅₄ values over time. Decrease in SUVA₂₅₄ indicates a decrease in aromaticity and increase in bioavailability............................................................... 92

Figure 4.48 Overall percent change in Sᵣ from t₀ to t₂₅ days for all incubation treatments............................................................... 93

Figure 4.49 ANOVA on rates of change in Sᵣ across all incubation treatments. Positive rates indicate decrease in Sᵣ values over time, which would indicate an increase in high molecular weight DOM fraction.............. 94

Figure 4.50 [DOC], [NO₃⁻-N], protein-like fluorescence, Q, air temperature, and litter fall mass during autumn 2014 at ST3. Grab samples for [DOC], [NO₃⁻-N], and protein-like fluorescence analysis were collected three times per week............................................................... 95
Figure 4.51 [DOC], [NO$_3^-$-N], protein-like fluorescence, Q, air temperature, and litter fall mass during autumn 2014 at ST12. Grab samples for [DOC], [NO$_3^-$-N], and protein-like fluorescence analysis were collected three times per week. ................................................................. 96

Figure 4.52 Autumn 2014 water temperature, air temperature, DO, litter fall mass, turbidity and FDOM at ST12. DO, turbidity, water temperature, and FDOM were measured in situ. ................................................................. 97

Figure 4.53 Autumn 2015 Q, litter fall mass, DOC load, and NO$_3^-$-N load at ST3. DOC and NO$_3^-$-N axes are zoomed in to accentuate baseflow loads. ..... 98

Figure 4.54 Autumn 2015 Q, litter fall mass, in situ [DOC], and grab and storm [DOC], protein- and humic-like fluorescence, SUVA$_{254}$, and $S_R$ at ST3. ........................................................................................................ 99

Figure 4.55 Autumn 2015 NO$_3^-$ crash and recovery at ST3 and ST12 with litter leaf mass and ST12 Q to depict the influence of leaf fall and hydrology on autumn stream chemistry dynamics. Note the order of magnitude difference in load scales. ........................................................................ 100

Figure 4.56 Autumn 2015 Q, litter fall mass, DOC load, and NO$_3^-$-N load at ST12. 101

Figure 4.57 Autumn 2015 Q, litter fall mass, in situ [DOC], and grab and storm [DOC], protein- and humic-like fluorescence, SUVA$_{254}$, and $S_R$ at ST12. ........................................................................................................ 102

Figure 4.58 Autumn 2015 water temperature, air temperature, DO, litter fall mass, turbidity and FDOM at ST12. DO, turbidity, water temperature, and FDOM were measured in situ. ................................................................. 103
Figure 5.1 **Temporal controls:** The solute patterns here represent the diel patterns throughout the seasons. In the afternoon, DOC, DO, and temperature peak and NO$_3$-N is at its minimum. The discharge line represents the moisture conditions of the season throughout the year. Diel patterns of discharge are not depicted, but the maximum would be in the morning and the minimum would occur in the afternoon. The amplitude of diel patterns is greatest during the spring. DOC and NO$_3$-N have weak or non-existent patterns during the summer, while DO, temperature, and discharge patterns have the lowest amplitude in the summer. The amplitude of diel patterns of all parameters increase during autumn, but not to springtime levels. NO$_3$-N geos through a depression during autumn leaf fall, when more labile DOC enters the stream. DOC, NO$_3$-N, and discharge patterns disappear during mid-winter. NO$_3$-N patterns are the first to return in later winter.

Figure 5.2 **Spatial controls:** Above is a sketch of our watershed with embellished spatial differences in stream size and canopy cover. Hydrologic connectivity and canopy cover are greater in the smaller watershed. Solar radiation and stream residence time are greater throughout the larger watershed. These conditions allow for stronger biotic control of diel patterns of DOC, NO$_3$-N, and DO in the second order stream. At the first order stream, hydrology mutes biotic diel patterns. An earlier autumn decline in stream NO$_3$-N is caused by hydrologic disconnect between the stream and watershed sources of NO$_3$-N.

Figure A1 Time series of DO, temperature, NO$_3$-, DOC, FDOM, and Q of the 2015 dataset for ST12.

Figure A2 The axes in this ST12 March 2015 graph use the common scales used throughout the year.

Figure A3 The axes in this ST12 April 2015 graph use the common scales used throughout the year.

Figure A4 The axes in this ST12 May 2015 graph use the common scales used throughout the year.

Figure A5 The axes in this ST12 June 2015 graph use the common scales used throughout the year.

Figure A6 The axes in this ST12 July 2015 graph use the common scales used throughout the year.
Figure A7 The axes in this ST12 August 2015 graph use the common scales used throughout the year. 

Figure A8 The axes in this ST12 September 2015 graph use the common scales used throughout the year. 

Figure A9 The axes in this ST12 October 2015 graph use the common scales used throughout the year. 

Figure A10 The axes in this ST12 November 2015 graph use the common scales used throughout the year. 

Figure A11 The axes in this ST12 December 2015 graph use the common scales used throughout the year. 

Figure A12 The axes in this ST12 January 2016 graph use the common scales used throughout the year. 

Figure A13 The axes in this ST12 February 2016 graph use the common scales used throughout the year. 

Figure A14 The axes in this ST12 March 2016 graph use the common scales used throughout the year. 

Figure A15 The axes in this ST12 April 2016 graph use the common scales used throughout the year. 

Figure A16 DO, temperature, DOC, FDOM, and Q all exhibited patterns at ST12 in June 2015 but NO$_3^-$-N did not. June was a stormy month and patterns are weaker than May. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. 

Figure A17 DO, temperature, DOC, FDOM, and Q all exhibited patterns at ST12 in July 2015 but NO$_3^-$-N did not. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. 

Figure A18 NO$_3^-$-N cycling returned with low amplitude during August 2015 and DO, temperature, DOC, FDOM, and Q all exhibited patterns at ST12. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A19 All solutes exhibited diel patterns at ST12 in September 2015. High FDOM compared to the rest of the year may be due to drought antecedent conditions. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ................. 156

Figure A20 As [NO$_3^-$-N] recovers, diel cycling of NO$_3^-$ resumes during November 2015. All other solutes exhibited diel patterns at ST12 during this time. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ....................... 157

Figure A21 All solutes exhibited weak diel patterns at ST12 during December 2015. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ....................... 158

Figure A22 Only DO and temperature exhibited discernable diel patterns at ST12 during January 2016. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ................. 159

Figure A23 NO$_3^-$-N, DO and temperature exhibited discernable diel patterns at ST12 during February 2016. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ....................... 159

Figure A24 All solutes exhibited strong diel patterns with large amplitudes, except for Q amplitude, during April 2016 at ST12. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ............................................ 160

Figure A25 Time series of NO$_3^-$-N, DOC, and Q of the 2015 dataset for ST3. ............ 162

Figure A26 No diel patterns were present at ST3 during March 2015. The axes in this graph use the common scales used throughout the year. ....................... 162

Figure A27 The axes in this ST3 April 2015 graph use the common scales used throughout the year. .......................................................... 163

Figure A28 The axes in this ST3 May 2015 graph use the common scales used throughout the year. .......................................................... 163
Figure A29 The axes in this ST3 June 2015 graph use the common scales used throughout the year. ................................................................. 164

Figure A30 The axes in this ST3 July 2015 graph use the common scales used throughout the year. ................................................................. 164

Figure A31 The axes in this ST3 September 2015 graph use the common scales used throughout the year. ................................................................. 165

Figure A32 The axes in this ST3 October 2015 graph use the common scales used throughout the year. ................................................................. 165

Figure A33 The axes in this ST3 November 2015 graph use the common scales used throughout the year. ................................................................. 166

Figure A34 The axes in this ST3 December 2015 graph use the common scales used throughout the year. ................................................................. 166

Figure A35 The axes in this ST3 February 2016 graph use the common scales used throughout the year. ................................................................. 167

Figure A36 The axes in this ST3 March 2016 graph use the common scales used throughout the year. ................................................................. 167

Figure A37 Diel solute patterns were diluted in June 2015 at ST3, likely due to the occurrence of my storm events. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ................................................................. 168

Figure A38 No diel patterns were presence at ST3 during July 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ...................... 168

Figure A39 Only Q exhibited diel patterns at ST3 during September 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ...................... 169

Figure A40 Only Q exhibited diel patterns at ST3 during November 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ...................... 169

Figure A41 No diel patterns existed at ST3 during December 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year. ...................... 170
Figure A42 Time series graph of ST12 2015 DOC, NO$_3^-$-N, and groundwater levels from two wells. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A43 ST12 DOC, NO$_3^-$-N, and groundwater levels during March 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A44 ST12 DOC, NO$_3^-$-N, and groundwater levels during April 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A45 ST12 DOC, NO$_3^-$-N, and groundwater levels during June 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A46 ST12 DOC, NO$_3^-$-N, and groundwater levels during July 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A47 ST12 DOC, NO$_3^-$-N, and groundwater levels during September 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A48 ST12 DOC, NO$_3^-$-N, and groundwater levels during November 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A49 ST12 DOC, NO₃⁻-N, and groundwater levels during December 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A50 ST12 DOC, NO₃⁻-N, and groundwater levels during January 2016. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A51 ST12 DOC, NO₃⁻-N, and groundwater levels during February 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A52 ST12 DOC, NO₃⁻-N, and groundwater levels during March 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A53 ST12 DOC, NO₃⁻-N, and groundwater levels during April 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A54 ST3 2015 groundwater, DOC, and NO₃⁻-N time series. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A55 ST13 DOC, NO₃⁻-N, and groundwater levels during June 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A56 ST3 DOC, NO$_3$-N, and groundwater levels during July 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph................................. 178

Figure A57 ST3 DOC, NO$_3$-N, and groundwater levels during September 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.............................................. 179

Figure A58 ST3 DOC, NO$_3$-N, and groundwater levels during October 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph...................................................... 179

Figure A59 ST3 DOC, NO$_3$-N, and groundwater levels during November 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph...................................................... 179

Figure A60 ST3 DOC, NO$_3$-N, and groundwater levels during November 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph...................................................... 180

Figure A61 ST3 DOC, NO$_3$-N, and groundwater levels during February 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.. 181

Figure A62 ST3 DOC, NO$_3$-N, and groundwater levels during March 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.. 182
Figure B1 Q, leaf litter mass, weekly rates of change in NO$_3^-$ and DOC loads, and monthly rates of change in NO$_3^-$ and DOC loads at ST3 during autumn 2015. ................................................................. 184

Figure B2 Fulvic-like components of our PARAFAC model with DOC, Q, and litter mass at ST3 during autumn 2015. ................................................................. 185

Figure B3 Protein-like component of our PARAFAC model with DOC, Q, and litter mass at ST3 during autumn 2015. ................................................................. 186

Figure B4 Humic-like components of our PARAFAC model with DOC, Q, and litter mass at ST3 during autumn 2015. ................................................................. 187

Figure B5 Q, leaf litter mass, weekly rates of change in NO$_3^-$ and DOC loads, and monthly rates of change in NO$_3^-$ and DOC loads at ST12 during autumn 2015. ........................................................................... 188

Figure B6 Fulvic-like components of our PARAFAC model with DOC, Q, and litter mass at ST12 during autumn 2015. ................................................................. 189

Figure B7 Protein-like component of our PARAFAC model with DOC, Q, and litter mass at ST12 during autumn 2015. ................................................................. 190

Figure B8 Humic-like components of our PARAFAC model with DOC, Q, and litter mass at ST12 during autumn 2015. ................................................................. 191

Figure C1 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the STR incubation..................................................... 193

Figure C2 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the STRNO3 incubation.............................. 194

Figure C3 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the ION incubation................................. 195

Figure C4 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the IONNO3 incubation.......................... 196

Figure C5 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the LEAF incubation............................. 197

Figure C6 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the LEAFNO3 incubation............................. 198
Figure C7 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the SOIL incubation. ............................................. 199

Figure C8 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the SOILNO3 incubation. .................................. 200

Figure C9 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the GLU incubation. ....................................... 201

Figure C10 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the GLUNO3 incubation. .............................. 202

Figure C11 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the SA incubation. ....................................... 203

Figure C12 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the SANO3 incubation. .............................. 204
ABSTRACT

Biotic and abiotic factors both play critical roles in the cycling of organic matter and nutrients in aquatic ecosystems. Understanding the relative control of these factors on solute fate and transport in fluvial systems is important for understanding how climatic changes can affect water quality. Many processes that control solute cycling in streams occur at sub-daily scales, making high-frequency, in situ, sub-hourly measurements important for capturing the response of dissolved organic matter and nutrients to changes in the strength of controlling processes. The tightly coupled aquatic and terrestrial environments present in headwater streams make them particularly useful systems for studying high-frequency changes in water chemistry.

In this study, we examined the patterns of dissolved organic carbon, nitrate, dissolved oxygen, temperature, dissolved organic matter fluorescence, and stream discharge using in-stream measurements at sub-hourly to monthly time scales to understand the daily and seasonal controls of aquatic organic matter and nutrient processing. We also conducted a laboratory incubation to measure the effects of dissolved organic carbon and nutrient treatments on consumption of carbon and nitrogen in our system. Our main objectives were to identify: 1) the relative controls of diel biotic and abiotic processes on stream dissolved organic carbon and nitrate-N; and 2) the mechanisms controlling rapid autumnal changes in dissolved organic carbon and nitrate-N in stream runoff.

We found that hydrology plays a key role in transporting solutes to a forested headwater stream in the Piedmont Region, Maryland; however, once solutes reach the
stream biotic controls dominate the stream solute patterns. Biology is an important regulator of diel patterns of stream water dissolved organic carbon and nitrate concentrations during springtime and autumn leaf fall. Diel cycling is most apparent during the spring prior to leaf out when the water temperature is increasing. Where patterns were evident, nitrate (annual average in second order stream: 17:00) and discharge (17:28) reached their minimums during the afternoon within a few hours of the peaks in dissolved oxygen (13:16), temperature (15:17), dissolved organic carbon (16:06), and dissolved organic matter fluorescence (17:23). Larger amplitudes of dissolved oxygen, nitrate, dissolved organic carbon, and dissolved organic matter fluorescence correspond with larger daily temperature changes.

Laboratory incubations showed increased consumption of nitrogen in the presence of labile carbon, but not in the presence of labile carbon plus nutrients, which indicates our system is carbon limited. Autumn dissolved organic carbon and nitrate dynamics also indicate our system is carbon limited. Increased rates of leaf litter fall corresponded with increased consumption of stream nitrate leading to a late October depression, or annual minimum, in nitrate concentration. Storm events accelerated the recovery of stream nitrate to early autumn concentrations as nitrate was mobilized and transported from soils to the stream. Hydrology is important for solute transport to and export from the stream. Autotrophic activity dominates on the daily scale, while heterotrophic activity controls seasonal responses in organic matter and nutrient cycling in this forested watershed. Carbon and nitrogen dynamics have been studied in other forested systems, as well, but the controlling processes vary among these watersheds. Our results highlight the importance of understanding controlling processes
within specific watersheds when making large scale predictions of the potential export of carbon and nitrogen from forested systems.
Chapter 1

INTRODUCTION

Carbon is the currency of ecosystems; primary producers use energy from the sun to produce organic matter. Dissolved organic matter (DOM), a large portion of which is dissolved organic carbon (DOC), consists of many different chemical classes, of which the chemical properties dictate the function and fate of a particular DOM molecule within the watershed (Nebbioso & Piccolo, 2013). DOC exerts a strong control over many environmental and ecological processes (Stanley et al., 2012). DOM concentration and composition vary spatially and temporally throughout a watershed depending on available sources of and hydrologic flow paths associated with DOM (Jenkins et al., 1994). DOM concentration decreases with source depth within a watershed; surficial sources likely contain high molecular weight (HMW) and humic organic matter, while deeper groundwater sources are composed of more bioavailable organic matter (Inamdar et al., 2012).

While source determines the composition of DOM, biotic and abiotic processes control the transport and transformation of DOM as it moves through watersheds. Factors such as physiographic region, stream order, land use, and season can affect which processes will be dominant. The pathway DOM travels through a watershed will determine the degree of processing DOM experiences before reaching the stream (Jenkins et al., 1994; Stanley et al., 2012). For example, dry periods will cause DOM to accumulate in soils due to lower decomposition rates and increased cell lysis;
accumulated DOM is then flushed to the stream with the next wetting event (Kalbitz et al., 2000).

In aquatic ecosystems, DOM concentration and composition change at varying rates. Diel, or 24 hour, patterns are evident over time scales of weeks to months but are created by sub-hourly changes in watershed processes (Nimick et al., 2011). Rapid changes in organic matter composition and concentration have been referred to as “hot moments” (McClain et al., 2003, Vidon et al. 2010). One mechanism reported to trigger such a “hot moment” is autumn leaf fall. Small, headwater streams are key to studying these changes as they exhibit a greater link with the terrestrial environment resulting in a more rapid response to watershed changes (Bass et. al, 2011).

Diel studies have found varying patterns in several water quality parameters based on land use (Duncan et al., 2015; Kaplan & Bott, 1982; Looman et al., 2016; Pellerin et al., 2012; Spencer et al., 2007; Wilson & Xenopoulos, 2013; Worrall et al., 2015). Wilson and Xenopoulos (2013) found hydrologic controls, driven by differences between day and nighttime evapotranspiration rates, created DOC maxima at night in watersheds along an agricultural gradient, while Kaplan and Bott (1982) found that microbial processes created DOC maxima in late afternoon in an agricultural creek within a forested riparian zone. Primary production is driven by temperature with the highest rates of production during warmer temperatures. When primary production is the driving diel process, stream nitrate (NO$_3^-$) concentrations will be lowest and DOC concentrations will be highest in the late afternoon (Kaplan & Bott, 1982), and diel dissolved oxygen (DO) patterns have the largest amplitude when primary production rates are high (Guasch et al., 1998). In forested watersheds, the largest NO$_3^-$ fluctuations will occur during the spring when the forest canopy is still
developing, which allows more sunlight to reach the stream (Nimick et al., 2011); in other systems, the amplitude of diel cycling of NO$_3^-$ and DOC will be greatest during the summer (Kaplan & Bott, 1982). Spencer et al. (2007) demonstrated the complexity of diel DOM patterns through the use of several spectrophotometric measurements. While bulk DOC concentration did not exhibit a diel pattern, DOC absorbance metrics exhibited diel patterns driven by primary production and fluorescence metrics indicated the presence of photo-chemical degradation in a California river (Spencer et al., 2007). The findings of that study emphasize the usefulness of high-frequency in situ sensors for understanding the complex controlling factors behind diel solute variations in streams.

Many studies have examined the coupled processing of DOC and NO$_3^-$ in aquatic systems and show that increases in labile DOM rapidly increase NO$_3^-$ consumption rates (Bernhardt & Likens, 2002; Sebestyen, Shanley, Boyer, Kendall, & Doctor, 2014; Strauss & Lamberti, 2000). During autumn leaf fall, labile DOM is introduced to the stream in the form of leaf litter, which stimulates rapid heterotrophic consumption of DOC and NO$_3^-$ . In forested catchments, up to one third of daily autumn DOC concentrations can be attributed to leaching from leaves in the stream (Meyer et al., 1998), and additions of labile carbon and nutrients to stream waters have been shown to stimulate complex responses in heterotrophic communities based on amount of carbon and nitrogen available in the stream (Bernhardt & Likens, 2002; Sebestyen et al., 2014; Strauss & Lamberti, 2000). Singh et al. (2014) found high “protein-like,” or more bioavailable, DOM in stream water during autumn and hypothesized the main contribution of this “fresher” DOM was input from fallen
leaves, which indicates the need for high frequency data to capture rapid stream water chemistry changes during autumn leaf fall events.

Rapid responses in stream water chemistry to changes in DOM such as autumn leaf fall affect water quality downstream and in the future. Climate change in the eastern United States has yielded more intense storms and more extreme temperatures (Melillo et al., 2014), which will contribute to rapid changes in OM composition and concentration. Increased precipitation will lead to increased DOC export (Kaushal et al., 2014). Climate change will impact the timing and relative influence of biotic and abiotic watershed processes. Understanding rapid DOM composition and concentration responses to biogeochemical processes in a watershed are important for predicting how watershed DOM quality and quantity may change in the future.

Identifying and understanding the influence of diel processes and autumn leaf fall on stream water DOM requires high-frequency measurements. New technologies are allowing scientists to more accurately and quickly measure concentrations of DOC and NO$_3^-$-N (Avagyan et al., 2014; Bass et al., 2011; Spencer et al., 2007) in concert with historically measured in situ parameters, such as DO and temperature. Tracking changes in DOM and nutrient concentration and composition in concert with parameters such as DO, which is indicative of biological activity, will provide insight into how aquatic ecosystems will respond to climate change.

Here, we used high-frequency, in situ water chemistry data and discharge measurements coupled with baseflow grab samples and automated storm samples to study the controlling mechanisms behind diel processes of DOC and NO$_3^-$ and the impacts of autumn leaf fall on stream chemistry in a forested watershed in Cecil County, MD. Additionally, a laboratory incubation study was performed to examine
controls on NO$_3^-$ processing by microbes native to our stream. Our main objectives were: 1) to study changes in dissolved organic carbon and nitrate in stream runoff across multiple spatial and temporal scales; and 2) to determine the watershed processes that control these changes in stream chemistry. Our study questions were: 1) what are the diel, or daily, patterns of dissolved organic carbon and nitrate in stream runoff; 2) what are the relative controls of biology and hydrology on these diel patterns; and 3) what are the mechanisms controlling rapid autumnal changes in dissolved organic carbon and nitrate in stream runoff.

We hypothesized that heterotrophic communities dominate our watershed and will drive diel patterns, especially during summer low flows. We further hypothesized that DOC concentrations will decrease and NO$_3^-$-N concentrations will increase during the day as DOM is consumed during mineralization processes. During autumn leaf fall, we hypothesized that the increase in DOC concentration and labile DOM fraction will be rapidly transformed by heterotrophs.
Chapter 2
LITERATURE REVIEW

This section highlights the biotic and abiotic processes and factors that have previously been found to contribute to diel patterns and autumnal processing of DOC and NO$_3^{-}$-N in stream waters. Stream water chemistry is often affected by coupling of hydrologic and biologic processes. Understanding each process separately is important for teasing apart their influence.

2.1 Importance of Sub-hourly, in situ Measurements

Previous studies have emphasized the importance of high-frequency data for understanding watershed processes. Many small streams have a residence time of less than one day and much of the DOC that enters a stream is lost in as little as two days (Moody & Worrall, 2015). High frequency measurements can lead to a better understanding of biotic and abiotic diel processes. Traditional stream studies rely on weekly grab samples for tracking changes in stream chemistry, but weekly sampling can underestimate DOC concentrations by 30 – 78% (Bass et al., 2011; Jollymore, et al., 2012). In-stream sensors have been shown to detect water chemistry changes before hydrologic changes are detected (Grayson & Holden, 2012). In situ sensors can measure multiple parameters that indicate concentration and composition changes in DOC and nutrients throughout the day. In some systems, bulk DOC concentration do not exhibit a diel cycle while chromophoric dissolved organic matter (CDOM),
dissolved organic matter fluorescence (FDOM), and certain spectral slopes will exhibit patterns (Spencer et al., 2007).

Many papers have demonstrated the importance of calibrating in situ UV-vis spectrometers to lab grab sample chemistry, using absorbance measurements at multiple wavelengths, to more accurately estimate the concentrations of solutes such as DOC and NO₃⁻-N (Avagyan et al., 2014; Carey et al., 2014; Carter et al., 2012; Fasching et al., 2015; Pellerin et al., 2012, 2016). Many initial studies used absorbance measurements at only one or two wavelengths as a proxy for DOC concentration. Popular wavelength choices include 254, 340, and 400 nm. Grayson and Holden (2012) found significant differences in the DOC concentrations predicted using the absorbance at 340 nm and 400 nm. Carter et al. (2012) compared lab measured DOC concentrations to values estimated using a single wavelength (254 nm) calibrated to the DOC from specific stream and a globally calibrated two wavelength model. The dual wavelength model, although not site specific, better predicted DOC concentrations in five of six cases (Carter et al., 2012). UV-vis measurements must also be compensated for interference in absorbance values by suspended particles in the water column. Avagyan et al. (2014) found a significant difference in the absorbance values for measurements on unfiltered and filtered aliquots of the same stream water samples.

Use of in situ sensors in small watersheds is especially important since aquatic and terrestrial processes are more tightly coupled within small watersheds (Worrall et al., 2015). Duncan et al. (2015) found that stream NO₃⁻ cycles were driven by cycling in and transport of nitrogen from the riparian zone. Seasonal changes in riparian nitrogen processing triggered a rapid increase from annual minimum stream NO₃⁻
concentration to summer maximum in only four days (Duncan et al., 2015). The deeper understanding of biogeochemical cycling that high-frequency, in-stream measurements can provide will increase understanding of ecosystem function, which in turn will allow scientists to better predict ecosystem responses to anthropogenic and climatic disturbances (Nimick et al., 2011; Pellerin et al., 2016).

2.2 Abiotic Processes

DOC degradation is influenced by several physical and chemical processes including time, light exposure, air temperature, organic matter composition, and initial concentration of DOC (Moody & Worrall, 2015). This section highlights daily cycles of abiotic processes that exhibit control over or give evidence to patterns of stream DOM and NO$_3^-$ composition and concentration.

2.2.1 Temperature and light

Solar radiation, which affects air and water temperature and temporal light sequence, exerts a dominant control on diel processes. Shallow, unshaded streams will experience the strongest temperature affects, especially during the summer when solar radiation reaching the stream, heat exchange with the atmosphere, and the temperature gradient between surface and groundwater are largest (Nimick et al., 2011). Kaplan and Bott (1982) found DOC concentration to have a positive relationship with both light and temperature. Photic processes have been shown to convert up to 95% of DOC to CO$_2$ (Cory et al., 2014), with DOC minimums during the late afternoon (Worrall et al., 2015). Moody and Worrall (2015) found that DOC concentration decreased over 10 times more during normal light and dark cycles than during constantly dark conditions. Peatland water samples exhibit no change in DOC
concentrations when kept in the dark (Jones et al., 2015). Multiple studies have found that photo-degradation rates will be higher for DOM with a higher initial DOC concentration (Jones et al., 2015; Moody & Worrall, 2015) or more chromophoric composition (Cory et al., 2014). Other physical and chemical properties that exhibit cycles due to underlying temperature changes include gas solubility, stream bed hydraulic conductivity, and sorption of metals and metalloids to organic matter (Nimick et al., 2011).

2.2.2 Dissolved gases

Like temperature and light, concentrations of dissolved gases influence other physical and chemical parameters in streams. Oxygen and carbon dioxide control pH and redox state of stream waters, both of which exhibit important geochemical controls within the aquatic environment; as dissolved CO$_2$ concentration increases, pH decreases (Nimick et al., 2011). Gas solubility is temperature dependent, as explained by Henry’s law: independent of biotic factors, concentrations of dissolved gases such as O$_2$ and CO$_2$ will be highest at night when stream water is coolest. Diel patterns of DO and CO$_2$ in stream waters will vary seasonally. Gas dissolution will be near saturation during wintertime when temperatures are low, which will cause a lack of diel patterns; however, during the summer and fall, CO$_2$ and DO have been found to be higher during the day than at night (Guasch et al., 1998). During the summer, a negative correlation of CO$_2$ with light and temperature and DO above equilibrium at night indicates high primary productivity (Guasch et al., 1998). A DO peak in the late afternoon is indicative of photosynthetic activity in the stream. Temperature and solar radiation will drive primary productivity throughout the day which will peak a few hours after the peak in temperature (Nimick et al., 2011).
2.2.3 Hydrology

Once again temperature is the main factor that influences abiotic processes. During low-flow, which typically occurs during summer months, evapotranspiration (ET) rates have a strong effect on discharge cycles; while ET is high during the day, discharge decreases (Groffman et al., 2012). The largest water table fluctuations in a forested Piedmont headwater stream, similar to our watershed, occurred during the summer months and were linked to evapotranspiration rates (Duncan et al., 2015). Wilson and Xenopoulos (2013) found the largest fluctuations in discharge in watersheds with the greatest percentages of forest cover. This pattern of high discharge at night and decreasing discharge throughout the day exists, but to a lesser extent, during the spring and autumn in watersheds unaffected by snowmelt (Nimick et al., 2011).

2.2.4 Effects of abiotic factors on solutes

Hydrologic cycles can control patterns of DOM quantity and composition. Studies have found a strong link between hyporheic DOM (Fasching et al., 2015) and groundwater chemistry in small streams during baseflow (Inamdar et al., 2013). Since lower transpiration rates allow more water to flow through surficial DOM pools, DOC concentrations are expected to be higher at night and lower during the day in systems where hydrology exerts the dominant control (Wilson & Xenopoulos, 2013). In some forested systems, riparian DOC has been found to be less aromatic than shallow groundwater DOC, which can have an impact on what type of high-frequency measurements are required to fully capture diel DOC patterns (Inamdar et al., 2012; Spencer et al., 2007).
Nutrients, such as NO$_3^-$ can also exhibit hydrologically driven diel cycles. Duncan et al. (2015) found summer stream NO$_3^-$ to exhibit a diel pattern with the highest concentrations during the day. Overall, NO$_3^-$ concentrations were highest in the summer due to reduced denitrification rates in riparian soils (Duncan et al., 2015). The amplitude of cycling was largest during the summer due to larger evapotranspiration rates. Daytime NO$_3^-$ peaks occur due to a greater contribution of deeper groundwater sources to the stream during low flow conditions (Duncan et al., 2015).

Abiotic and biotic processes may have coupled effects on DOM and nutrient cycling and many biotic processes are influenced by abiotic processes. Low stream discharge and velocity create more favorable conditions for heterotrophs, which consume DOC and NO$_3^-$ (Bernhardt et al., 2005). Conversely, dilution effects can decrease heterotrophic activity and increase labile DOM subsidies, especially during storms (Fasching et al., 2015). Abiotic factors, such as light and temperature, drive rates of autotrophic production and heterotrophic consumption which control the amplitude of concentration changes of NO$_3^-$ and DOC during baseflow periods (Nimick et al., 2011).

2.3 Biologic Processes

Light and temperature are the underlying drivers for biotic processes. Temperature controls toxicity, the kinetics of metabolic processes such as autotrophy and heterotrophy, the overall productivity of biota, and increases dissolution of gases throughout the night (Nimick et al., 2011). Forested watersheds receive more sunlight during the springtime, prior to leaf out; springtime warming of water stimulates higher rates of primary production, which leads to the largest seasonal amplitudes in diel
NO$_3^-$ cycles with concentrations declining throughout the day as assimilation by autotrophs increases as stream temperature increases (Nimick et al., 2011). Concentrations of dissolved gases can be indicators of biologic activity in aquatic environments. If hydrology is the dominant process, gas solubility will decrease during the day due to solar radiation warming the stream water. Both photosynthesis and respiration occur at higher rates during the day, but photosynthesis is the dominant process, so CO$_2$ will be consumed and DO will be produced, while at night the opposite pattern will be observed due to a lack of photosynthetic activity (Nimick et al., 2011).

2.3.1 Effects of biotic factors on solutes

Several studies have found biotic controls on diel patterns of DOC and NO$_3^-$ concentration. Allan and Castillo (2007) report that algal production accounts for up to 37% of stream water DOC increase, which peaks in the early afternoon. Kaplan and Bott (1982) found DOC to be produced within the aquatic environment through algal production; net production contributed 20% of daily DOC export and created a late afternoon peak in the DOC concentration. In contrast, Looman et al. (2016) observed heterotrophic metabolism to control DOC cycling; CO$_2$ maxima coincided with the peak in photosynthetically active radiation (PAR) during the late afternoon indicating DOC was consumed throughout the day. While autotrophically produced DOC will peak in the late afternoon, NO$_3^-$ will reach its minima in the afternoon due to assimilation by autotrophs (Nimick et al., 2011; Pellerin et al., 2012).
2.4 Comparing abiotic vs. biotic influence of DOM cycling

Not all streams experience diel cycling of DOC or NO$_3^{-}$. In streams where diel cycling of DOC or NO$_3^{-}$ is present, the timing and shape of the patterns depend on the dominant drivers of the system in question (Nimick et al., 2011). Kaushal et al. (2014) identified biotic processes to be the dominant control on DOM cycling in agricultural systems through the use of high-frequency DO, NO$_3^{-}$, groundwater, microbial activity, photodegradation, and FDOM measurements. Moody and Worrall (2015) looked at the relative degradation rates of DOC from a peatland headwater catchment during ambient light and dark environments and found that degradability of DOC varied seasonally and was most likely dependent on photic processes rather than strong biologic controls. In the case of Moody and Worrall (2015), separate rate laws were needed to describe the change in DOC over time during the day vs. the night. Several studies have looked beyond DOC concentration by examining diel changes in DOM composition.

While the bulk DOC concentration in the agricultural watershed studied by Spencer et al. (2007) did not exhibit a diel pattern, the absorbance across the spectral range, FDOM, and several other spectral characteristics, which are indicators of DOM composition, did exhibit daily patterns. Absorbance values increased throughout the day, while FDOM peaked in the morning; these opposing patterns are indicative of photo-chemical bleaching of DOM (Spencer et al., 2007). Fasching et al. (2015) used spectrofluorometric measurements to link higher DOC concentration in the stream to terrigenous DOM sources and identified the presence of higher molecular weight DOM during high flows in an alpine watershed. In contrast, Wilson and Xenopoulous (2013) found that daytime DOC was lower in concentration and more terrestrial throughout several agricultural watersheds. The watersheds with the most forest cover
exhibited the largest daily change in stream DOC concentration, driven by evapotranspiration; the highest concentrations of DOC occurred at night when evapotranspiration rates were lower allowing more water to flow through organic rich upper soil horizons and transport more DOM to the stream (Wilson & Xenopoulos, 2013). The presence of varying DOC sources across differing watersheds highlights the importance for tracking several metrics of DOM composition and concentration when exploring local diel patterns.

2.5 Leaf chemistry and decomposition

It is well known among stream ecologists that leaf decomposition will increase with greater nitrogen availability, and leaves with initially higher nitrogen and carbon concentrations will break down more rapidly (Allan & Castillo, 2007). The lignin fraction of leaves has a higher amount of complexed nitrogen, which can lead to increased processing of lignin, especially during the first six to twelve weeks of in-stream leaf processing (Suberkropp et al., 1976). The rate of overall processing will affect the changes in concentration of major macromolecules in leaves during decomposition (Suberkropp et al., 1976).

Leaching is the first stage in litter decomposition and can decrease leaf mass 30% by the second day of leaching (Cuss & Guéguen, 2015); however, decomposition rates will vary with leaf species (Suberkropp et al., 1976). It has been well established that there are positive correlations between the amount of water-soluble substances in litter and the amount of litter decomposition (Nykvist, 1959). Up to 50% of the dry mass of leaves is water soluble (Cuss & Guéguen, 2015) and soluble compounds, such as reducing sugars and polyphenols, are most rapidly broken-down during the first two weeks of in-stream decomposition (Suberkropp et al., 1976). Leaf leachate dissolved
organic matter can satiate up to half of the carbon demand and community respiration of bacterial communities in third order streams (Cuss & Guéguen, 2015).

### 2.5.1 Tracking decomposition

Optical measurements are useful for studying leaf litter decomposition and chemical effects on bioavailability for microbial consumption. Protein-like fluorescence is used as an indicator of labile DOM quality since protein-like fluorescence decreases with DOC concentration during the biotransformation of DOM (Cuss & Guéguen, 2015; Fellman et al., 2008). The specific absorbance at a chosen wavelength normalized by carbon concentration (SUVA) is an optical property of DOM that is useful in predicting the total carbon mineralization of leachates from multiple leaf species, but only in the presence of excess nutrients (Wieder et al., 2008). Wymore et al. (2015) demonstrated the important influence leaf litter DOC has on stream ecosystems by correlating optical properties with changes in litter chemistry throughout DOC decomposition and using sediment incubations to study the effect of litter DOM on heterotrophic respiration. The following correlations were made: 1) aromaticity was negatively correlated with fluorescence index (FI) but positively correlated with SUVA; 2) bioavailability was positively correlated with FI but negatively correlated with SUVA; 3) the T280 (tryptophan- and tyrosine-like or protein-like) fluorescence peak associated with labile amino acids was positively correlated with bioavailability and DOC uptake rates; and 4) decomposition decreased with increasing tannin and lignin concentrations. Litter respiration rates were negatively correlated with FI and positively correlated with SUVA

254 and T280, even though these properties had been shown to exhibit the opposite correlations with stream water in past studies (Wymore et al., 2015). The fact that the optical
characteristics and bioavailability of litter DOC were opposite the optical characteristics and bioavailability typically found for stream DOC indicates that differences in source DOM biogeochemistry can be investigated using optical properties. Leaf litter containing higher recalcitrant compounds had lower DOC concentrations and mass loss during leaching, while rapidly decomposing leaves leached 40-50% more DOC and total dissolved nitrogen and increased respiration by 25-50% greater than the recalcitrant DOC (Wymore et al., 2015).

2.6 **Microbial processing and nutrients in streams**

Heterotrophic microbial communities tend to be carbon limited but other nutrient limitations can occur; autotrophic communities are more often nutrient limited since they convert nutrients into organic matter (Allan & Castillo, 2007). For decades, scientists have known that addition of leaf litter to a stream can rapidly increase DOC concentration (up to 10 times initial concentrations) and decomposition of labile carbon and nitrogen lead to rapid bacterial population growth, which can alter ecosystem dynamics, within the first few days of decomposition (Wetzel & Manny, 1972). DOC can account for 30-75% of the total energy input into stream ecosystems (Bernhardt & Likens, 2002). Carbon and nitrogen cycles in nutrient depleted ecosystems are closely linked and DOC availability will control stream microbial biomass (Bernhardt & Likens, 2002).

Bernhardt and Likens (2002) added DOC in the form of potassium acetate to a predominately heterotrophic, forested stream, while keeping a nearby reference stream undisturbed, to study the effects of carbon availability on rates of \( \text{NO}_3^- \) and ammonium \( \text{NH}_4^+ \) consumption since previous studies had shown many microbial communities are carbon limited. An increase in labile carbon in the treatment stream decreased the
concentration of NO$_3^-$ in the stream water, created shorter uptake lengths for NO$_3^-$ and NH$_4^+$, and increased the metabolism rates above those of the reference stream (Bernhardt & Likens, 2002). The DOC addition, however, did not increase denitrification; the primary effect of the DOC treatment was to stimulate bacterial growth, which increased the rates of respiration and assimilative nitrogen demand (Bernhardt & Likens, 2002). Bernhardt and Likens (2002) predicted that the increase in labile DOC in the stream caused rapid DOC removal, which increased dissolved inorganic nitrogen (DIN) consumption leading to increased microbial biomass, activity, and respiration. These findings may not be directly translatable to autumn leaf fall since leaves contain less labile forms of carbon than the carbon source added by Bernhardt and Likens (2002), but they indicate the importance of nutrient stoichiometry for microbial processes.

Overall microbial activity in stream sediments will increase when DOC concentration increases, and respiration has been found to increase four to six times that of control sediment rates when carbon concentrations are artificially increased (Strauss & Lamberti, 2000). While overall activity increases, nitrification can decrease and rates will decrease more in the presence of labile carbon such as glucose versus more recalcitrant maple leaf leachate DOC, even when additional nutrients are added along with glucose (Strauss & Lamberti, 2000). In contrast, if NH$_4^+$ alone is added to stream sediments, nitrification rates can increase by 40% by decreasing C:N and NH$_4^+$ competition and increasing the availability of NH$_4^+$ to nitrifying bacteria (Strauss & Lamberti, 2000). Glucose addition decreases nitrification by increasing C:N and NH$_4^+$ and decreasing the availability of NH$_4^+$ for microbial processing (Strauss & Lamberti, 2000).
The importance of leaf surface area on the rates of leaf decomposition were demonstrated by Tant et al. (2013). Bacteria dominate on fine particles (less than 1 mm diameter) and fungi dominate on coarse particles (greater than 1 mm diameter). While the dominating species is not affected by nutrient enrichment, both bacteria and fungi population size increased more on the coarse particles under nutrient enrichment conditions (Tant et al., 2013). Respiration rates also increase far more (up to 300%) on the coarse particles than the fine particles (only 50% increase) and greater changes in nutrient ratios occur on the larger particles due to increased consumption of carbon, nitrogen, and phosphorous (Tant et al., 2013).

2.7 Leaf fall and stream chemistry

A stream model of nutrient uptake and mineralization during leaf decay shows that stream nutrient concentrations can substantially decrease due to microbial uptake during the initial stages of litter decomposition; however, mineralization during later decomposition stages can cause a rebound in nutrient concentrations and the initial nutrient concentrations will affect these in-stream dynamics (Webster et al., 2009). Many scientists have studied the complex effects of litter decomposition in forested streams (Bernhardt & Likens, 2002; Meyer et al., 1998; Sebestyen et al., 2014; Strauss & Lamberti, 2000).

A key study conducted in forested Coweeta streams, demonstrated the importance of leaf litter to stream chemistry and ecosystem metabolism. Meyer et al. (1998) compared water chemistry in an unaltered reference stream to a litter-excluded treatment stream. They found that litter exclusion decreased the concentration, export, and in-stream generation of DOC and concluded that the benthic leaf litter stock directly influences mean stream DOC concentration by contributing up to 50% of the
daily DOC exports generated within the stream (Meyer et al., 1998). Benthic control on stream DOC was also evident in the greater separation of DOC concentrations in the reference and treatment stream during storm events, which was likely due to the flushing of DOC leached from benthic organic matter in the hyporheic zones and stream banks (Meyer et al., 1998). Meyer et al. (1998) also found a lack of change in DOC concentration in the treatment stream during the spring and summer, emphasizing the important effects of leaf litter on stream DOC chemistry.

During peak leaf fall, baseflow NO$_3^-$ can decrease by an order of magnitude and in-stream transformations, such as nitrification and heterotrophic cycling of NO$_3^-$, can retain up to 72% of the NO$_3^-$ (Sebestyen et al., 2014). Autumn storms can trigger NO$_3^-$ concentrations to recover by increasing stream NO$_3^-$ up to 25 times the pre-storm concentrations (Sebestyen et al., 2014). Stream dissolved organic nitrogen, however, increases during leaf fall and can remain high for two months after peak litter fall since in-stream litter will continue to act as a DOM source (Sebestyen et al., 2014).

Mineau et al. (2013) studied the importance of DOM nutrient content and inorganic nutrient availability for the bioavailability of DOM using additions of DOM and nutrient treatments to a bottle incubation and comparing a reference stream and a nutrient enriched stream. Nitrogen enrichment increased DOC consumption by 14% more than reference stream consumption rates, but DOM consumption was greatest during enrichment of both nitrogen and phosphorous, and nitrogen and phosphorous co-limitation was the primary driver of DOM processing (Mineau et al., 2013). Mineau et al. (2013) also found that nutrient availability will have a greater impact on DOM processing than the nutrient content of the DOM itself since the nutrient enrichment allows microbes to utilize more recalcitrant DOM pools; the nutrient
treatment DOM exhibited an increase in humic-like DOM consumption and decrease in protein-like DOM consumption over time.

Previous studies in a forested Maryland watershed (the study site for this research) have shown the importance of studying stream chemistry during autumn leaf fall and have potentially pointed to leaf fall as a controlling factor in certain seasonal in-stream changes. Surficial DOM sources, including litter leachate, have higher DOC concentrations (Inamdar et al., 2013) and median DOC concentration is higher in litter compared to most other DOM sources (Inamdar et al., 2012). Singh et al. (2014) found that seasonal DOM separation is most pronounced in litter leachate during storm events and especially for fall litter samples. Litter leachate DOC concentration, protein-like DOM fluorescence (values up to 30% for maple, beech, and polar leachate), and stream water DOC concentration all exhibit sharp increases during the early autumn; however, stream water does not exhibit the increase in labile DOM signature (Singh et al., 2014).
Chapter 3
METHODS

3.1 Site Description

We studied stream water carbon and nitrogen dynamics in the Fair Hill Natural Resource Management Area in Cecil County, MD, (39°42′N, 75°50′W), which is located within the Piedmont region and experiences a humid climate with well-defined seasons (Inamdar et al., 2011). The study area sits on the Mt. Cuba Wissahickon formation, which mainly contains schist and Glenelg series soils, which are deep and well-drained (Singh, Inamdar, & Mitchell, 2014). The watershed is mainly deciduous forest (Singh, Inamdar, & Mitchell, 2014). Within the study area, there are two nested watersheds, 12 ha (first order stream) and 79 ha (second order stream), which drain into the Big Elk Creek. The Big Elk Creek basin is located within the Chesapeake Bay watershed. Sampling locations include the outlet of the 12 ha catchment (ST3) and the outlet of the 79 ha catchment (ST12) (Fig. 3.1).

3.2 Field Methods

In order to examine the daily and seasonal changes in stream water DOC and NO₃⁻-N concentrations, we deployed in situ sensors to measure DOC and NO₃⁻-N concentrations at sub-hourly frequency. Sub-hourly changes in stream stage, DO, temperature, FDOM, and turbidity were measured to examine the relative roles of hydrology and biology in changes in DOC and NO₃⁻-N concentrations. We hypothesized that heterotrophic communities dominate our watershed and will drive
DOC concentrations to decrease and NO$_3^-$-N concentrations to increase during the course of a day as DOM is consumed during mineralization processes. During autumn, we hypothesized that leaf fall will increase DOC concentration and labile DOM fraction in the stream, which would be transformed by heterotrophs leading to a rapid decrease in stream NO$_3^-$-N concentrations.

### 3.2.1 In-stream sensors

The s::can spectro::lyser measures the full UV-Vis spectrum (200 to 750 nm) and calculates parameters such as DOC (ppm) and NO$_3^-$-N (ppm). The YSI EXO 2 sonde consists of several probes, which measure DO (% saturation), temperature (°C), turbidity (FNU), and FDOM (QSU). The s::can spectro::lyser and EXO 2 sonde were deployed at the outlet of the 79 ha watershed and measured stream parameters every 30 minutes. An additional spectro::lyser is deployed at the outlet of the 12 ha watershed. All sensor data was downloaded to a computer every two weeks and the EXO 2 sonde was calibrated every two months. The spectro::lysers and EXO 2 sonde were cleaned every week. Global Water Inc. level loggers and pressure transducers recorded stream stage every 20 minutes and groundwater level every 30 minutes. At the 12 ha outlet, stream discharge estimates were calculated using a Parshall flume. The dimensions of the flume are known and can be used in concert with stream stage values to calculate the volume of water flowing through the flume at a given time. At the 79 ha outlet, stream discharge is estimated using a rating curve calculated from paired pressure transducer and acoustic Doppler velocity meter measurements in a culvert. The groundwater levels were calculated as depth below the ground level using site-specific calibrations.
3.2.1.1 Sensor calibration

A partial least squares regression (PLSR) code developed in R 3.2.5 (R Core Team, 2016) by Etheridge et al. (2014) that utilizes the pls package (Mevik et al., 2011) was applied the spectro::lyser absorbance data and laboratory analyzed chemistry data to create a calibration for the spectro::lyser DOC and NO$_3$-N values. PLSR is a chemometric technique that uses the full absorbance spectrum to find a model with components that describe the maximum correlation with solute concentrations (Etheridge et al., 2014). Laboratory DOC and NO$_3$-N concentration values from grab and storm samples were temporally matched with turbidity compensated absorbance spectra measured within 15 min of baseflow samples and 10 min of stormflow samples. The optimal number of components for each of our models were chosen based on the coefficient of variance (CV) and adjusted CV for models with up to 10 components. Our goal was to choose the number of components that resulted in the smallest CV. Models were created for DOC and NO$_3$-N separately with one model for each solute at each of our two sensor deployments, ST3 and ST12. A combination of lab data from 2014 and 2015 was applied for certain solutes at certain sites depending on the availability of lab data and the ability of the model to predict known values.

In some cases the number of components chosen did not lead to the smallest CV but did lead to the largest difference among the lower CV values and the CV for the next lowest number of components, or if the CV increased and decreased again with more components, so as to not over parameterize the model. For example, in the ST3 DOC 2015 calibration, a 7 component model had a CV of 0.9916 which was less than both the 6 component model CV (1.064) and 8 component CV (1.0153). A second example is the calibration for ST12 NO$_3$-N 2015. For this calibration the
difference between CVs when choosing a 7 component (CV = 0.04893) model over a 6 component model (CV = 0.06341) was 0.01448, which is a much greater difference than between the 7 component model CV and the 8 component model CV (CV = 0.0381) which was 0.00583. The lab data was regressed on the PLSR calibration data and the spectro::lyser calculated concentrations to assess if the PLSR method of calibration improved the accuracy of our data.

The following models were created: 1) 7 component DOC model for ST3; 2) 7 component NO₃-N model for ST3; 3) 7 component DOC model for ST12; 4) 7 component NO₃-N model for ST12. The R² (0.9448) and adjusted R² (0.9441) values for the ST12 NO3 2015 model are very similar. The relative closeness in the R² and adjusted R² values is present for all calibration models used for this research and indicates there was little introduction of overparameterization in the calibration by using the PLSR approach.

The accuracy of the PLSR calibration could not be compared to the accuracy of the spectro::lyser’s internal global calibration. The only lab values for DOC and NO₃-N that temporally matched the PLSR and spectro::lyser values were from 2014. The 2014 data record had inconsistencies due to recent deployment of the sensors, which limited our use of the 2014 time series. There were also less than 15 data points available for comparison (between calibrated and lab values), which would decrease the power of the linear regression. The PLSR modeling steps included the output of a linear regression of the PLSR calculated concentrations with the lab concentrations originally supplied to create the model. These lab values were generated from samples collected fewer than 15 min before or after an absorbance measurement taken by the
spectro::lyser. The PLSR models show very good fit as reflected in the $R^2$ values (Table 3.1) for the linear regressions of each model (Figs. 3.2, 3.3, 3.4, 3.5).

### 3.2.2 Water sampling

Grab samples were taken at different intervals throughout the study period to calibrate sensor data as well as allow for additional laboratory analyses. Throughout the year, weekly baseflow samples were collected during routine maintenance site visits. Data from the weekly baseflow grab samples were used in both the diel and autumn leaf fall study. Grab samples were collected in 250 mL Nalgene bottles. Automated water samples (*Teledyne ISCO*) were collected in 500 mL HDPE *ISCO* bottles. All collection bottles were pre-washed with 10% HCl (v/v) and glass storage vials were combusted at 500 °C for four hours.

Autumn leaf fall baseflow grab samples were taken three times per week from October 17 to December 12, 2014 to capture changes in DOC and NO$_3$-N concentrations during the autumn leaf fall period. Grab samples were collected once per week during autumn 2015 since the sensors were well established at this point and could better capture the rapid changes in stream chemistry during leaf fall.

### 3.2.3 Chemical analyses

All grab samples were filtered with *Whatman* 0.7 µm pore size glass fiber filters to separate the dissolved and particulate phases. The filtered solution was divided into aliquots for chemical analyses, total carbon and nitrogen analysis, and fluorescence spectrometry. Samples collected prior to June 2015 were sent to the University of Delaware Soils Lab for analysis of base cations, NO$_3$-N, NH$_4$+ -N, carbon to nitrogen ratio (C:N), pH, and electrical conductivity. DOC and TNb
concentrations for samples collected before June 2015 were analyzed on an Elementar TOC analyzer; DOC and TN for samples collected in June 2015 onward were analyzed on a Shimadzu TOC-L, TNM-L. Samples collected from June 2015 forward were analyzed with a Seal AQ2 discrete colorimetric analyzer for NO$_3^-$-N, NH$_4^+$-N, and phosphate-P. A Horiba Aqualog® fluorometer was used to produce fluorescence Excitation-Emission Matrices (EEMs). The excitation wavelengths range from 700-240 nm at 4 nm increments and the emission wavelengths were measured from 700-240 nm with an average interval of 4.66 nm. Several daily checks were performed to ensure consistent performance of the instrument including a manufacturer’s excitation check, emission check, cuvette check, and Raman water scan. Additionally, we applied the Aqualog® post-processing steps to correct for inner filter effects and apply 1st and 2nd order Rayleigh Masking. The sum of slit width was 10 nm. Post-processing of absorbance and fluorescence data was performed in Matlab (Version R2015b V 8.6.0) to calculate several metrics. Absorption coefficients at 250, 254, 280, 320, 350, 365, 375, 436, 465, and 665 nm were isolated and spectral slope 1 (275-290 nm) and spectral slope 2 (350-400 nm) were used to calculate the spectral slope ratio ($S_R$). MATLAB scripts also calculated the fluorescence metrics (HIX, BIX, FI) and ran the fluorescence data through a PARAFAC model as described in (Singh et al., 2013, 2014).

3.3 Exploration of diel patterns of stream water DOC and NO$_3^-$-N

In order to test the hypothesis that heterotrophic metabolism will drive DOC concentrations to decrease and NO$_3^-$-N concentrations to increase during the day as DOM is consumed during mineralization processes, calibrated spectro:lyser data and quality checked EXO 2 data from January 2015 through March 2016 were plotted.
This data set allowed us to explore the seasonality of diel patterns throughout one full calendar year plus strengthen our springtime observations, since the period prior to leaf out has been shown to be most important for diel patterns in forested watersheds. Diel patterns in DOC and NO$_3^-$-N at ST3 and DOC, discharge, NO$_3^-$-N, DO, water temperature, and FDOM at ST12 were identified. Discharge data at both sites and groundwater level from two wells (LW2 and LW3, Fig. 3.1) were also plotted to link diel changes in hydrology to diel changes in stream water chemistry. The maximum and minimum values and the times at which each occurred were determined for each day that patterns were evident during base flow periods. DO minimum values and times were not determined due to lack of clear daily minimum in DO patterns likely driven by nighttime temperature effects on DO solubility (Fig. 3.6). There is also much uncertainty in the maximum and minimum times for groundwater level data due to slower changes in the data during the day, likely due to longer residence time of groundwater (Fig. 3.7). Groundwater data was plotted with lower values, which indicate water level is closer to the ground surface, at the top of the axis to mimic how the groundwater would look as viewed in a cross-section of the soil profile. Monthly averages of daily maximum and minimum values were calculated and are reported in Tables 4.1 – 4.4, 4.6. The daily and monthly averages reported for ST3 NO$_3^-$-N and DOC were calculated from four hour moving average values rather than 30 min concentration values since there was too much noise in the data to find clear maximum and minimum concentration values (Table 4.5). Relative controls of biology and hydrology on the diel cycling of DOC and NO$_3^-$ were determined by examining the relationship between these patterns and the temporal patterns of discharge,
groundwater level, DO, and temperature. Due to the size of the diel dataset, additional graphs are presented in Appendix A.

3.4 Exploration of DOC and NO$_3^-$-N Dynamics during Autumn Leaf Fall

In order to test the hypothesis that autumn stream NO$_3^-$-N would decrease due to increased DOC concentration and the labile DOM fraction in stream waters during leaf fall, litter fall rates were calculated from average weekly leaf masses collected by Parr (Unpublished Data). Baskets were distributed throughout the 12 ha watershed to collect litter fall. Leaf litter was collected and weighed weekly. Average weekly litter fall rates were estimated for the whole watershed area. Time series of discharge, leaf fall rates, NO$_3^-$-N concentration and load, DOC concentration and load, rates of change in DOC and NO$_3^-$-N concentration, EEMs metrics, DO, FDOM, turbidity, and water temperature were graphed. DOC and NO$_3^-$-N loads were calculated by linearly interpolating discharge values for the times at which calibrated DOC and NO$_3^-$-N concentrations were measured by the spectro::lyser. The interpolated discharge values were multiplied by the solute concentrations to find the load for each solute at each spectro::lyser measurement time step. Visual comparisons of the data for the 12 ha and 79 ha sites during autumn 2014 and 2015 were made. Grab and sensor values were used to identify the changes in concentrations of stream parameters throughout the autumn. Additional autumn data is presented in Appendix B.

3.4.1 Stream water incubations

Bottle incubations of our stream water were performed to assess the controls of several factors on carbon and nitrogen consumption in our stream during autumn. Approximately 40 L of stream water were collected within the 12 ha catchment during
September 2015 for laboratory incubations. Stream water from the 12 ha catchment was filtered to 0.7 µm and divided amongst 12 treatments with three replicates each. Six base treatments were created and each base treatment was repeated with the addition of a nutrient solution (NO3). The base treatments were: 1) stream water (STR); 2) stream water plus glucose (GLU); 3) stream water plus ions (see below for details) (ION); 4) stream water plus leaf leachate (LEAF); 5) stream water plus soil extract (SOIL); and 6) stream water plus surface area (SA) (Table 3.2).

The intent of the nutrient addition was to add extra NO3⁻ to the system with a NO3⁻:PO4³⁻ similar to autumn values in our watershed. The nutrient solution contained 1 ppm NO3⁻ and 0.05 ppm PO4³⁻ and was autoclaved at 121 °C on a liquid cycle for 20 minutes to reduce microbial contamination. The cation salt solution contained 3.5 ppm Ca (CaCl₂·2H₂O), 1.5 ppm Mg (MgSO₄), 1.5 ppm K (KHCO₃), and 5.5 ppm Na (NaHCO₃) to mimic historic autumn mean concentrations measured in the 12 ha catchment.

The carbon treatments (GLU, LEAF, SOIL) were used to explore the effect of carbon availability on NO3⁻ consumption. Typical baseflow concentrations of DOC in the study watershed range from ~0.5 – 3.0 ppm. Glucose was added as a 10 ppm carbon solution to push the microbes in the system to the extreme compared to what is usually available. LEAF and SOIL were added at concentrations slightly higher than baseflow to test the effect of increased concentrations of naturally occurring forms of DOC on NO3⁻ consumption.

Leaf leachate was created from a mix of fresh litter from poplar, beech, oak and maple leaves to mimic DOC input during autumn leaf fall. Leaves were ground in a coffee grinder and homogenized with a mortar and pestle. Four grams of each leaf
species were added to 0.5 L Nanopure® water and the bottle was shaken for one hour. The leaf mixture was centrifuged for 10 minutes at 3500 rpm and filtered at 0.7 µm. The leachate solution was measured on the s::can spectro::lyser to estimate DOC concentration and diluted to ~ 6 ppm DOC for the LEAF and LEAFNO3 treatments.

Soil DOC extract was created from upland humus and A horizon soils from our watershed to act as a natural, yet less bioavailable than leaf leachate, form of DOC found in our system. The soils were sieved at 2 mm and homogenized. Two hundred and nineteen grams of soil was added to 0.5 L of Nanopure® water and shaken for one hour. The soil extract solution was centrifuged for 10 minutes at 3500 rpm and filtered at 0.7 µm. The extract solution was measured on the s::can spectro::lyser to estimate DOC concentration and diluted to ~ 5 ppm DOC for the SOIL and SOILNO3 treatments.

The surface area treatment consisted of the addition of plastic squares that added ~ 60 cm² of surface area to 1 L of stream water. Increased surface area has been shown to increase respiration rates (Tant et al., 2013). Two controls were created along with the 36 treatments 1) Nanopure® water which acted as a treatment blank and 2) Nanopure® water with 60 cm² plastic added to control for any inputs from the plastic in the surface area treatments.

The incubation was performed in closed bottles in the dark at room temperature over the course of 25 days. Bottles were shaken daily. Sampling occurred at t = 0, 1, 2, 4, 6, 10, 15, 25 days. On sampling days, 90 mL of water were removed from each treatment and filtered to 0.7 µm. Filtered aliquots were analyzed for TN, DOC, NO₃⁻-N, sum of protein-(SumPL), humic- (SumHL), and fulvic-like fluorescence (SumFL), specific absorbance at 254 nm (SUVA₂₅₄), and spectral slope
Variability in the time series trends for several parameters compared between treatment replicates convoluted interpretations of time series plots for the incubation (Appendix C). We focused on the percent change and rate of change for each parameter over the course of the incubation so that statistically relationships could be determined between the means (ANOVA). The percent change of each parameter from \( t_0 \) to \( t_{25} \) was calculated for each bottle (replicate) of each treatment. ANOVA and Tukey post-hoc test (means and standard deviations are reported in results as M and SD, respectively) were used to assess the difference between the mean percent change across the 12 treatments for each parameter (TN, DOC, NO\(_3\)-N, SumPL, SumHL, SumFL, SUVA\(_{254}\), and \( S_R \)). The rate of change of each parameter was determined using first order decay rates calculated as the slope of log transformed data (\( x = -\ln(\text{parm}_t/\text{parm}_0) \), \( y = \text{time} \)). Due to instrumental issues, lack of replicate data for some treatments impeded our ability to analyze the difference between slopes using ANCOVA. ANOVA and Tukey post-hoc test were used to analyze the difference between the mean rates across treatments for each parameter. C:N ratios were calculated for all carbon and carbon plus nutrient treatments. Bioavailable dissolved organic carbon (BDOC) was calculated for leaf leachate, soil extract, and glucose using the following equation

\[
BDOC_{\text{DOM}} = \frac{\text{Conc}_{\text{Tot}} \cdot \text{Vol}_{\text{Tot}} \cdot \text{BDOC}_{\text{Tot}} - \text{Conc}_{\text{STR}} \cdot \text{Vol}_{\text{STR}} \cdot \text{BDOC}_{\text{STR}}}{\text{Conc}_{\text{DOM}} \cdot \text{Vol}_{\text{DOM}}} \tag{1}
\]

in which DOM is the carbon source in question, STR is stream water, Conc is concentration, and Vol is volume. The BDOC\(_{\text{STR}}\) and BDOC\(_{\text{Tot}}\) were calculated using lab measured DOC and the following equation.

\[
\%BDOC = \frac{[\text{DOC}]_{T_0} - [\text{DOC}]_{T_{25}}}{[\text{DOC}]_{T_0}} \times 100 \tag{2}
\]
Figure 3.1 Map of our watershed with sampling sites marked. ST3 is the outlet of the 12 ha catchment and ST12 is the outlet of the 79 ha catchment where the scan spectrolysers are deployed. LW2 and LW5 are groundwater wells.

Table 3.1 $R^2$ and adjusted $R^2$ values for the four models used to calibrate the sensor data used throughout this thesis, which show no evidence of the issue of multicollinearity among variables sometimes evident when using multiple regression analyses.

<table>
<thead>
<tr>
<th>Model</th>
<th>R2</th>
<th>Adj. R2</th>
<th>% Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST3 DOC</td>
<td>0.9705</td>
<td>0.9703</td>
<td>0.02</td>
</tr>
<tr>
<td>ST3 NO$_3$-N</td>
<td>0.9325</td>
<td>0.9318</td>
<td>0.08</td>
</tr>
<tr>
<td>ST12 DOC</td>
<td>0.9833</td>
<td>0.9831</td>
<td>0.02</td>
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<tr>
<td>ST12 NO$_3$-N</td>
<td>0.9697</td>
<td>0.9693</td>
<td>0.04</td>
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</table>
Figure 3.2 PLSR calculated DOC values regressed on lab DOC for ST3 2015 data.

Figure 3.3 PLSR calculated NO$_3^-$-N values regressed on lab NO$_3^-$-N for ST3 2015 data.
Figure 3.4 PLSR calculated DOC values regressed on lab DOC for ST12 2015 data.

Figure 3.5 PLSR calculated NO$_3^-$-N values regressed on lab NO$_3^-$-N for ST12 2015 data.
Figure 3.6 DO, temperature, NO$_3$-N, DOC, FDOM, and Q all exhibited diel patterns at ST12 in April 2015. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 3.7 Comparison of diel patterns of groundwater and stream discharge. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Table 3.2 Laboratory incubation treatment abbreviations and descriptions.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Bottle Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>STR</td>
<td>Stream water</td>
</tr>
<tr>
<td>SA</td>
<td>Stream water, plastic (to add surface area)</td>
</tr>
<tr>
<td>ION</td>
<td>Stream water, ions</td>
</tr>
<tr>
<td>LEAF</td>
<td>Stream water, leaf leachate</td>
</tr>
<tr>
<td>SOIL</td>
<td>Stream water, soil extract</td>
</tr>
<tr>
<td>GLU</td>
<td>Stream water, glucose</td>
</tr>
<tr>
<td>STRNO3</td>
<td>Stream water, nutrients</td>
</tr>
<tr>
<td>SANO3</td>
<td>Stream water, plastic, nutrients</td>
</tr>
<tr>
<td>IONNO3</td>
<td>Stream water, ions, nutrients</td>
</tr>
<tr>
<td>LEAFNO3</td>
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<tr>
<td>SOILNO3</td>
<td>Stream water, soil extract, nutrients</td>
</tr>
<tr>
<td>GLUNO3</td>
<td>Stream water, glucose, nutrients</td>
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Chapter 4
RESULTS

4.1 Diel Variations in Stream Water DOC and NO$_3^-$-N

Diel DOC and NO$_3^-$-N patterns were most pronounced at ST12 during the spring. Diel DOC and NO$_3^-$-N patterns were much less evident at ST3 throughout the year. Winter 2016 was warmer than winter 2015 and had a pronounced effect on the strength of the diel patterns at both catchments (Fig. 4.1).

4.1.1 Seasonal diel signals throughout the watershed

Diel patterns in DOC, NO$_3^-$-N, discharge, DO, FDOM, and temperature were evident at ST12 throughout 2015 and the spring of 2016 except during January and February 2016 for DOC, July 2015 for NO$_3^-$-N, and July 2015 through February 2016 for discharge (Tables 4.1 – 4.3). The most prominent patterns emerged in the springtime and the least diel variation occurred during summer for DOC (largest change in April 2015: 0.803 ppm in ~ 7 hrs), NO$_3^-$-N (largest change in April 2016: 0.116 ppm in ~ 10 hrs), and temperature (largest change in April 2016: 6.29 °C in ~ 8.5 hrs). The largest maximum DO saturation occurred in spring (Table 4.2). Baseflow diel patterns in stream discharge were not consistently evident in July 2015 through February 2016, mostly due to instrument error or flow below the detection capabilities of the stream level logger. Discharge patterns had greater amplitudes during spring 2015 than spring 2016 (Table 4.3). Where quantifiable, groundwater level variations exhibited a similar pattern to discharge with maximum stream discharge coinciding
with minimum depth to ground water (Table 4.4). Daily FDOM changed ~ 1.5 – 2 QSU except in September 2015 for which the average change was 4.763 QSU in 9 hrs (Table 4.4). Diel patterns were much less evident at ST3. DOC and NO$_3^-$-N only exhibited strong diel patterns in April 2015 and March 2016 with weaker but still quantifiable patterns in September and October 2015 (Table 4.5). Discharge only exhibited diel behavior during April, September, October, November 2015, and March 2016 at ST3 (Table 4.6).

Limited sensor data was available at ST12 until March 2015. During March 2015, NO$_3^-$-N peaked midmorning (8:14) and reached a minimum in the evening (17:24) (Fig. 4.2). Discharge also peaked in the morning (7:26) and reached its maximum during the evening (17:01). Conversely, March 2015 DOC peaked in the evening (18:54) and reached a minimum late morning (9:44). DO and temperature also peaked in the afternoon (13:41 and 15:28, respectively) and temperature minimums occurred midmorning (7:25). Diel patterns of all parameters increased in magnitude during April 2015 (Fig. 4.3). The timing of the maximum and minimum values during April was relatively similar to those in March (Tables 4.1 – 4.3). May 2015 patterns for all solutes were still strong but to a lesser extent than April (Fig. 4.4). Patterns became muted in June 2015, a month that experienced many storms. After June, the watershed began to dry up (Fig. 4.5) and solute patterns were muted during July, August, and part of September 2015. The amplitude of diel patterns in DOC, FDOM, NO$_3^-$-N increased during October 2015 but not to the same extent as the spring. Average daily changes in DOC, FDOM, and NO$_3^-$-N were 0.803 ppm, 1.807 QSU, 0.097 ppm, respectively, in April and 0.570 ppm, 1.051 QSU, 0.056 ppm, respectively, in October (Fig. 4.6). Diel variations in DOC, discharge, NO$_3^-$-N, and
groundwater level at ST12 ceased, and all other patterns were greatly muted, during the winter until February 2016 when NO$_3$-N was the first to return to exhibiting unmuted diel patterns. During February and March 2016, storm events caused little disturbance to the daily variations of NO$_3$-N at ST12 (Figs. 4.7, 4.8). Temperature had a strong effect on the variations of NO$_3$-N and DOC at ST12. When the daily fluctuations in temperature increased in March 2016, the magnitude of the NO$_3$-N and DOC variations also increased (Fig. 4.9).

Specific conductance and solar radiation were visually examined to explore further evidence of hydrologic or biologic controls on NO$_3$-N and DOC in the second order stream (ST12). Specific conductance was plotted with NO$_3$-N, discharge and groundwater for May, September, and November 2015, and March 2016. Daily changes in conductance at ST12 followed the same general pattern, with maximum and minimum values falling within two hours of NO$_3$-N maximum and minimum values in May 2015, December 2015, and March 2016 (Figs. 4.10, 4.11, 4.12). During September 2015, conductance and NO$_3$-N were out of phase with one another (Fig. 4.13). In November 2015, the conductance pattern was still out of phase with NO$_3$-N until the 11/19/15 storm, which brought the conductance and NO$_3$-N patterns back into phase (Fig. 4.14).

Daily solar radiation values (MJ m$^{-2}$ day$^{-1}$) were plotted with DOC, NO$_3$-N, water temperature, discharge, and groundwater for March, April, May 2015, and February, March, April 2016. Frequent storms during March 2015 muted any effect radiation may have had on diel patterns of DOC and NO$_3$-N (Fig. 4.15) and a correlation between radiation and strength of diel patterns was not observed in May 2015, February 2016, April 2016 (Figs. 4.16, 4.17, 4.18); however, in April 2015 and
March 2016, the amplitude of DOC patterns was noticeably muted on days with low solar radiation (Figs. 4.19, 4.20).

Diel patterns were much less evident at ST3. There were only enough data available during April, September and October 2015 and March 2016 to calculate monthly averages of the daily maximum and minimum values of the moving averages of NO$_3$-N and DOC (Table 4.5). The September and October 2015 NO$_3$-N and DOC data contained fewer than 10 points to include in the monthly average calculations. Discharge data for April, September, October, and November 2015, and March 2016 was available for monthly average calculations and November 2015 had fewer than 10 daily maximum and minimum values available for the monthly average calculation (Table 4.6). Few months exhibited diel patterns at ST3 due to high variability in the sub-hourly measurements of NO$_3$-N and DOC (Fig. 4.21). Seasonal differences in diel patterns at ST3 were similar to those at ST12; at ST3, the largest amplitude patterns occurred during the spring, several parameters exhibited weak or no patterns during the summer, and low amplitude patterns reemerged during autumn (Tables 4.5, 4.6). In April 2015 at ST3, NO$_3$-N and stream discharge peaked in the early morning (5:27) and reached a minimum in the early afternoon (13:18), while DOC peaked at night (22:50) and reached a minimum in the morning (5:01) (Fig. 4.22). The timing of patterns was similar in May 2015 but the amplitude of the DOC signal decreased and the NO$_3$-N signal was weaker (Fig. 4.23). Distinct diel patterns in DOC and NO$_3$-N at ST3 were not evident from June through August 2015. Similar to ST12, distinguishable diel patterns in DOC and NO$_3$-N returned during autumn at ST3 (Fig. 4.24).
Due to longer residence time, the groundwater levels took longer to return to baseflow than stream water after storms (Fig. 4.25). Where groundwater diel patterns were evident, however, they gave some insight to the relative control of hydrology on diel variations in stream DOC and NO$_3$-N. Diel groundwater patterns were not evident in either well until April 2015 (Figs. 4.26, 4.27, Table 4.4). The amplitude of diel patterns in groundwater depth increased in late May 2015, likely due to increased evapotranspiration rates during leaf out (Fig. 4.28). Increased groundwater depth, and the loss of LW2 data for two months, during the period from July 2015 through September 2015 was indicative of drought during this time (Fig. 4.5). Exact timing of the maximum and minimum values for groundwater level could not be determined due to relatively constant depth values during portions of the day. Average times for the flat line periods were used. Generally, periods of minimum groundwater depths (when groundwater was closest to the surface) began within a couple hours of NO$_3$-N maximums and DOC minimums during the early morning. For example, in May 2015 at ST12, groundwater was closest to the surface (minim depth) on average at 5:33 for LW2 and 6:33 for LW5, NO$_3$-N peaked at 6:43, and DOC was at its lowest at 6:40 (Fig. 4.29). The timing of the DOC minimum, NO$_3$-N peak, and minimum groundwater depth continued to occur during the morning throughout the year. In August 2015, at ST12, DOC was lowest at 8:44, NO$_3$-N peaked at 9:44, and the groundwater was closest to the land surface (minimum depth) at 6:41 for LW2 and 8:31 for LW5 (Fig. 4.30). During October 2015, DOC was lowest at 8:12, NO$_3$-N peaked at 7:22, and the groundwater was closest to the land surface (minimum depth) at 6:06 for LW5 (Fig. 4.31). All other groundwater exploration graphs can be found in Appendix A.
Overall, analysis of diel patterns revealed that NO$_3^-$-N and discharge followed roughly the same pattern with peaks in the morning. DOC, FDOM, temperature, DO peaked in the afternoon when NO$_3^-$-N, was lowest. When the amplitude of temperature patterns increased, the amplitude of NO$_3^-$-N, DOC, FDOM, and DO increased. Patterns were strongest during the spring.

4.1.2 Differences in 2015 and 2016 winters and the strength of diel patterns

While the timing of the diel signals remained similar between spring 2015 and spring 2016, there was one key difference (Fig. 4.1). The average minimum temperature in March was much warmer in 2016 (2015 max: 7.58, min: 2.56; 2016 max: 11.10, min: 6.15). February 2016 temperatures were similar to March 2015 temperatures (Table 4.2). The warmer winter was accompanied by larger amplitude in the diel signals of NO$_3^-$-N, DOC, and FDOM in March 2016 (Figs. 4.2, 4.9). Even though diel patterns at ST3 were less present throughout the year and weaker than those at ST12, when diel patterns did occur at ST3, they were stronger in spring 2016. March 2015 data for ST3 was too variable to discern any clear diel signals in NO$_3^-$-N or DOC (Fig.4.21), but both solutes had clear signals in March 2016, especially after the temperature increase around 3/8/16 (Fig. 4.32). Despite the increased strength in diel solute signals due to warmer winter temperatures, the March 2016 storm caused a greater disturbance in the diel NO$_3^-$-N signal at ST3 than at ST12 due to weaker signals at the ST3 outlet (Fig. 4.33).

4.2 Incubation

Six treatments were intended to add varying carbon compositions to the stream water to understand the role of carbon composition on NO$_3^-$ consumption. These
treatments were STR, STRNO3, GLU, GLUNO3, LEAF, LEAFNO3 and the final C:N ratios of the overall incubated bottle were calculated. The GLU treatment had the largest C:N and STRNO3 had the lowest (Table 4.7). BDOC was also calculated for all treatments that added carbon to the system. For the types of external carbon sources added to the stream water, glucose DOC was most bioavailable and the soil extract DOC was least bioavailable (Table 4.8).

4.2.1 Nitrogen consumption

TN decreased in almost all treatments (Fig. 4.34). Where the overall percent change was not a decrease, it was usually due to skew caused by one of the three data points. There is no effect of surface area, ionic strength, or addition of nutrients on TN concentration compared to consumption in STR. TN consumption was significantly increased in the presence of “labile” carbon. Stream TN decreased by 14.6 ± 5.2% and was significantly different from LEAF (M = -52.0, SD =6.3) and GLU (M = -51.0, SD = 5.2). The percent change in TN caused by LEAF and GLU was significantly different from all treatments except each other and GLU percent change was not significantly different from GLU NO3 (M = -22.0, SD = 5.6).

The rate of consumption of TN in STR was 0.0006 ± 0.0021 –ln(ppm) day\(^{-1}\). Most treatments had rates of consumption or production near zero (Fig. 4.35). GLU had the highest rate of consumption and GLU (M = 0.0133, SD = 0.0021) and LEAFNO3 (M = -0.0183, SD = 0.0026) were the only treatments significantly different from STR. While the LEAFNO3 results indicated a high rate of TN production in this treatment, the percent change in TN value indicated that overall TN was consumed between \(t_0\) and \(t_{25}\). The discrepancy is likely due to the high variability in the TN measurements throughout the incubation and between replicates.
Stream NO$_3^-$-N increased by 0.8 ± 7.5% and was produced at an average rate of 0.0020 ± 0.0041 –ln(ppm) day$^{-1}$. There was no effect of surface area or ionic strength on the percent change in NO$_3^-$-N compared to STR. Labile carbon plus nutrients increased consumption of NO$_3^-$-N significantly compared to STR control (LEAFNO3 M = -70.3, SD = 7.5; GLUNO3 M = -70.4, SD = 6.1), while more recalcitrant carbon plus nutrients significantly increased NO$_3^-$-N production (STRNO3 M = 63.7, SD = 7.5; SOILNO3 M = 78.1, SD = 7.5) (Fig. 4.36). LEAF and GLU could not be compared to the other treatments with ANOVA due to only having one accurate replicate NO$_3^-$-N measurement for each treatment; however if the available values are representative of each treatment’s effect on NO$_3^-$-N, the treatments would have significantly increased the negative percent change in NO$_3^-$-N compared to all other treatments each other and their nutrient counterparts (green dots, Fig. 4.29).

Most treatments did not cause a large rate of change in NO$_3^-$-N over the course of the incubation (Fig. 4.37). STRNO3 and SOILNO3 exhibited an increasing trend in the rate of production of NO$_3^-$-N, but these treatments did not create a significant difference in the rate of NO$_3^-$-N processing when compared to STR. LEAFNO3 (M = 0.0242, SD = 0.0041) and GLUNO3 (M = 0.0103, SD = 0.0033) significantly increased the rate of consumption of NO$_3^-$-N. Again, LEAF and GLU could not be statistically compared to the other treatments; however if the available values are representative of each treatment’s effect on rate of change in NO$_3^-$-N, both treatments would have significantly increased the rate of consumption compared to all treatments except GLUNO3 and maybe LEAFNO3 (green dots, Fig. 4.30).
4.2.2 Carbon concentration and composition

DOC decreased in all treatments and 16.2 ± 3.2% of stream DOC was consumed at a rate of 0.0368 ± 0.0018 –ln(ppm) day⁻¹ (Fig. 4.38). Labile carbon addition caused the largest decrease in DOC from t₀ to t₂₅. LEAF (M = -57.5, SD = 3.2), LEAFNO3 (-46.2, SD = 4.0), GLU (M = -69.7, SD = 3.3), and GLUNO3 (M = -55.1, SD = 3.2) significantly increased consumption compared to STR, SOIL, and SOILNO3 (M = -32.6, SD 3.2).

GLU increased the consumption rate of DOC the most (Fig. 4.39) and was significantly different from all other treatments. LEAF (M = 0.0413, SD = 0.0018), GLU (M = 0.0664, SD = 0.0018), LEAFNO3 (M = 0.0325, SD = 0.0022), GLUNO3 (M = 0.0466, SD = 0.0018), and SOILNO3 (M = 0.0161, SD = 0.0018) significantly increased DOC consumption rates compared to STR. LEAF DOC consumption rate was significantly different from all treatments except GLUNO3 and LEAFNO3 and vice versa. BDOC calculations show that lability of DOM solutions increases from soil extract to leaf leachate to glucose (Table 4.9). SANO3 may have leached DOC, even after subtracting plastic control values, since the rate shows production and the variability in the percent change crosses zero into positive response (Figs. 4.38, 4.39).

All treatments exhibited consumption of protein-like (PL) fluorophores (Fig. 4.40). STR SumPL decreased by 45.0 ± 7.9 % at a rate of 0.0234 ± 0.006 –ln(ppm) day⁻¹. Ionic strength, surface area, and nutrient addition alone had no effect on the change in SumPL compared to stream water. Only LEAFNO3 (M = -87.2, SD = 7.9) and GLUNO3 (M = -93.2, SD = 7.9) SumPL percent change are significantly different from STR, and LEAF (M = -89.9, SD = 9.7), LEAFNO3, GLU (M = -81.6, SD = 7.9), and GLUNO3 are different from STRNO3 (M = -28.9, SD = 9.7). LEAF (M = 0.0919, SD = 0.0073), LEAFNO3 (M = 0.0827, SD = 0.0060), GLUNO3 (M = 0.0633, SD =
increased consumption rates significantly compared to STR and most of the rest of the treatments (Fig. 4.41). LEAF and LEAFNO3 rates of change are significantly different from everything except GLUNO3 and each other. GLUNO3 is different from everything except LEAF, LEAFNO3, GLU and SANO3 and had the largest absolute change during the course of the incubation. GLUNO3 had a higher initial SumPL fluorescence than other treatments except GLU, but GLU did not significantly change the rate of consumption, which may be due to measurement errors. GLU t₀ samples may have undergone DOM cycling before chemical measurements were made (see discussion for more information).

No treatment caused the percent change or rate of change of humic-like (HL) fluorescence to be significantly different from STR (Figs. 4.42, 4.43). For both percent change and rate of change, the SumHL values have high variability across all treatments. Stream SumHL increased by 2.0 ± 7.9% at a rate of 0.0004 ± 0.0021 – ln(ppm) day⁻¹. An increase would be expected for these treatments as labile DOM was processed, more recalcitrant DOM was left behind.

Many treatments did not cause a change in fulvic-like (FL) fluorescence over time (Fig. 4.44). Stream SumFL decreased by 1.5 ± 3.7% at a rate of 0.0008 ± 0.0014 –ln(ppm) day⁻¹ and the percent change was only significantly different from LEAF (M = -27.1, SD = 3.7). LEAF was significantly different from STR and everything else except SANO3 (M = -0.5, SD = 6.4) and LEAFNO3 (M = -10.5, SD = 3.7). Most rates of change of SumFL do not vary significantly from one another across treatments (Fig. 4.45). LEAF and GLUNO3 produced rates of change in SumFL significantly different from everything including each other. LEAF (M = 0.0144, SD = 0.0014) significantly increased the rate of consumption of FL fluorophores compared to STR, and
GLUNO3 ($M = -0.0139$, $SD = 0.0014$) significantly increased the production of FL fluorophores compared to STR.

$SUVA_{254}$ remained the same or increased over time in most treatments (Fig. 4.46). Stream $SUVA_{254}$ increased by $14.7 \pm 15.7\%$ at a rate of $0.0087 \pm 0.0025 - \ln(ppm)$ day$^{-1}$. LEAF and GLU caused a much larger increase over time but only GLU ($M = 109.4$, $SD = 12.8$) was significantly different from STR. The rate of change of $SUVA_{254}$ over the course of the incubation varied greatly among treatments (Fig 4.47). STR rate of change was significantly different from SA ($M = 0.0074$, $SD = 0.0020$), LEAF ($M = -0.0244$, $SD = 0.0025$), GLU ($M = -0.0542$, $SD = 0.0020$), SANO3 ($M = 0.0114$, $SD = 0.0025$), GLUNO3 ($M = -0.0335$, $SD = 0.0020$). SA and SANO3 significantly increased the rate of $SUVA_{254}$ decrease over the course of the incubation compared to STR, while LEAF, GLU and GLUNO3 all significantly increased the rate of increase in $SUVA_{254}$ over the course of the incubation. GLU increased the rate of increase of $SUVA_{254}$ the most, GLUNO3 had the next strongest effect on increasing $SUVA_{254}$ value rates followed by LEAF.

The spectral slope ratio, which is inversely related to DOM molecular weight (Helms et al., 2008), decreased over time in all treatments (Fig 4.48) and all treatments had rates of decreased spectral slope ratio over time (Fig 4.49) STR $S_R$ decreased by $12.9 \pm 2.2\%$ at a rate of $0.0038 \pm 0.0007 - \ln(ppm)$ day$^{-1}$. GLU ($M = -32.4$, $SD 2.2$) and GLUNO3 ($M = -40.6$, $SD 2.2$) significantly changed the percent change in $S_R$ compared to STR. GLUNO3 ($M = 0.0105$, $SD = 0.0007$) increased the rate of decreased $S_R$ the most and is the only treatment that significantly changed the rate of change in spectral slope ratio compared to STR.
4.3 Coupled Changes in Stream Water NO\textsubscript{3}⁻-N and DOC during Autumn

4.3.1 Tracking patterns with grab samples

Autumn 2014 spectro\textregistered:lyser data was limited due to trial and error as we set up our sensor deployments. Three times weekly grabs samples were taken in conjunction with the in situ sensor measurements. At ST3, the data shows evidence that a NO\textsubscript{3}⁻-N depression, or minimum in stream concentrations, may have occurred during the period of missing data between 10/21/14 and 11/1/14 since lab values during this time were below detection (0.02 ppm NO\textsubscript{3}⁻-N) (Fig. 4.50). The exact date the ST3 depression began to recover is unclear due to missing spectro\textregistered:lyser data, but stream NO\textsubscript{3}⁻-N concentrations began to recover around 11/8/14 and reached early autumn concentrations after the 11/17/14 storm. DOC data that is available indicates an increase in concentration throughout autumn at ST3 with the highest concentrations mostly during the period of missing data (10/21/14 – 11/1/14). Autumn leaf fall during 2014 peaked within the first few days of November. Protein-like fluorescence values were variable throughout autumn (Fig. 4.50).

The stream water NO\textsubscript{3}⁻-N depression at ST12 began between 10/3/14 and 10/6/14. The lab measured NO\textsubscript{3}⁻-N was below detection after 10/6/14. NO\textsubscript{3}⁻-N recovery began around 11/13/14, was accelerated by the 11/17/14 storm, and began to plateau in in mid-December (Fig. 4.51). Baseflow DOC increased until about mid-October, may have plateaued during the NO\textsubscript{3}⁻-N depression, and then decreased through the end of autumn.

4.3.2 High-frequency measurements capture autumn NO\textsubscript{3}⁻ depression

The DO and water temperature time series for autumn 2014 are of good quality but do not show any unexpected or novel patterns (Fig. 4.52). The water temperature
decreased throughout autumn with decreasing air temperature which caused DO saturation to increase. The turbidity and FDOM datasets were not of good quality. During the weeks preceding the 2014 NO₃⁻-N depression, baseflow turbidity increased but this is likely due to the increased hydrologic activity during that time. FDOM, which here reflects humic-like fluorescence, increased leading up to the depression, but this is also likely linked to hydrologic activity and increasing overall DOC concentration during that period.

Leaf fall at ST3 in autumn 2015 also peaked in early November. The timing of the NO₃⁻-N depression was similar to 2014 and occurred around 10/20/15. While the 11/19/15 storm accelerated the stream water NO₃⁻-N recovery, early autumn concentrations were not reestablished until about a month after the depression in early December (Fig. 4.53). During the recovery period, storms increased NO₃⁻-N and DOC concentrations, but NO₃⁻-N loads show a linear decline between each storm at ST3. DOC decreased quickly over the course of approximately one week, starting on 9/19/15. During this time NO₃⁻-N increased slightly. Change in load for both DOC and NO₃⁻-N over time did not exhibit much deviation from zero on a monthly basis and the weekly pattern is generally similar for both analytes. Most positive rates of change occurred during storm flow and most negative rates of change occurred during extended baseflow periods. During the NO₃⁻-N depression, baseflow protein- and humic-like fluorescence increased slightly (Fig. 4.54). There is too much variability and too infrequent sampling of optical metrics such as SUVA₂₅₄ and Sᵣ for a discernible pattern to be observed. Baseflow changes in the fluorescence components of the PARAFAC model follow the changes in the pattern of bulk DOC concentration throughout autumn at both drainage locations.
The ST12 NO₃⁻-N decline began much sooner than the ST3 decline (Fig. 4.55). ST12 NO₃⁻-N started to decrease gradually at the end of August, reached its minimum around 10/26/15, and started to recover after the 11/19/15 storm (Fig. 4.56). The NO₃⁻-N recovery at ST12 took almost as long as the original decline. Storm events accelerated the increase in NO₃⁻-N after the depression for a few days to a week, but after that time, the concentrations decreased again until the next storm. Due to the lack of baseflow data (in situ or more frequent grab samples) in early to mid-autumn, it is difficult to track the more subtle changes in DOC concentration to confirm or deny if DOC increased after leaf fall began. As winter began and bulk DOC concentration decreased so did protein- and humic-like fluorescence, while SUVA₂₅₄ and S₉ increased (Fig. 4.57). Baseflow FDOM and turbidity began to decrease approximately one week before the NO₃⁻-N depression (Fig. 4.58). Water temperature decreased throughout autumn, which caused DO saturation to increase. The rates of change of NO₃⁻-N and DOC concentrations did not exhibit patterns over monthly or daily time scales (Appendix B). The rates of change of both solutes were generally the same throughout autumn 2015 and, generally, decreased during baseflow and increased during storms.
Figure 4.1 Warmer temperatures during winter 2016 vs. winter 2015 increased the magnitude of diel patterns in stream solutes during spring 2016.
Table 4.1 Summary of the ST12 monthly averages of the daily values and timing of maximum and minimum DOC and NO$_3$-N, the amplitude of the change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.

<table>
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<tr>
<th>Month</th>
<th>Max DOC (ppm)</th>
<th>Min DOC (ppm)</th>
<th>Max Time</th>
<th>Min Time</th>
<th>Change (ppm)</th>
<th>Time of Change (hrs)</th>
<th>Max NO$_3$-N (ppm)</th>
<th>Min NO$_3$-N (ppm)</th>
<th>Max Time</th>
<th>Min Time</th>
<th>Change (ppm)</th>
<th>Time of Change (hrs)</th>
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<td>17:24</td>
<td>0.032</td>
<td>9:10</td>
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Table 4.2 Summary of the ST12 monthly averages of the daily values and timing of the maximum DO, maximum and minimum temperature, amplitude of temperature change over the course of a day, and how long the temperature change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.

<table>
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<th>Max Temp (°C)</th>
<th>Min Temp (°C)</th>
<th>Max Time</th>
<th>Min Time</th>
<th>Change (°C)</th>
<th>Time of Change (hrs)</th>
<th>Max DO (% Sat)</th>
<th>Max Time</th>
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<td>2.12</td>
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Table 4.3 Summary of the ST12 monthly averages of the daily values and timing of the maximum and minimum Q and FDOM, amplitude of change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.

<table>
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<th>Max Q (L s⁻¹)</th>
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<th>Max Time</th>
<th>Min Time</th>
<th>Change (ppm)</th>
<th>Time of Change (hrs)</th>
<th>Max FDOM (QSU)</th>
<th>Min FDOM (QSU)</th>
<th>Max Time</th>
<th>Min Time</th>
<th>Change (QSU)</th>
<th>Time of Change (hrs)</th>
</tr>
</thead>
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<td>8:37</td>
<td>1.807</td>
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<td>NA</td>
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Table 4.4 Summary of the monthly averages of the daily values and timing of the maximum and minimum ground water depth at two wells, amplitude of change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.

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<th>Min Time</th>
<th>Change (ppm)</th>
<th>Time of Change (hrs)</th>
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<th>Min LW5 depth (m)</th>
<th>Max Time</th>
<th>Min Time</th>
<th>Change (ppm)</th>
<th>Change (QSU)</th>
<th>Time of Change (hrs)</th>
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Table 4.5 Summary of the ST3 monthly averages of the daily values and timing of maximum and minimum DOC and NO$_3$-N, the amplitude of the change over the course of a day, and how long the change took during 2015 and spring 2016. Values used to determine the maximum and minimum daily concentrations of DOC and NO$_3$-N were moving averages calculated for every four hours, or eight measurements, of concentration data. Red values indicate data or patterns were present for fewer than 10 days during that month.

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<th>Min Time</th>
<th>Change (ppm)</th>
<th>Time of Change (hrs)</th>
<th>Max DOC (ppm)</th>
<th>Min DOC (ppm)</th>
<th>Max Time</th>
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<th>Change (ppm)</th>
<th>Time of Change (hrs)</th>
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</table>
Table 4.6 Summary of the ST3 monthly averages of the daily values and timing of the maximum and minimum Q, amplitude of Q change over the course of a day, and how long the change took during 2015 and spring 2016. Red values indicate data or patterns were present for fewer than 10 days during that month.

<table>
<thead>
<tr>
<th>Month</th>
<th>Max Q (L s⁻¹)</th>
<th>Min Q (L s⁻¹)</th>
<th>Max Time</th>
<th>Min Time</th>
<th>Change (ppm)</th>
<th>Time of Change (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar 2015</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Apr 2015</td>
<td>3.33</td>
<td>2.96</td>
<td>4:34</td>
<td>14:36</td>
<td>0.37</td>
<td>10:01</td>
</tr>
<tr>
<td>May 2015</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Jun 2015</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Jul 2015</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Aug 2015</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sep 2015</td>
<td>0.56</td>
<td>0.34</td>
<td>0.68</td>
<td>0.29</td>
<td>0.22</td>
<td>0.39</td>
</tr>
<tr>
<td>Oct 2015</td>
<td>0.51</td>
<td>0.43</td>
<td>0.60</td>
<td>0.30</td>
<td>0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>Nov 2015</td>
<td>0.59</td>
<td>0.54</td>
<td>0.66</td>
<td>0.28</td>
<td>0.04</td>
<td>0.38</td>
</tr>
<tr>
<td>Dec 2015</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Jan 2016</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Feb 2016</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mar 2016</td>
<td>1.21</td>
<td>1.07</td>
<td>7:17</td>
<td>17:31</td>
<td>0.13</td>
<td>10:13</td>
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<tr>
<td>Apr 2016</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Figure 4.2 Diel patterns at ST12 were observed in NO$_3^-$, DO, temperature, and DOC in March 2015. FDOM appeared to also exhibit a diel pattern, but not enough data was present to definitively conclude if a pattern was present. Diel Q cycling is weak during the early spring. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.3 DO, temperature, NO$_3^-$, DOC, FDOM, and Q all exhibited patterns at ST12 in April 2015. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.4 DO, temperature, NO$_3^-$, DOC, FDOM, and Q all exhibited patterns at ST12 in May 2015 but the amplitudes of the patterns were less than April. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.5 Groundwater data in both wells indicate a drought occurred throughout later summer and early autumn 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.6 The strength of NO$_3^-$ and DOC patterns increased during October 2015 compared to summer months. DO and temperature amplitudes were similar to the preceding months. Discharge measurements were inaccurate during this time and inaccurate data was reported as a minimum value. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.7 After a February 2016 storm, NO$_3^-$ diel patterns recovered more rapidly than Q. Diel DOC patterns were not observed during this month. The grey box indicates the storm period and immediate return to diel NO$_3^-$ patterns as baseflow resumes. Solid lines are midnight. Dotted lines are noon.
Figure 4.8 A March storm had little effect on the diel patterns of NO$_3$-N during spring 2016. The grey box indicates the storm period and highlights that the NO$_3$-N pattern goes through a brief dilution at the peak of the storm but recovers the diel pattern in less than 24 hrs. Solid lines are midnight. Dotted lines are noon. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.9 Stronger and warmer temperature signals led to increased amplitude in NO$_3$-N and DOC patterns during March 2016. Solid lines are midnight. Dotted lines are noon. Black arrow highlights the temperature increase. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.10 ST12 diel conductance pattern in phase with NO$_3$-N, water temperature, discharge, and groundwater during May 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.11 ST12 diel conductance pattern in phase with NO$_3$-N, water temperature, discharge, and groundwater during December 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.12 ST12 diel conductance pattern in phase with NO$_3$-N, water temperature, discharge, and groundwater during March 2016. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.13 ST12 diel conductance pattern out of phase with NO$_3$-N, water temperature, and groundwater during September 2015. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.14 ST12 diel conductance pattern out of phase with NO\textsubscript{3}\,-N, water temperature, and groundwater during November 2015 until the 11/19/15 storm which brought conductance back into phase with the other parameters. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.15 Daily solar radiation values plotted with ST12 March 2015 time series of NO\textsubscript{3}\,-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.16 Daily solar radiation values plotted with ST12 May 2015 time series of NO$_3^-$-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.17 Daily solar radiation values plotted with ST12 February 2016 time series of NO$_3^-$-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.18 Daily solar radiation values plotted with ST12 April 2016 time series of NO$_3^-$-N, DOC, discharge, and groundwater. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.19 Daily solar radiation values plotted with ST12 April 2015 time series of NO$_3^-$-N, DOC, discharge, and groundwater. Weaker diel patterns of DOC occur on low solar radiation days (grey box) compared to high solar radiation days (green box). Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.20 Daily solar radiation values plotted with ST12 March 2016 time series of NO$_3$-N, DOC, discharge, and groundwater. Weaker diel patterns of DOC occur on low solar radiation days (grey box) compared to high solar radiation days (green box). Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.21 High variability in scan data mutes March 2015 NO$_3$-N and DOC patterns at ST3. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.22 13 Diel NO$_3$-N and DOC patterns emerge strongly at ST3 in April 2015. Discharge begins to show clear diel patterns. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.

Figure 4.23 14 Diel patterns are still evident for NO$_3$-N and DOC at ST3 in May 2015 but daily magnitudes are less than those in April. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.
Figure 4.24 Weak diel patterns in DOC and NO₃⁻-N return in October after being muted during summer drought. Red scale values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A.

Figure 4.25 Comparison of diel patterns of groundwater and stream discharge. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.26 Diel patterns of DOC, NO$_3^-$-N, and groundwater level are non-existent at ST3 during March 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.27 Diel patterns of DOC, NO$_3^-$-N, and groundwater level begin to emerge at ST3 during April 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.28 The amplitude of diel patterns in groundwater level increased at ST3 during May 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure 4.29 Diel patterns of DOC, NO₃⁻-N, and groundwater levels at ST12 during May 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.30 Diel patterns of DOC, NO$_3$-N, and groundwater levels at ST12 during August 2015. For groundwater levels, red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. All other red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.31 Diel patterns of DOC, NO$_3$-N, and groundwater levels at ST12 during October 2015. For groundwater levels, red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. All other red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.32 Diel patterns in $\text{NO}_3^-$-$\text{N}$ and DOC show an increase in strength during March 2016 at ST3 during the same time water temperature was shown to increase the strength of diel patterns at ST12, indicated by the blue arrow. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure 4.33 Since diel pattern of NO$_3^-$-N and DOC at ST3 is not as strong as ST12, the winter 2016 storms increase the time it takes for diel variations in NO$_3^-$-N to resume at ST3 compared to at ST12. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year as depicted in Appendix A. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Table 4.7 Carbon to nitrogen ratios for each treatment of the lab incubation whose purpose was to add carbon and/or nitrogen to the system.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td>STR</td>
<td>4</td>
</tr>
<tr>
<td>STRNO3</td>
<td>2</td>
</tr>
<tr>
<td>GLU</td>
<td>32</td>
</tr>
<tr>
<td>GLUNO3</td>
<td>14</td>
</tr>
<tr>
<td>LEAF</td>
<td>20</td>
</tr>
<tr>
<td>LEAFNO3</td>
<td>9</td>
</tr>
<tr>
<td>SOIL</td>
<td>16</td>
</tr>
<tr>
<td>SOILNO3</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 4.8 The bioavailability of each carbon treatment solution and the overall BDOC. BDOC\textsubscript{DOM} is the bioavailability of the treatment solution and %BDOC is the overall BDOC of the treatment when added to stream water.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%BDOC</th>
<th>BDOC\textsubscript{DOM} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream water</td>
<td>16.19</td>
<td>N/A</td>
</tr>
<tr>
<td>Glucose</td>
<td>69.69</td>
<td>1959</td>
</tr>
<tr>
<td>Leaf leachate</td>
<td>57.54</td>
<td>1050</td>
</tr>
<tr>
<td>Soil extract</td>
<td>23.84</td>
<td>333</td>
</tr>
</tbody>
</table>

Figure 4.34 Overall percent change in total nitrogen from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.35 ANOVA on rates of consumption or production of TN across all incubation treatments. Positive rates indicate consumption.
Figure 4.36 Overall percent change in NO$_3$-N from $t_0$ to $t_{25}$ days for all incubation treatments. Green dots for LEAF and GLU represent the value of the one replicate bottle which had data for those treatments.
Figure 4.37 ANOVA on rates of consumption NO$_3^-$-N across all incubation treatments. Positive rates indicate consumption. Green dots LEAF and GLU represent the value of the one replicate bottle which had data for those treatments.
Figure 4.38 Overall percent change in DOC from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.39 ANOVA on rates of consumption of DOC across all incubation treatments. Positive rates indicate consumption.
Figure 4.40 Overall percent change in protein-like fluorescence from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.41 ANOVA on rates of consumption of protein-like fluorescence across all incubation treatments. Positive rates indicate consumption.
Figure 4.42 Overall percent change in humic-like fluorescence from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.43 ANOVA on rates of consumption of humic-like fluorescence across all incubation treatments. Positive rates indicate consumption.
Figure 4.44 Overall percent change in fulvic-like fluorescence from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.45 ANOVA on rates of consumption of fulvic-like fluorescence across all incubation treatments. Positive rates indicate consumption.
Figure 4.46 Overall percent change in SUVA$_{254}$ from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.47 ANOVA on rates of change in SUVA\textsubscript{254} across all incubation treatments. Positive rates indicate decrease in SUVA\textsubscript{254} values over time. Decrease in SUVA\textsubscript{254} indicates a decrease in aromaticity and increase in bioavailability.
Figure 4.48 Overall percent change in $S_R$ from $t_0$ to $t_{25}$ days for all incubation treatments.
Figure 4.49 ANOVA on rates of change in $S_R$ across all incubation treatments. Positive rates indicate decrease in $S_R$ values over time, which would indicate an increase in high molecular weight DOM fraction.
Figure 4.50 [DOC], [NO$_3^-$-N], protein-like fluorescence, Q, air temperature, and litter fall mass during autumn 2014 at ST3. Grab samples for [DOC], [NO$_3^-$-N], and protein-like fluorescence analysis were collected three times per week.
Figure 4.51 [DOC], [NO$_3$-N], protein-like fluorescence, Q, air temperature, and litter fall mass during autumn 2014 at ST12. Grab samples for [DOC], [NO$_3$-N], and protein-like fluorescence analysis were collected three times per week.
Figure 4.52 Autumn 2014 water temperature, air temperature, DO, litter fall mass, turbidity and FDOM at ST12. DO, turbidity, water temperature, and FDOM were measured in situ.
Figure 4.53 Autumn 2015 Q, litter fall mass, DOC load, and NO$_3^-$-N load at ST3. DOC and NO$_3^-$-N axes are zoomed in to accentuate baseflow loads.
Figure 4.54 Autumn 2015 Q, litter fall mass, in situ [DOC], and grab and storm [DOC], protein- and humic-like fluorescence, SUVA_{254}, and S_{R} at ST3.
Figure 4.55 Autumn 2015 NO$_3^-$ crash and recovery at ST3 and ST12 with litter leaf mass and ST12 Q to depict the influence of leaf fall and hydrology on autumn stream chemistry dynamics. Note the order of magnitude difference in load scales.
Figure 4.56 Autumn 2015 Q, litter fall mass, DOC load, and NO$_3^-$-N load at ST12.
Figure 4.57 Autumn 2015 Q, litter fall mass, in situ [DOC], and grab and storm [DOC], protein- and humic-like fluorescence, SUVA$_{254}$, and $S_q$ at ST12.
Figure 4.58 Autumn 2015 water temperature, air temperature, DO, litter fall mass, turbidity and FDOM at ST12. DO, turbidity, water temperature, and FDOM were measured in situ.
Chapter 5

DISCUSSION: COUPLED RESPONSES OF AND CONTROLS ON STREAM WATER CARBON AND NITROGEN

5.1 Diel Patterns in Stream Water Carbon and Nitrogen

The timing of the maximum and minimum values of many of the parameters we measured, coupled with the seasonal variations in the strength of the diel patterns, indicate biotic factors exhibit the strongest control on the diel patterns of NO$_3^-$-N and DOC in our watershed. While we hypothesized the biotic control on diel patterns would be heterotrophy, we find autotrophy to be the dominant control on diel patterns of DOC and NO$_3^-$-N. The minimum NO$_3^-$-N concentration coincides with the maximum DO saturation, temperature, and DOC concentration at both sites, indicating that autotrophic assimilation of NO$_3^-$ and production of DOC is the driving process behind diel patterns of DOM and nutrients in our stream. These findings are consistent with those of Kaplan & Bott (1982), Pellerin et al. (2012), Roberts & Mulholland (2007) and others (Nimick et al., 2011) (Tables 5.1, 5.2). Kaplan & Bott (1982) observed DOC peaks in the late afternoon which were driven by temperature. Algal production accounted for as much as 20% of DOC production in agricultural Piedmont streams with forested riparian zones from late March until early June and again in November (Kaplan & Bott, 1982). Pellerin et al. (2012) observed baseflow diel patterns in NO$_3^-$ after snowpack ablation with minimum NO$_3^-$ during the late afternoon. They attributed the NO$_3^-$ variations to autotrophic assimilation prior to leaf out in their forested headwater catchment. Roberts & Mulholland (2007) found gross primary production explained 91% of the variance of NO$_3^-$ amplitude with the highest net uptake of NO$_3^-$ during spring causing the daily afternoon NO$_3^-$ minimum values to
occur within 2 hours of our springtime minimums. Several other studies conducted in forested catchments observed diel patterns in NO$_3^-$ during springtime prior to leaf out (Nimick et al., 2011).

In forested watersheds, the maximum solar radiation reaching the stream likely occurs during the springtime while the canopy is still open. We observed the largest amplitude of change in daily NO$_3^-$-N, DOC, discharge, and DO patterns during springtime and when temperature fluctuations were greatest. Roberts & Mulholland (2007) found primary production rates were highest in late March and early April during the open-canopy period. We observed increased strength of spring patterns on days with more solar radiation. In April 2015 and March 2016, diel DOC patterns were muted on days with low solar radiation (Figs. 4.19, 4.20). This potential link between solar radiation and DOC cycling is further evidence that autotrophy is likely the dominant control on diel patterns in Fair Hill, especially during the spring prior to leaf out.

The apparent relationship between the strength of diel changes in DOC and NO$_3^-$-N with diel changes in temperature or solar radiation are consistent with the findings of Aubert & Breuer (2016). While Aubert & Breuer (2016) observed diel NO$_3^-$-N patterns with maximum and minimum timings shifted several hours compared to ours, they found similar drivers behind the patterns. Their study stream is much larger and the watershed contained 35 % arable land, 9 % grassland, 8% infrastructure, and only 48 % forest cover. The land use and physical stream characteristic differences between Fair Hill and the Aubert & Breuer (2016) watershed likely contribute to the differences in timing of daily NO$_3^-$-N maximum and minimum values. Aubert & Breuer (2016) observed NO$_3^-$-N peaks during the early evening in
June and at midnight during August. They attributed their much earlier than pre-dawn peaks to the mixed land use of their watershed. Aubert & Breuer (2016) also found temperature amplitude and maximum daily solar radiation to be drivers behind diel patterns of NO$_3^-$-N. Algal blooms were observed and can explain the asymmetry of the diel NO$_3^-$-N patterns; NO$_3^-$-N increased more quickly throughout the day than it decreased, which is likely do to metabolic denitrification beginning during the night in this watershed (Aubert & Breuer, 2016).

Rusjan & Mikoš (2010) also determined primary production to be the controlling mechanism behind diel patterns of NO$_3^-$-N in a large hilly, forested watershed in Slovenia. Solar radiation peaks between 12:00 and 14:00 likely contributed to water temperature peaks, which occurred 3-5 hours later (Rusjan & Mikoš, 2010). Dissolved oxygen saturation peaked an hour earlier than solar radiation. Similar to our observations at Fair Hill, NO$_3^-$-N in the Slovenian watershed was negatively correlated with water temperature, but NO$_3^-$-N concentration minimums generally occurred later (18:00 – 21:00) compared to our observations (~15:00 – 20:00 in the second order stream), which is likely due to differences in watershed size. Seasonal changes in the timing of maximum and minimum NO$_3^-$-N concentrations were likely due to changes in the relationship between terrestrial NO$_3^-$-N sources and autotrophic assimilation of NO$_3^-$-N (Rusjan & Mikoš, 2010).

The seasonal variation in the strength of diel patterns we observed is consistent with the findings of Worrall et al. (2015) who found that seasonal differences, not daytime differences, explained the most variance in DOC concentration. For the 1674 km$^2$ catchment with heath, bog, and mountainous DOC sources studied by Worrall et al. (2015), seasonal differences explained 28.2% of the variance while daytime
differences only explained 0.04% of the variations in DOC concentrations. Rusjan & Mikoš (2010) found that 81% of the seasonal variance in diel NO₃⁻-N amplitudes was caused by changes in water temperature amplitudes. Roberts & Mulholland (2007) also found that in-stream metabolism rates explained 81% of the seasonal variation of DIN retention. Seasonal changes in diel patterns in Fair Hill are likely due to a combination of seasonal biotic and hydrologic changes. Changes in solar radiation reaching the stream as leaf out occurred reduced the amplitude of diel temperature changes which in turn dampened autotrophic assimilation of NO₃⁻-N. Terrestrial biotic uptake of NO₃⁻-N also likely increased during the summer. High evapotranspiration rates decreased daytime hydrologic transport of NO₃⁻-N to the stream. The decreased in-stream assimilation of NO₃⁻-N and increased terrestrial assimilation of NO₃⁻-N coupled with lower daytime hydrologic transport of NO₃⁻-N to the stream all contributed to the decrease in diel patterns from spring to summer in the Fair Hill streams (Fig. 5.1).

April NO₃⁻-N, DOC, and temperature patterns were the strongest in our watershed. Diel patterns in stream NO₃⁻-N and DOC were weak or nonexistent during the summer, and diel amplitudes of NO₃⁻-N and DOC increased from summer to autumn; though, the autumnal amplitudes did not increase to the same level as spring. The increase in amplitude of diel NO₃⁻-N and DOC patterns during autumn is likely due to increased hydrologic connectivity to NO₃⁻ and DOC sources throughout the watershed compared to the dry summer months, as indicated by increased groundwater depth during summer (Fig. 4.5). Increased solar radiation during leaf fall and larger amplitude in daily temperature fluctuations may also have contributed to the increase in solute signal amplitudes from summer to autumn (Tables 4.1 – 4.3, 4.5,
As shown in March 2016 (Fig. 4.9), when the daily temperature change increased, the magnitude of DOC and NO$_3^-$-N diel patterns also increased. The increase in amplitude of diel patterns of temperature, DOC, and NO$_3^-$-N during autumn could be associated with autumnal algal blooms. A study across distinct geographic areas in the United States found that in watersheds where algal biomass was greatest during the spring, biomass was generally lowest during the summer and made a reappearance during autumn (Lee et al., 2012). Roberts & Mulholland (2007) found high net NO$_3^-$ uptake to occur during both spring and autumn in a first order deciduous forest; however, heterotrophic respiration rates dominated primary production rates during autumn. These results give further support that autumnal processing of NO$_3^-$ is likely due to the interplay of several factors.

Hydrology does not exhibit a dominant control on the diel patterns in this forested Piedmont stream. The relationship between the maximum and minimum values of solute concentrations and water levels are opposite those found in watersheds with dominant hydrologic controls. In Fair Hill, maximum discharge and minimum depth to groundwater (when groundwater is closest to the land surface) coincide with the maximum daily NO$_3^-$-N and minimum DOC concentrations. In contrast, Wilson & Xenopoulos (2013) studied diel patterns of DOC in six Canadian watersheds that fell along a 21-73% agricultural land use gradient. Throughout all of the study sites, DOC and discharge were lowest in the afternoon. The greatest amplitude of change in both DOC and discharge were greatest in the watersheds with more forest cover due to larger amplitudes in evapotranspiration rates in forested watersheds. Wilson & Xenopoulos (2013) attributed the resultant diel stream water DOC variations to changes in evapotranspiration rates. At night, evapotranspiration
rates are lower allowing more water to flow through more organic rich upper soil
horizons of riparian areas, transporting more DOC to the stream; during the day, a
higher proportion of stream water comes from deeper groundwater sources where
DOC concentrations are lower (Inamdar et al., 2012; Wilson & Xenopoulos, 2013).

Duncan et al. (2015) found hydrology to drive the diel patterns of NO$_3^-$-N in a
south-facing, forested Piedmont headwater stream with the highest NO$_3^-$-N
concentrations during the day, especially during the summer. High evapotranspiration
rates led deep groundwater to contribute to the greatest proportion of streamflow
during the afternoon. Riparian soils had the highest NO$_3^-$-N during summer. Duncan et
al. (2015) speculated that dry watershed conditions created a more oxic riparian
environment, which caused denitrification rates to decrease allowing more NO$_3^-$
transport to the stream. High daytime evapotranspiration rates drove groundwater level
and stream discharge to be the lowest later in the day, causing NO$_3^-$-N peaks to
coincide with the lowest discharge due to a greater contribution of riparian
groundwater during low flows (Duncan et al., 2015). Flewelling et al. (2014) also
found hydrology to control diel patterns of stream NO$_3^-$-N. In the agricultural, coastal
plain watershed studied by Flewelling et al. (2014), stream NO$_3^-$-N did not exhibit diel
patterns during springtime. Diel changes in NO$_3^-$-N during summer corresponded with
changes in stream discharge, which were driven by evapotranspiration. Discharge and
NO$_3^-$-N peaked during the late morning (~10:00) (Flewelling et al., 2014).

The lowest NO$_3^-$-N stream water concentrations, lowest discharge, and
maximum depth to groundwater occur in the afternoon, when stream water DOC is
lowest, in our Piedmont watershed. While both Wilson & Xenopoulos (2013) and
Flewelling et al. (2014) found hydrology to be the dominant driver of diel variations in
stream water chemistry, we find that the control of hydrology on diel solute patterns is important but not dominant in our watershed. The relationship between NO$_3^-$-N and water levels in our watershed indicates NO$_3^-$-N is being transported to the stream with groundwater, similar to (Flewelling et al., 2014). As the stream discharge increases so does NO$_3^-$-N concentration; however, the relationship between DOC and water levels is not the same as Wilson & Xenopoulus (2013). We would also expect to see similar seasonal amplitude changes in discharge and/or groundwater and stream NO$_3^-$-N and DOC if hydrology was the controlling mechanism behind diel patterns. Our observations reveal the opposite response, however. Diel discharge and groundwater amplitude increases during the summer when daytime evapotranspiration rates increase, but diel NO$_3^-$-N and DOC patterns become weaker during the summer (Figs. 4.3, 4.27, 4.30, A16, A45, A46). Heffernan & Cohen (2010) observed nighttime increases in NO$_3^-$-N due to primary production and were able to show that hydrologic dispersion had no control over diel NO$_3^-$-N patterns. Similar to our observations, Rusjan & Mikoš (2010) observed seasonal changes in both discharge and stream NO$_3^-$-N amplitudes with discharge amplitudes increasing and NO$_3^-$-N amplitudes decreasing during the summer. Rusjan & Mikoš (2010) also concluded that primary production was the controlling mechanism behind their diel NO$_3^-$-N patterns.

Diel conductance patterns have been shown to be indicative of groundwater input to a stream (Hayashi et al., 2012; Robson et al., 1992). The conductance data for our second order stream shows diel conductance patterns are in phase with diel NO$_3^-$-N during spring, late autumn, and winter. During the late summer drought, September conductance patterns are completely out of phase with NO$_3^-$-N (Fig. 4.13). This is likely indicative of a shift flow paths and in NO$_3^-$-N source during the drought since
conductance patterns were in phase with NO$_3^-$-N again after the 11/19/15 storm flushed soil NO$_3^-$-N to the stream accelerating the stream NO$_3^-$-N recovery. Rusjan & Mikoš (2010) hypothesized that during wetter watershed conditions, transport of terrestrial sources of NO$_3^-$-N controls the amount of in-stream NO$_3^-$-N that is available for microbial processing; however, as the watershed dries up, NO$_3^-$-N dynamics shift from a terrestrial control to an in-stream control. Our results indicate hydrology is important for transporting NO$_3^-$ to the stream; however, once the NO$_3^-$ reaches the stream, autotrophic assimilation of the NO$_3^-$ to produce DOC becomes the dominant driver of diel patterns.

Diel patterns of DOC and NO$_3^-$-N were strongest and most evident at the outlet of the second order stream in the 79 ha catchment (ST12) compared to patterns at the outlet of the first order stream in the 12 ha catchment (ST3), which is likely due to several factors. There is less canopy cover in the 79 ha catchment year round, which would allow for greater solar radiation to reach the stream. Since biotic factors play a dominant role in creating the diel patterns of stream DOC and NO$_3^-$-N, changes in solar radiation throughout the day likely contribute to the patterns of DOC and NO$_3^-$-N. Hydrology also likely plays a role in the differences in the strength of the diel patterns in the 12 and 79 ha watersheds. Visual observations indicate streamflow and drainage area are higher in the 12 ha catchment, especially during summer baseflow periods, indicating that the first order stream is more hydrologically connected to the 12 ha catchment than the second order stream is to the 79 ha catchment. The less frequent occurrence of diel patterns in DOC and NO$_3^-$-N at the more hydrologically connected first order stream, may be indicative of hydrologic controls muting the biologic controls on diel variations in stream water chemistry. The fact that the 79 ha
catchment is less hydrologically connected comes into play during autumn (Fig. 5.2) and contributes to the strength of diel patterns of DOC and NO$_3^-$-N by allowing autotrophs more time to assimilate stream NO$_3^-$-N to produce DOC before these solutes are hydrologically exported.

Bernhardt & Likens (2002) found that denitrification in a forested stream was nitrogen limited due to observations of increased rates of denitrification in the presence of NO$_3^-$ addition. The fact that the 13 ha, forested headwater catchment studied by Bernhardt & Likens (2002) was N limited leads us to speculate our study watershed is enriched in nitrogen, which could explain why we see evidence of autotrophic assimilation NO$_3^-$ in our watershed even when carbon is limited. For example, while diel patterns in the 79 ha catchment were indicative of autotrophic dominance, heterotrophic bacteria must also be present since DOC did not always exhibit a pattern when NO$_3^-$-N did (Figs. 4.7, 4.8). This suggests autotrophic organisms were still consuming NO$_3^-$ but the DOC produced was rapidly being consumed elsewhere. The incubation results indicated our system is carbon limited, which is additional evidence that heterotrophic activity is present in our streams.

5.2 Incubations Indicate Carbon Limitation

Our incubation results indicate our system is carbon limited. More nitrogen was consumed in the presence of labile carbon and consumption of nitrogen was not significantly different from STR when labile carbon was added with additional nutrients, which decreased the C:N ratio in those treatments. These results indicate that the increased availability of carbon is what stimulated the nitrogen consumption. Increased rates of heterotrophic consumption of nitrogen in the carbon treatments is consistent with literature which shows higher initial concentrations of nitrogen and
carbon will increase breakdown rates of OM (Allan & Castillo, 2007; Guenet et al., 2010; Suberkropp et al., 1976). Labile carbon increased overall TN consumption (GLU and LEAF) and the rate of TN consumption (GLU) while carbon plus nutrients (LEAFNO3 and GLUNO3) caused TN production. Strauss & Lamberti (2000) observed increased rates of production when they decreased C:N.

We observed increased rates of DOC and NO$_3^-$-N consumption in the labile carbon treatments, which is consistent with Bernhardt & Likens (2002) who found a stream that underwent labile carbon addition experienced rapid DOC removal, which increased DIN consumption and increased microbial biomass, activity, and respiration. Our incubation showed that mixed leaf leachate from our watershed has a %BDOC value three times that of the stream water alone. By increasing the labile carbon availability, we increased the NO$_3^-$ consumption rates. Strauss & Lamberti (2000) found that nutrient addition without carbon increased NO$_3^-$ production rates by 40% by decreasing C:N and NH$_4^+$ competition and increasing the availability of NH$_4^+$, which could explain why we observed increased rates of production of NO$_3^-$-N in STRNO3 and SOILNO3 (Table 4.8). Increased NO$_3^-$-N consumption rates in the labile carbon treatments is key to understanding our watershed is carbon limited. When carbon and nutrients are added together the consumption rate is still significantly different from the STR treatment, but less DOC is consumed in LEAFNO3 than LEAF. LEAFNO3 likely reduced consumption relative to LEAF since the C:N of LEAFNO3 is less than LEAF. If our system is carbon limited, the C:N is too low relative to the C:N preferred by heterotrophs. Adding LEAF increased the C:N to a more favorable level for heterotrophic metabolism. The additional nitrate added by LEAFNO3 compared to LEAF lowered the C:N in LEAFNO3 to a value closer to
STR, which only provides enough carbon for slower metabolic rates. By increasing the C:N in LEAF, this treatment stimulated the carbon limited microbes and increases consumption rates.

Protein-like fluorescence is used as an indicator of labile DOM quality since PL fluorescence decreases with DOC concentration during the biotransformation of DOM (Cuss & Guéguen, 2015; Fellman et al., 2008). The observed trend of decreasing PL fluorescence over time in the labile carbon treatments is consistent with the idea that more labile DOM will be consumed first, as demonstrated by Wymore et al. (2015) who found respiration rates increased when SUVA\textsubscript{254} increased. SUVA\textsubscript{254} is negatively correlated with bioavailability (Weishaar et al., 2003), so increased SUVA\textsubscript{254} values during respiration indicates less bioavailable DOM is left behind during consumption. SumPL increased with lability except for with GLU but glucose does not fluoresce and GLU DOC\textsubscript{t0} indicates rapid consumption occurred before lab analyses captured true t\textsubscript{0} data (Figs. C9, C10). Addition of leaf litter to a stream can rapidly increase DOC concentration (up to 10 times initial concentrations) and decomposition of labile carbon and nitrogen lead to rapid bacterial population growth (Wetzel & Manny, 1972). We speculate rapid bacterial population growth occurred in our glucose treatments, as glucose was the most labile carbon form added, which may have led to the inconsistent DOC data at the beginning of the incubation.

Bioavailability is negatively correlated and aromaticity is positively correlated with SUVA\textsubscript{254} (Weishaar et al., 2003; Wymore et al., 2015). Our SUVA\textsubscript{254} results indicate GLU carbon is very bioavailable and the bioavailability of LEAF as indicated by SUVA\textsubscript{254} is comparable to STR. As SUVA\textsubscript{254} increases, relative DOC decreases which means a larger portion of the remaining DOC is more recalcitrant. Increased
SUVA\textsubscript{254} values may indicate increased consumption rate of non-CDOM. Increasing SUVA\textsubscript{254} throughout the incubation, and especially in the presence of labile carbon, is consistent with our findings that protein-like fluorophores were consumed most in the labile carbon treatments over the course of the incubation. It is important to note that SUVA has been found to be useful in predicting the total carbon mineralization of leachates from multiple leaf species only in the presence of excess nutrients (Wieder et al., 2008). SUVA\textsubscript{254} is a useful parameter for characterizing changes in our stream DOM since we are carbon limited, not nutrient limited.

The spectral slope ratio decreased over time in all treatments, which indicates the fraction of HMW DOM increased. These results are consistent with the presence of heterotrophic consumption during which lower molecular weight DOM is more likely to be consumed (van Hees et al., 2005). The addition of glucose and glucose with nutrients are the only treatments that were significantly different from STR and they caused a much larger increase in HMW fraction in the remaining water.

The results from the laboratory incubation provide insight into DOM and nutrient processing in our stream system. The incubation indicates the heterotrophic population in our streams is carbon limited; rates of consumption increased in the presence of additional carbon. The carbon limited nature of our system affects the stream water dynamics during autumn leaf fall.

5.3 Changes in Carbon Input to Stream Waters Stimulate Heterotrophic Activity during Autumn

We hypothesized that increased DOC concentration and labile DOM fraction during autumn leaf fall would decrease stream NO\textsubscript{3}⁻-N concentrations. A NO\textsubscript{3}⁻-N depression did occur during autumn 2014 and autumn 2015. The depression occurred
around the same time (late October) at the 12 and 79 ha stream locations in both 2014 and 2015. The decline in the second order stream began sooner than the decline in the first order stream during 2015. At both sites, during both years, the recovery in stream NO$_3^-$-N was accelerated by a storm event. There is stronger evidence in the 12 ha catchment in 2014 for an increase in DOC during the leaf fall period. Sensor data for both years is too spotty for in the 79 ha catchment to establish if there was an increase in DOC or not. In the 12 ha catchment, during 2015, there was a decline in DOC concentration in late September. This decline may have been caused by drier watershed conditions leading up to autumn 2015. The drier conditions caused hydrologic disconnect between the stream and DOM sources in the watershed. DOC recovered immediately following a storm on 9/30/15.

As we see with our data, autumn storms have been shown to increase DOC and NO$_3^-$ export and can lead to NO$_3^-$ to recover to as much as 25 times pre-storm concentrations (Sebestyen et al., 2014). The post-storm NO$_3^-$-N concentrations in the 12 ha catchment were ~10 times the lowest recorded concentration and ~7 times the lowest recorded concentration in the 79 ha catchment. In the 79 ha catchment storm events accelerated the increase in NO$_3^-$-N for as much as one week, but after that time, the concentrations decreased again until the next storm. This is likely due to the lag time between the storm event and when soil water NO$_3^-$ reaches the stream. The more immediate return to a decline in NO$_3^-$-N concentration after a storm event in the 12 ha catchment compared to the 79 ha catchment is due to differences in hydrologic connectivity. The 12 ha catchment is more hydrologically linked with watershed DOM and nutrient sources, while in the 79 ha catchment drains a larger area and will experience more delayed solute signals. Once the C:N balance is reestablished, NO$_3^-$
likely is retained by in-stream processes until the next storm (Sebestyen et al., 2014). The larger hydrologic disconnection between the second order stream and the 79 ha watershed compared to the first order stream and the 12 ha watershed could have contributed to the early start to the NO$_3^-$-N decline in the second order stream.

Groundwater data indicates the watershed began to dry out during the late summer (Fig. 4.5). This drought would have created a disconnect between the second order stream and riparian zone NO$_3^-$ pools causing the decline in stream NO$_3^-$-N concentrations. The importance of the hydrologic connection, or disconnection, in the 79 ha watershed was reflected in the diel patterns of NO$_3^-$-N, as well. Since NO$_3^-$-N increases with increasing discharge, it is likely that NO$_3^-$-N is transported to the stream from near stream source areas, similar to the findings of Sebestyen et al. (2014). See section 5.4 for a more detailed discussion of effects of the spatial and temporally differences between the two catchments on stream water chemistry.

Alternatively, increased autotrophic assimilation during autumn could explain the differences in the stream water NO$_3^-$-N decline between the first and second order stream. A study across distinct geographic areas in the United States found that in watersheds where algal biomass was greatest during the spring, biomass was generally lowest during the summer, but a reappearance during autumn (Lee et al., 2012). During autumn, the amplitude of diel patterns of temperature, DOC, and NO$_3^-$-N all increase compared to summer in Fair Hill. The late summer drought triggered early senescence of leaves before the normal leaf fall period, which would have increased the solar radiation reaching the streams. Since the canopy is less dense over the second order stream, this could explain the earlier decline in stream NO$_3^-$-N concentrations in the second versus first order stream. The second order stream is shallower and
experiences lower flow rates, as well. Low flows have been attributed to higher rates of benthic metabolism (Bernhardt et al., 2005).

Our field results for autumn indicate our stream is carbon limited. Leaf leachate dissolved organic matter can satiate up to half of the carbon demand and community respiration of bacterial communities in third order streams (Cuss & Guéguen, 2015). Increased NO$_3^-$ consumption in mid-October was triggered by an increase in stream DOC concentration, which would have led to increased consumption of both DOC and DIN (Bernhardt & Likens, 2002). Bernhardt & Likens (2002) observed a decrease in NO$_3^-$-N (from ~ 40 μg L$^{-1}$ to < 10 μg L$^{-1}$) and increased metabolic rates in a forested first-order stream in New Hampshire which underwent a labile carbon treatment. Meyer et al. (1998) showed that benthic breakdown of leaf litter could contribute up to 50% of DOC exports in a stream. Similarly, Sebestyen et al. (2014) discussed how in-stream transformations such as heterotrophic cycling can retain over 70% of in-stream NO$_3^-$-N. In a forested headwater catchment in Vermont, Sebestyen et al. (2014) found NO$_3^-$-N to decrease from 5.4 to 0.7 mmol L$^{-1}$ during peak leaf fall. Sebestyen et al. (2014) speculated that increased organic matter input during autumn leaf fall resulted in stream nitrogen limitation. Nitrogen limitation coupled with increased DOM availability may have led to decreased nitrification during autumn leaf fall (Sebestyen et al., 2014).

While lab fluorescence and FDOM (increasing FDOM leading up to the NO$_3^-$-N depression corresponds to increasing overall DOC during that time) data do not provide any insight into what may trigger the NO$_3^-$-N depression in our watershed, leaf mass does. The depression at both sites occurred while mean daily leaf litter fall rates increased rapidly. The first recorded, mid-September litter fall rate was 1.35 g
leaves m$^{-2}$ d$^{-1}$, the week of the NO$_3^-$-N depression litter fall was 3.42 g leaves m$^{-2}$ d$^{-1}$, and litter fall was 1.67 g leaves m$^{-2}$ d$^{-1}$ the day before the 11/19/15 storm accelerated NO$_3^-$-N recovery. Our incubation study showed that litter leachate NO$_3^-$-N consumption rate is ~30 times greater than the consumption of stream water alone. The increased carbon input during leaf fall stimulated rapid heterotrophic consumption of NO$_3^-$-N and DOC.

We speculate our watershed is carbon rather than nitrogen limited due to regional atmospheric deposition of nitrogen. Our study site received 7.62 kg N ha$^{-1}$ during atmospheric deposition during 2011 (4 kg NH$_4^+$-N ha$^{-1}$, 1.54 kg NO$_3^-$-N ha$^{-1}$, and 2.08 kg dissolved organic nitrogen ha$^{-1}$) (Inamdar et al., 2015). The Piedmont watershed, Pond Branch, where Duncan et al. (2015) studied diel NO$_3^-$ patterns receives 9 kg N ha$^{-1}$ yr$^{-1}$ of DIN through deposition. While the deposition at Pond Branch watershed is twice the DIN deposition estimated at our watershed, Duncan et al. (2015) observed lower stream water NO$_3^-$-N concentrations. Nitrate-N concentrations at Pond Branch range from 0.04 - 0.14 mg NO$_3^-$-N L$^{-1}$, while we observed baseflow NO$_3^-$-N concentrations from 0.05 – 0.43 mg NO$_3^-$-N L$^{-1}$. The forested stream in VT studied by Sebestyén et al. (2014) has been shown to have mean baseflow NO$_3^-$-N concentrations less 0.93 mg NO$_3^-$-N L$^{-1}$. On the other hand, NH$_4^+$ stream studied by (Bernhardt & Likens, 2002) has much lower NO$_3^-$-N concentrations (40-60 μg L$^{-1}$ NO$_3^-$-N).

Other watershed characteristics are also important for determining the relative concentrations of stream water NO$_3^-$-N among seemingly similar watersheds (e.g. forested, headwater catchments). The watershed in which Rusjan & Mikoš (2010) observed diel patterns of NO$_3^-$-N has a high density of black alder trees in the riparian
zone. Black alder has a symbiotic relationship with nitrogen fixing bacteria, which can lead to excess nitrogen in riparian soils. This excess nitrogen is available for leaching to the stream (Rusjan & Mikoš, 2010). Flewelling et al. (2014) also discussed the importance of understanding different source pools in different watersheds. When the water table increases during storm events, shallow groundwater contributes to a larger portion of stream water. As the water table relaxes, deep groundwater will contribute to a greater proportion of streamflow which can have implications for solute transport to streams (Flewelling et al., 2014; Inamdar et al., 2012). Subtle differences in location of terrestrial pools of NO$_3^-$-N, whether created by natural differences such as riparian tree species or anthropogenic differences such as atmospheric deposition, can create large differences in stream water chemistry dynamics. As discussed above, the forested Piedmont watershed studied by Duncan et al. (2015) receives twice the atmospheric deposition of DIN as the Piedmont Fair Hill watershed, yet the maximum baseflow stream NO$_3^-$-N concentrations observed at Fair Hill are almost triple those observed by Duncan et al. (2015).

5.4 Conceptual Models of Carbon and Nitrogen Dynamics in a Forested Piedmont Headwater System

Several spatial and temporal aspects of a forested, headwater catchment in the Piedmont Region, MD, influence whether hydrologic or biotic processes dominate solute dynamics in a first and second order stream. These factors include: 1) hydrologic connectivity; 2) solar radiation and temperature; 3) stream characteristics; and 4) season.

The first order stream draining a 12 ha catchment has higher streamflow and drains a greater proportion of the surrounding catchment than the second order stream.
These factors created a greater hydrologic connection between the watershed and the first order stream. The 12 ha catchment also has denser forest cover, which decreases the solar radiation reaching the first order stream. Larger stream width, longer residence time, and increased solar radiation throughout the 79 ha watershed increases the rates of microbial processing in the second order stream. Water temperature and rainfall change throughout the seasons. Temperature affects metabolism rates and rainfall affects solute transport to and export form the streams.

5.4.1 Temporal controls of solute patterns

Temperature, solar radiation, and rainfall create temporal differences in the relative strength of hydrologic and biologic effects on solute cycling (Fig. 5.1). More solar radiation reaches a forested stream during springtime prior to leaf out. The amplitude of diel patterns of temperature, DOC, DO, and NO$_3^-$-N are largest during the spring. As solar radiation reaching the stream decreases during the summer, most patterns become diluted. During autumn, leaf fall allows more solar radiation to reach the stream and autumn storms increase transport of DOC and NO$_3^-$-N to the stream. Diel patterns in DOC and NO$_3^-$-N become stronger in autumn vs. summer. Autumn leaf fall increases the labile carbon available to heterotrophic communities and stream NO$_3^-$-N is rapidly consumed as the newly available DOC is consumed, creating a NO$_3^-$-N depression. In addition to providing NO$_3^-$-N for diel autotrophic cycling, increased solute transport during autumn storms contributes to the stream NO$_3^-$-N recovery to early autumn concentrations.
5.4.2 Spatial controls of solute patterns

Physical differences between the 12 ha and 79 ha watershed create differences in solute signals in the first and second order streams (Fig. 5.2). The smaller stream is more hydrologically connected to the catchment and has more canopy cover. The larger stream is less hydrologically connected allowing for disconnect between the stream and riparian NO$_3^-$ sources during dry periods. Summer disconnection between NO$_3^-$ sources and the stream triggered an earlier decline in NO$_3^-$-N concentrations in the second order stream than the first order stream during late summer/early autumn 2015. The hydrologic disconnect in the 79 ha catchment gives way for stronger diel patterns in the second order stream by allowing more time for autotrophic metabolism to act on a NO$_3^-$-N molecule. The first order stream lacks clear diel patterns in DOC and NO$_3^-$-N throughout the year most likely due to more continuous transport of DOC and NO$_3^-$-N to the stream from watershed sources.
Table 5.1 Table of previous findings of diel patterns of DOC. Site description, summary of patterns, and controlling mechanisms behind the patterns are included. The text for the process controlling the patterns is color coded bases on which process was dominant: blue indicates hydrology dominated, green indicates biotic processes dominated, and purple indicates a mix of controls were found.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Watershed Type</th>
<th>Solute Pattern</th>
<th>Amount of Change</th>
<th>Study Period</th>
<th>Process</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>21-73% cropland gradient</td>
<td>Lowest in afternoon</td>
<td>Greatest amplitude at more forested sites</td>
<td>July</td>
<td>ET rates drive greater diel variations in discharge and DOC; Higher night DOC due to lower ET rates → more water flows through more organic rich upper soil horizons</td>
<td>Wilson &amp; Xenopoulos 2013</td>
</tr>
<tr>
<td>DOC</td>
<td>Piedmont; ag with forested riparian; up to 3rd order</td>
<td>Highest in late afternoon</td>
<td>Late March to early June; Nov.</td>
<td>Algal production (accounts for 20% of DOC export)</td>
<td>Kaplan &amp; Bott 1982</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>2nd order; rainforest; 11.4 km</td>
<td>Highest in evening (19:30)</td>
<td>Mid-autumn</td>
<td>PAR decreases just before peak DOC; algal production</td>
<td>Looman et al. 2016</td>
<td></td>
</tr>
<tr>
<td>CDOM</td>
<td>Hyper-eutrophic; 25,107 km</td>
<td>Highest in early evening</td>
<td>~4.2-4.6 to 5.4-5.8 m$^{-1}$</td>
<td>July</td>
<td>No pattern in bulk DOC concentration; linear regression with Chlorophyll suggests CDOM pattern due to phytoplankton growth and other processes</td>
<td>Spencer et al. 2007</td>
</tr>
</tbody>
</table>
Table 5.2 Table of previous findings of diel patterns of NO$_3^-$-N. Site description, summary of patterns, and controlling mechanisms behind the patterns are included. The text for the process controlling the patterns is color coded bases on which process was dominant: blue indicates hydrology dominated, green indicates biotic processes dominated, and purple indicates a mix of controls were found.

<table>
<thead>
<tr>
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<th>Solute Pattern</th>
<th>Amount of Change</th>
<th>Study Period</th>
<th>Process</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$</td>
<td>37 ha; south-facing; forested Piedmont headwater</td>
<td>Highest during day</td>
<td>In 4 days: from 0.04 mg L$^{-1}$ NO$_3^-$-N to 0.14 mg L$^{-1}$ NO$_3^-$-N</td>
<td>Summer</td>
<td>High ET led GW to contribute the most streamflow; Riparian soils have highest nitrate during summer due to dry conditions, high oxygen, low denitrification so more nitrate is transported to the stream during the summer</td>
<td>Duncan et al. 2015</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>Forested headwaters, generally</td>
<td>Lowest in late afternoon</td>
<td></td>
<td>Spring</td>
<td>Autotrophy prior to leaf out</td>
<td>Nimick, Gammons, Parker 2011</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>40.5 ha; forested, headwater; second-order; VT</td>
<td>Lowest in late afternoon</td>
<td>1-2 μmol L$^{-1}$</td>
<td>Baseflow after snow melt</td>
<td>Pattern independent of Q during baseflow suggests autotrophic assimilation prior to leaf out</td>
<td>Pellerin et al. 2012</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>3.4 km$^2$ mostly forested with ag and development</td>
<td>Max between 21:15-01:30</td>
<td></td>
<td>Summer</td>
<td>Solar radiation, groundwater depth, soil moisture</td>
<td>Aubert &amp; Breuer 2016</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>Forested, 1$^{st}$ order</td>
<td>Minimu in afternoon (~16:00)</td>
<td></td>
<td>Strongest during spring</td>
<td>Primary production explained 91% of NO$_3^-$ amplitude variance</td>
<td>Roberts &amp; Mulholland 2007</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>42 km$^2$ forested, hilly</td>
<td>Minimu between 18:00-21:00</td>
<td></td>
<td>Strongest during spring</td>
<td>Assimilation of NO$_3^-$ through primary production; NO$_3^-$ negatively correlated with temperature</td>
<td>Rusjan &amp; Mikoš</td>
</tr>
</tbody>
</table>
Figure 5.1 Temporal controls: The solute patterns here represent the diel patterns throughout the seasons. In the afternoon, DOC, DO, and temperature peak and NO$_3^-$-N is at its minimum. The discharge line represents the moisture conditions of the season throughout the year. Diel patterns of discharge are not depicted, but the maximum would be in the morning and the minimum would occur in the afternoon. The amplitude of diel patterns is greatest during the spring. DOC and NO$_3^-$-N have weak or non-existent patterns during the summer, while DO, temperature, and discharge patterns have the lowest amplitude in the summer. The amplitude of diel patterns of all parameters increase during autumn, but not to springtime levels. NO$_3^-$-N goes through a depression during autumn leaf fall, when more labile DOC enters the stream. DOC, NO$_3^-$-N, and discharge patterns disappear during mid-winter. NO$_3^-$-N patterns are the first to return in later winter.
Figure 5.2 **Spatial controls:** Above is a sketch of our watershed with embellished spatial differences in stream size and canopy cover. Hydrologic connectivity and canopy cover are greater in the smaller watershed. Solar radiation and stream residence time are greater throughout the larger watershed. These conditions allow for stronger biotic control of diel patterns of DOC, NO$_3^-$-N, and DO in the second order stream. At the first order stream, hydrology mutes biotic diel patterns. An earlier autumn decline in stream NO$_3^-$-N is caused by hydrologic disconnect between the stream and watershed sources of NO$_3^-$-N.
Chapter 6

CONCLUSIONS

The relative control of biology and hydrology on solute patterns in our watershed varies spatially and temporally. While we hypothesized that heterotrophic organisms are dominate in our watershed, our findings suggest autotrophy is most important at the daily scale and heterotrophy plays a greater role in the seasonal patterns of solutes. When diel patterns of NO$_3^-$-N and DOC are present in our watershed, they are driven by autotrophic production. The strongest expression of diel patterns occurs during the spring prior to leaf out. Temperature, and likely solar radiation, exhibits the strongest control over the diel patterns. The larger the amplitude of the temperature patterns during a given day, the larger the amplitude of DO, DOC, and NO$_3^-$-N. A warmer winter during 2016 increased the strength of spring diel patterns compared to 2015 in both the first and second order streams. Patterns are strongest and most consistent in the second order stream.

The fact that hydrology is not the dominant control of diel solute patterns was evident during February and March 2016 storms. During these storms NO$_3^-$-N returned to a diel pattern in less than 24 hrs, before baseflow discharge has resumed. In contrast, throughout the rest of the year, NO$_3^-$-N follows the pattern of the hydrograph and diel patterns in NO$_3^-$-N do not resume until baseflow has been reestablished. Hydrology is important for transporting NO$_3^-$-N to the stream at the diel scale and during stormflow, however, and plays an important role in controlling stream NO$_3^-$-N patterns on a seasonal scale. Hydrologic disconnect in the 79 ha
watershed during a drought, caused an early decline in autumn NO₃⁻-N in the second order stream. After the NO₃⁻-N depression, NO₃⁻-N concentrations returned to early autumn concentrations through mobilization of flow paths and increased transport of NO₃⁻ to the stream during storms.

While hydrology influences seasonal stream solute concentrations, biotic processes play an important role in autumn stream DOC and NO₃⁻-N signals. Autotrophic activity continues to drive the daily patterns of DOC and NO₃⁻-N during autumn, as demonstrated in Fig. 4.17, but heterotrophic activity becomes an important factor in the larger seasonal scale signals of DOM and nutrients in stream water. Input of fresh leaf litter during early autumn increases the availability of labile DOC in our carbon-limited stream, which triggers increased rates of heterotrophic consumption. The coupled consumption of the newly available carbon and preexisting NO₃⁻ creates a NO₃⁻ depression within the stream ecosystem.

The presence of both autotrophic and heterotrophic controls on DOM and nutrient cycling in our watershed gives further evidence that our stream is carbon limited. The main evidence is reflected in the laboratory incubation results which showed increased nitrogen consumption in the presence of additional labile carbon sources but not in the presence of added carbon and nutrients. The higher C:N in the carbon only treatments shifted the C:N to allow for more consumption of nutrients by increasing the availability of carbon for microbes. The increased consumption of NO₃⁻ during autumn leaf fall also contributes to the hypothesis that our system is carbon limited. Furthermore, during the winter-spring transition, microbial processing began to increase as stream water temperature increased. During February 2016, diel patterns of NO₃⁻-N and DO suggest the presence of autotrophic assimilation even though diel
patterns of DOC do not exist. We speculate that the DOC produced by autotrophs is being rapidly consumed by the heterotrophs, but the rate of heterotrophic consumption outweighs the rate of autotrophic production of DOC during this time due to overall carbon limitation in this stream system. We did not see this carbon limitation during the spring since soils had thawed and DOC was available for transport to the stream.

We suggest future work include measuring PAR at the first and second order stream locations to confirm that the relative strength of patterns at the second order stream is driven by a greater amount of solar radiation reaching the stream. Future work could delve deeper into studying the effect of C:N ratios on the heterotrophic communities in our stream to explore the strength of carbon limitation in this system. We also suggest future work look into our speculations about carbon limitation being driven by high nitrogen deposition in this region. A future study could confirm this speculation by studying nitrogen isotopes in the watershed to determine what portion of stream nitrogen has an atmospheric source, similar to Duncan et al. (2015). Nitrogen isotopes could also provide insight into the connectivity between the stream and groundwater sources throughout the watershed. Studying the age of nitrogen in groundwater in our watershed may also indicate why our watershed has excess nitrogen. This watershed is historically agricultural with a forest stand less than 100 years old.

Solute sources and the transport of those solutes to the stream can depend on natural and anthropogenic factors. While the forested Piedmont watershed studied by Duncan et al. (2015) receives twice the atmospheric deposition of DIN as the Piedmont Fair Hill watershed, the maximum baseflow stream NO$_3$-N concentrations observed at Fair Hill are almost triple those observed by Duncan et al. (2015).
differences reflect the importance of terrestrial processing of and flow paths that can be taken by NO$_3^-$ and other solutes. The potential that atmospheric deposition has contributed to the controlling biogeochemical process within a small, forested watershed has tremendous significance. Anthropogenic activity has led to nitrogen pollution, which can occur through atmospheric deposition. Fertilizers have also lead to increased NO$_3^-$ in groundwater and drinking water sources which can lead to adverse effects such as harmful algal blooms and birth defects (Pellerin et al., 2016). As anthropogenically driven climate change continues, wet and dry deposition patterns will change. Climate change will impact the timing and relative influence of biotic and abiotic of watershed processes. Groffman et al. (2012) demonstrated the importance of long term watershed studies, especially those in watersheds of varying sizes and land use histories. Studies like ours that use high-frequency in situ data to understand the controlling processes on biogeochemical cycling of DOM and nutrients are key to understanding how water quality could shift as the climate continues to shift. Small headwater catchments are useful for studying these processes as they are fairly pristine and there is a tight link between aquatic and terrestrial processes. Small watersheds are also important for ecosystem function and contribute to sources of drinking water. Understanding the driving processes of and rapid changes in stream water chemistry in these watersheds will be important for predicting how water quality may change in the future.
REFERENCES


Tant, C. J., Rosemond, A. D., & First, M. R. (2013). Stream nutrient enrichment has a greater effect on coarse than on fine benthic organic matter. *Freshwater Science,


Appendix A

ADDITIONAL DIEL GRAPHS

Figure A1 Time series of DO, temperature, NO$_3^-$, DOC, FDOM, and Q of the 2015 dataset for ST12.
Figure A2 The axes in this ST12 March 2015 graph use the common scales used throughout the year.
Figure A3 The axes in this ST12 April 2015 graph use the common scales used throughout the year.
Figure A4 The axes in this ST12 May 2015 graph use the common scales used throughout the year.
Figure A5 The axes in this ST12 June 2015 graph use the common scales used throughout the year.
Figure A6 The axes in this ST12 July 2015 graph use the common scales used throughout the year.
Figure A7 The axes in this ST12 August 2015 graph use the common scales used throughout the year.
Figure A8 The axes in this ST12 September 2015 graph use the common scales used throughout the year.
Figure A9 The axes in this ST12 October 2015 graph use the common scales used throughout the year.
Figure A10 The axes in this ST12 November 2015 graph use the common scales used throughout the year.
Figure A11 The axes in this ST12 December 2015 graph use the common scales used throughout the year.
Figure A12 The axes in this ST12 January 2016 graph use the common scales used throughout the year.
Figure A13 The axes in this ST12 February 2016 graph use the common scales used throughout the year.
Figure A14 The axes in this ST12 March 2016 graph use the common scales used throughout the year.
Figure A15 The axes in this ST12 April 2016 graph use the common scales used throughout the year.
Figure A16 DO, temperature, DOC, FDOM, and Q all exhibited patterns at ST12 in June 2015 but NO₃⁻-N did not. June was a stormy month and patterns are weaker than May. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A17 DO, temperature, DOC, FDOM, and Q all exhibited patterns at ST12 in July 2015 but NO$_3^-$-N did not. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A18 NO$_3$-N cycling returned with low amplitude during August 2015 and DO, temperature, DOC, FDOM, and Q all exhibited patterns at ST12. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A19 All solutes exhibited diel patterns at ST12 in September 2015. High FDOM compared to the rest of the year may be due to drought antecedent conditions. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
As $\text{NO}_3^-\text{-N}$ recovers, diel cycling of $\text{NO}_3^-$ resumes during November 2015. All other solutes exhibited diel patterns at ST12 during this time. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A21 All solutes exhibited weak diel patterns at ST12 during December 2015. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A22 Only DO and temperature exhibited discernible diel patterns at ST12 during January 2016. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A23 NO$_3$-N, DO and temperature exhibited discernable diel patterns at ST12 during February 2016. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A24 All solutes exhibited strong diel patterns with large amplitudes, except for Q amplitude, during April 2016 at ST12. Solid lines are midnight. Dotted lines are noon. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A25 Time series of NO$_3^-$-N, DOC, and Q of the 2015 dataset for ST3.

Figure A26 No diel patterns were presence at ST3 during March 2015. The axes in this graph use the common scales used throughout the year.
Figure A27 The axes in this ST3 April 2015 graph use the common scales used throughout the year.

Figure A28 The axes in this ST3 May 2015 graph use the common scales used throughout the year.
Figure A29 The axes in this ST3 June 2015 graph use the common scales used throughout the year.

Figure A30 The axes in this ST3 July 2015 graph use the common scales used throughout the year.
Figure A31 The axes in this ST3 September 2015 graph use the common scales used throughout the year.

Figure A32 The axes in this ST3 October 2015 graph use the common scales used throughout the year.
Figure A33 The axes in this ST3 November 2015 graph use the common scales used throughout the year.

Figure A34 The axes in this ST3 December 2015 graph use the common scales used throughout the year.
Figure A35 The axes in this ST3 February 2016 graph use the common scales used throughout the year.

Figure A36 The axes in this ST3 March 2016 graph use the common scales used throughout the year.
Figure A37 Diel solute patterns were diluted in June 2015 at ST3, likely due to the occurrence of my storm events. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.

Figure A38 No diel patterns were presence at ST3 during July 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A39 Only Q exhibited diel patterns at ST3 during September 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.

Figure A40 Only Q exhibited diel patterns at ST3 during November 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.
Figure A41 No diel patterns existed at ST3 during December 2015. Red values indicate the scale is different than the common scale chosen to compare the magnitude of patterns throughout the year.

Figure A42 Time series graph of ST12 2015 DOC, NO₃⁻-N, and groundwater levels from two wells. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the land surface, representing the lowest depth, is at the top of the graph.
Figure A43 ST12 DOC, NO$_3$-N, and groundwater levels during March 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A44 ST12 DOC, NO$_3$-N, and groundwater levels during April 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A45 ST12 DOC, NO$_3$-N, and groundwater levels during June 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A46 ST12 DOC, NO$_3$-N, and groundwater levels during July 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A47 ST12 DOC, NO$_3$-N, and groundwater levels during September 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A48 ST12 DOC, NO$_3$-N, and groundwater levels during November 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A49 ST12 DOC, NO$_3^-$-N, and groundwater levels during December 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A50 ST12 DOC, NO$_3^-$-N, and groundwater levels during January 2016. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A51 ST12 DOC, NO₃⁻-N, and groundwater levels during February 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A52 ST12 DOC, NO$_3^-$-N, and groundwater levels during March 2016.
Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A53 ST12 DOC, NO$_3^-$-N, and groundwater levels during April 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A54 ST3 2015 groundwater, DOC, and NO$_3^-$-N time series. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A55 ST13 DOC, NO₃⁻-N, and groundwater levels during June 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A56 ST3 DOC, NO₃⁻-N, and groundwater levels during July 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A57 ST3 DOC, NO$_3$-N, and groundwater levels during September 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A58 ST3 DOC, NO$_3$-N, and groundwater levels during October 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A59 ST3 DOC, NO$_3^-$-N, and groundwater levels during November 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.

Figure A60 ST3 DOC, NO$_3^-$-N, and groundwater levels during December 2015. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A61 ST3 DOC, NO$_3^-$-N, and groundwater levels during February 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Figure A62 ST3 DOC, \( \text{NO}_3^- \)-N, and groundwater levels during March 2016. Negative LW2 values indicate the wetland where this well is installed was saturated. Red scale values indicate the scale is different throughout the year due to large seasonal changes in groundwater level. Note the values on the groundwater are flipped so that the lowest depth, representing the land surface, is at the top of the graph.
Appendix B

ADDITIONAL AUTUMN GRAPHS
Figure B1 Q, leaf litter mass, weekly rates of change in \( \text{NO}_3^- \) and DOC loads, and monthly rates of change in \( \text{NO}_3^- \) and DOC loads at ST3 during autumn 2015.
Figure B2 Fulvic-like components of our PARAFAC model with DOC, Q, and litter mass at ST3 during autumn 2015.
Figure B3 Protein-like component of our PARAFAC model with DOC, Q, and litter mass at ST3 during autumn 2015.
Figure B4: Humic-like components of our PARAFAC model with DOC, Q, and litter mass at ST3 during autumn 2015.
Figure B5 Q, leaf litter mass, weekly rates of change in NO$_3^-$ and DOC loads, and monthly rates of change in NO$_3^-$ and DOC loads at ST12 during autumn 2015.
Figure B6 Fulvic-like components of our PARAFAC model with DOC, Q, and litter mass at ST12 during autumn 2015.
Figure B7 Protein-like component of our PARAFAC model with DOC, Q, and litter mass at ST12 during autumn 2015.
Figure B8 Humic-like components of our PARAFAC model with DOC, Q, and litter mass at ST12 during autumn 2015.
Appendix C

INCUBATION TIME SERIES
Figure C1 Time series plots of TN, NO$_3$-N, DOC, fluorescence and UV metrics throughout the course of the STR incubation.
Figure C2 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the STRNO3 incubation.
Figure C3 Time series plots of TN, NO₃⁻-N, DOC, fluorescence and UV metrics throughout the course of the ION incubation.
Figure C4 Time series plots of TN, NO₃⁻-N, DOC, fluorescence and UV metrics throughout the course of the IONNO3 incubation.
Figure C5 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the LEAF incubation.
Figure C6 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the LEAFNO3 incubation.
Figure C7 Time series plots of TN, NO$_3$-N, DOC, fluorescence and UV metrics throughout the course of the SOIL incubation.
Figure C8 Time series plots of TN, NO$_3$-N, DOC, fluorescence and UV metrics throughout the course of the SOILNO3 incubation.
Figure C9 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the GLU incubation.
Figure C10 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the GLUNO3 incubation.
Figure C11 Time series plots of TN, NO$_3$-N, DOC, fluorescence and UV metrics throughout the course of the SA incubation.
Figure C12 Time series plots of TN, NO$_3^-$-N, DOC, fluorescence and UV metrics throughout the course of the SANO3 incubation.