THE IMPACT OF TERRESTRIAL DISSOLVED ORGANIC CARBON ON STREAM ECOSYSTEMS THROUGH AN INVESTIGATION OF HYDROLOGIC SOURCES

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ABSTRACT

THE IMPACT OF TERRESTRIAL DISSOLVED ORGANIC CARBON ON STREAM ECOSYSTEMS THROUGH AN INVESTIGATION OF HYDROLOGIC SOURCES

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In stream ecosystems, dissolved organic carbon (DOC) influences microbial heterotrophic production and nitrogen (N) cycling. Since the surrounding watershed supplies the majority of DOC, understanding the terrestrial DOC flux is critical to determining the influence of hydrologic sources on N removal. However, the linkages between terrestrial export and stream DOC concentration and composition are poorly understood. This dissertation seeks to identify hydrologic sources, determine spatial and temporal patterns of DOC lability, and investigate the impact of terrestrial C quality on denitrification rates in a headwater stream in Pennsylvania.

Stream water sources during baseflow and storm flow were identified using end-member mixing analyses. DOC concentrations predicted based on the derived source contributions were compared to observed concentrations to evaluate the importance of hydrology in controlling stream water DOC. Three baseflow groundwater sources were identified and one storm flow source. Higher observed DOC concentrations compared to predicted DOC concentrations suggest some mechanism of production during baseflow and the possibility of additional storm flow sources.

DOC lability among terrestrial source waters and in stream water was measured using bioreactors colonized by stream microorganisms. Labile BDOC concentrations varied among sources declining as water percolated through the soil. Dissimilar storm dynamics were observed between two biological reactivity classes, labile and semi-labile BDOC, indicating different source pools.

The impact of C quality on denitrification was evaluated using glucose amended stream water and storm-induced overland flow water in sediment perfusion core experiments. Glucose amendments had no effect on denitrification rates, but significantly greater denitrification was observed for the overland flow treatment suggesting either a threshold concentration of labile BDOC and/or that semi-labile BDOC has a greater stimulatory effect.

The results described demonstrate the importance of considering the interactive effects of transport and biogeochemical processing in stream ecosystems. I argue that the impact of terrestrial DOC on stream ecosystem function depends on both the rate of DOC supply and the effect of C lability on biogeochemical processing. The removal of N from the stream ecosystem via denitrification is therefore ultimately connected to the dynamic changes in BDOC delivery from terrestrial sources.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

INTRODUCTION

CHAPTER 1

CHAPTER 2

CHAPTER 3

INTRODUCTION

In stream ecosystems, dissolved organic carbon (DOC) plays an important role by supplying energy (Aitkenhead-Peterson et al. 2003, Kaplan et al. 2008, Findlay and Sinsabaugh 1999) and regulating microbial biomass and activity (Kaplan and Bott 1983). In addition to controlling heterotrophic production, C availability is also linked to other stream ecosystem functions such as in-stream nitrogen (N) cycling (Ziegler and Brisco 2004). Since N limits plant growth and excess N contributes to eutrophication, understanding the effect of C on rates of in-stream N-cycling is critical to determining possible environmental conditions that might enhance N-retention or removal mechanisms. Because of the potential significance of DOC to ecosystem-level processes, characterizing the DOC supply to streams is critical. In forested watersheds, the majority of DOC flowing downstream enters from the terrestrial environment via dynamic flow pathways through the subsurface (Fisher and Likens 1973, Meyer et al. 1998). Terrestrial sources vary in both DOC concentration and composition, and therefore, the concentration and composition of stream water DOC depends on the relative contributions of the terrestrial sources and the modifications that occur while en route to the channel along flow pathways. As such, hydrological processes partially control the supply of DOC to streams with distinct flow paths often determining the chemistry of the water discharged into the channel (Burns et al. 2003, Weiler et al. 2005). Therefore, determining variations in export of terrestrially derived DOC can be accomplished through characterization of terrestrial source waters and quantification of source contributions. Furthermore, the established linkages between DOC and N-cycling makes understanding the flux of terrestrial DOC critical to determining the influence of terrestrial sources on in-stream N-cycling rates which is important for water quality.

Despite the importance of hydrologic flow and of the dynamic aquatic-terrestrial linkages created by water movement through the terrestrial environment, stream ecosystems have typically been studied in reference to a longitudinal resource gradient (Vannote et al. 1980), but broadening the boundaries to encompass the surrounding terrestrial environment is necessary and often overlooked by limnologist (Hynes 1975). While much research has focused on export dynamics based on measurements from catchment outlets (Kaplan and Newbold 1993), less is known about the spatial and temporal variation in terrestrial source fluxes with few studies relating stream concentration to flow paths and sources (Christophersen et al. 1990, Easthouse et al. 1992, Mulholland 1993, Fisher et al. 1998, McGlynn and McDonnell 2003a). An understanding of DOC lability can then help to elucidate which terrestrial sources have the greatest influence on in-stream N-cycling by testing their influence on N transformation rates. Although, artificial and manufactured DOC sources have been used to test the effect of C on N transformation rates in stream sediments (Strauss and Lamberti 2002, Starry et al. 2005) and measurements of potential N transformation rates along natural gradients of DOC quality have been made (Barnes et al. 2012), the response of N transformations rates to different terrestrial source waters has not been studied. Therefore, the focus of this dissertation is to identify and characterize sources of terrestrial C and determine the impact on stream ecosystem function by investigating compositional changes under different hydrologic conditions and linkages between C and N cycles.

The following introduction provides a brief account of the current state of knowledge regarding watershed hydrology, sources of terrestrial C, and connections between C and N-cycling ending with a description of the goals, methods, findings, and implications of each additional chapter in this dissertation. The research chapters are then presented in manuscript form with introduction, methods, results, and discussion followed by a literature cited section, table and figure legends, tables, and figures.

Background

The main flow paths (Figure 1) that contribute to annual water yield are groundwater outflow, direct interception of precipitation, throughfall, subsurface storm flow, and overland flow (Bruijnzeel 2004). Groundwater is the saturated soil zone in which water can easily move lateral. In some cases, groundwater flows to the surface and runs off to the stream via spring seeps. Groundwater is assumed to be the main contributing source of water during base flow, but additional terrestrial sources become prevalent during storms. Direct interception occurs as precipitation falls directly on the channel, and throughfall contributions occur as rainwater leaches the overhanging riparian forest foliage. In the unsaturated soil zone, the majority of water moves vertically during periods between storms, but horizontal flow becomes dominant during storms due to impervious soil layers deflecting water laterally (Elsenbeer and Vertessy 2000).

Overland flow occurs when the rainfall rate exceeds the infiltration rate or when the soil is saturated and additional precipitation causes surface runoff.

The interest in identifying terrestrial sources of water has resulted in the quantification of runoff amounts, but the significance of different sources is still unclear (Burns et al. 2001, Hangen et al. 2001, McGlynn and McDonnell 2003a, Bernal et al. 2006, Inamdar and Mitchell 2006). Classical hydrology assumed that the quick rise in stream flow during storms was due to surface runoff (Betson 1964, Dunne and Black 1970b), but more recent results suggest other mechanisms control runoff to streams.

A mixing model using stream chemistry identified throughfall as a controlling source to catchment runoff in a forested watershed in New York (Inamdar and Mitchell 2006). Subsurface soil pathways have also been considered, but their importance has been debated because horizontal flow could be dampened by storage within the soil or triggered by a perched water table within riparian areas (Whipkey 1962, Dunne and Black 1970a,b, Weiler et al. 2005). For example, hydrologic investigations of a small forested watershed in eastern Tennessee showed that the development of a transient perched groundwater table generated large increases in stream flow via shallow subsurface flow (Mulholland 1993). Alternatively, McDonnell (1990) proposed a sequence of events including infiltration to deeper soil, expansion of groundwater into the unsaturated hillslope soils, and lateral rapid pipe flow. Others have shown flow in macropores to be significant in comparison to flow through the soil matric, conducting up to 85% of the ponded infiltration (Luxmoore et al. 1990, Mulholland et al. 1990).

Collectively these studies suggest large variability in hydrologic flow paths among watersheds. Despite considerable progress in determining the mechanisms generating stream flow in small catchments, there is still uncertainty as to the exact pathways of terrestrial water discharging into streams.

The dominance of a given flow path depends on a combination of soil hydraulic properties (soil heterogeneity, anisotropy or the changes in permeability with depth, and antecedent soil moisture) and rainfall characteristics (intensity, amount, and frequency) (Elsenbeer and Vertessy 2000). Soil characteristics such as porosity, grain size, and conductivity determine the possible infiltration rate, but the infiltration rate increases with increasing rainfall intensity then declines as soil pores fill with water (Freeze and Cherry 1979, Dunne et al. 1991).

The relationship between precipitation amount and the volume of subsurface flow is expected to be threshold-like, non-linear, and dependent on antecedent moisture conditions (McDonnell 2003, Weiler et al. 2005). For example, observations from a highly responsive, steep catchment located in New Zealand illustrated a delayed response in runoff from upland hillslopes as a result of disconnectivity during early portions of the hydrograph when soil moisture deficits where not satisfied (McGlynn and McDonnell 2003b). Alternatively, wetting of deeper soil horizons occurs due to poorly developed soil anisotropy favoring vertical flow followed by rapid flow along the bedrock topographic surface (Bonnell 2005). As such, bedrock topography may control the direction and accumulation of flow more so than the surface topography.

Consequently, subsurface storm flow can occur as a result of wetting from the top down caused by the input of rain or wetting of deeper subsoils via storm flow accumulation from adjacent hillslopes (Lin and Zhou 2008). These interactions between soil properties and storm characteristics determine the spatial and temporal variation in flow, but the elasticity of these controls makes determining source contributions to streams a formidable task.

The horizontal and vertical heterogeneity of soil characteristics makes predicting the spatial dynamics of hydrologic flow problematic, and dynamic changes in source areas during storms make forecasting the temporal variations in flow paths difficult. The ability to identify flow patterns, velocities, and pathways in the subsurface across space and time is limited, but the relative contribution of various sources to stream water can be quantified using an end-member mixing model analysis (EMMA).

EMMA assumes that stream water is a mixture of source solutions such that geochemical variations in terrestrial sources and mixing equations can be used to quantify the relative contributions of different sources to stream flow (Christophersen et al. 1990). EMMA can be applied to datasets of varying spatial and temporal extent and provides a tool for evaluating the linkages between small-scale source measurements and larger-scale catchment hydrology by determining the hydrological aspects of an ecosystem that are important to stream chemistry (Hooper 2001). Determining the spatial and temporal variations in hydrologic source contributions is necessary to predict changes in stream water chemistry and to characterize the export of terrestrial DOC.

The concentration of stream water DOC reflects a balance between terrestrial and in-stream inputs and downstream exports. Allochthonous and autothonous sources contribute to the pool of stream water DOC which is the main form of organic matter in aquatic ecosystems (Findlay and Sinsabaugh 1999). Every year approximately 0.2 Gt of DOC is transported to the oceans via rivers, the majority of which is soil derived (Meybeck 1982, Hedges et al. 1994, Hedges and Oades 1997).

Terrestrially derived DOC is produced when precipitation leaches vegetation (Willey et al 2000, Raymond 2005), infiltrates the forest floor and organic soil horizons flushing soluble organic matter from detritus, leaf litter, and roots (Nykvist 1962, McDowell and Likens 1988, Magill and Aber 2000), and activates microbial decomposition producing leachable metabolites (Brooks et al. 1999, Aitkenhead-Peterson et al. 2003). Autothonous DOC is primarily derived from algae and macrophytyes (Webster and Meyer 1997, Bertilsson and Jones 2003), but the majority of DOC flowing downstream in forested watersheds comes from terrestrial sources (Fisher and Likens 1973, Meyer et al. 1998).

Once terrestrial water enters the stream channel, DOC interacts with the physical environment and additional microbial decomposition occurs. DOC regulates microbial activity, playing an important role in nutrient retention and release. For example, the uptake of nitrogen and phosphorous that accompanies the degradation of DOC links nutrient availability to rates of DOC mineralization (Kragh and Sondergaard 2004). Conversely, the supply of microbially available C is one of many factors that potentially control nutrient cycling (Seitzinger 1988). The significance of DOC to a variety of ecosystem-level processes makes isolating the runoff from different terrestrial sources an important undertaking necessary for characterizing the supply and composition of DOC exported to streams.

The processes that generate and consume DOC in the soil influence the delivery of terrestrial DOC to streams, and the rate of water movement controls the exposure time to these mechanisms of production and retention. For example, the dissimilarities in microbial community structure and water residence time among different landscape types within a catchment influence the concentration of DOC delivered to the adjacent aquatic ecosystems (Lewis et al. 2006, Creed et al. 2008, Wilson and Xenopoulos 2008).

Because soil microorganisms are involved in the mobilization and processing of DOC (Gamboa and Galicia 2011, Zak et al. 2011), water movement through distinct landscape components process material differentially. High DOC exports have been attributed to flow through shallow pathways since DOC concentrations decline exponentially with depth in the soil due to decreasing bacterial activity and increasing chemical sorption (McDowell and Likens 1988, Guggenberger and Zech 1993).

Differences in microbial processing among flow paths also results in variation in the amount of biodegradable DOC (BDOC) exported to streams. For example, the fraction of labile DOC may range from 1 to 50% of the total DOC pool (Meyer 1994). Benthic microbial communities respond rapidly to terrestrial DOC (Kreutzweiser and Capell 2003), but differential responses have been seen with DOC extracted from different soil horizons indicating that BDOC can be important in regulating benthic microbial activity (Bott and Kaplan 1985, Kreutzweiser and Capell 2003). Therefore, environmental conditions in combination with residence time influence not only the concentration but also the BDOC exported from the terrestrial environment.

Despite the tight coupling between terrestrial biogeochemical processes, soil hydrology, and stream ecosystem function, the linkages between terrestrial export and stream water DOC concentration and composition are poorly understood. Hydrologic models, such as EMMA, provide quantitative estimates of the contributions from various terrestrial sources and can be used to estimate stream concentrations expected due solely to hydrologic processes. A comparison of non-conservative solute concentrations, such as DOC, to predicted concentrations based on the EMMA estimated source contributions helps to elucidate processes that are occurring along flow paths. For example, if the ratio of observed to predicted DOC concentrations is equal to 1, the variation in hydrologic flow paths controls the flux of DOC to the stream, whereas a ratio less than 1 suggests depletion occurring and greater than 1 suggests production occurring along the pathway between the source and the stream. Therefore, the ratio of observed to predicted concentrations can be used to evaluate the importance of hydrologic flow to terrestrial DOC export.

Allochthonous DOC export is linked to in-stream N transformations, and N-cycling is hypothesized to be influenced by C quality. Therefore, explaining how C supply varies holds practical and theoretical appeal. Nitrate (NO_3) is the most common form of N in the environment due to its high solubility and low adsorption. NO_3 is easily transported from terrestrial ecosystems to groundwater and then exported downstream contributing to eutrophication of coastal waters. The use of manufactured N fertilizers in agriculture and increased fossil fuel use has drastically increased the amount of reactive N entering aquatic ecosystems (Vitousek et al. 1997), and N export is expect to increase by as much as 30% over the next thirty years (Howarth et al. 2002). Since two thirds of the nation's coastal waters are already moderately or severely degraded from N pollution (Bricker et al. 1999), understanding the factors regulating rates of N transformations is critical for developing strategies to control landscape-scale N fluxes and effectively manage freshwater ecosystems.

Identifying the relative importance of N processing by specific landscape components and determining the factors that control N-cycling rates allow for scaling up site-level processes to ecosystem effects and can suggest where nitrate reduction mechanisms might be most effective. Denitrification, the reduction of NO_3 to $N_{2,}$ mitigates the amount of N transported downstream through the conversion of NO_3 to an inert gas that is abundant in the atmosphere.

Riparian zones have been identified as hot spots of N removal (Peterjohn and Correll 1984, Hill 1996, Newbold et al. 2010). As such, riparian forest buffers are used to reduce the transport of agrichemicals to streams. However, measurements of riparian zone N retention indicates variability in N removal rates within and among sites as well as variations in N exports (Hanson et al. 1994, Jordan et al. 1997, Johnston et al. 2001) with some studies showing substantial NO_3 entering streams despite intact riparian zones (Kemp and Dodds 2001). Therefore, an understanding of the factors that regulate in-stream denitrification is also needed to implement effective freshwater management practices.

Since denitrifiers are thought to be ubiquitous, denitrification rates should not be limited by the denitrifer community but instead by environmental conditions and resources (Firestone 1982, Kemp et al. 1990, Reddy and Delaune 2008). Factors that potential control denitrification include NO_3 concentration, prevalence of anoxic conditions, and the supply of microbially available C (Seitzinger 1988, Martin et al. 2001, Kemp and Dodds 2002, Royer et al. 2004). Due to the increases in anthropogenic inputs of reactive N, agricultural or urbanized watersheds will have excess NO_3. Additionally, oxygen levels should drop in areas of high microbial activity which would be expected to occur where available C is abundant. Therefore, the regulation of denitrification, a heterotrophic process, should be mainly due to the supply of microbially available C. Additionally, DOC quality could limit sediment denitrification rather than actual concentration (Royer et al. 2004).

Although the main factors that theoretically regulate rates of denitrification are known, the significance of these factors varies among streams and over time within the same stream (Pattinson et al. 1998). The magnitude by which in-stream denitrification may alleviate high NO_3 concentrations and the environmental conditions which enhance denitrification are still uncertain, but understanding the controls on in-stream denitrification is important because of its influence on NO_3 biogeochemistry and export.

Goals and Objectives

Despite significant advances in hydrologic sciences, there is still only a poor understanding of the specific hydrologic mechanisms acting within terrestrial soils and how water actually gets to the stream. Additionally, the sources of DOC and the pathway traveled to the stream are unknown. Stream energy budget studies (Webster and Meyer 1997) and nutrient budget studies (Likens and Bormann 1974) do not quantify specific inputs or sources directly, and uncertainties regarding inputs to streams are a result of uncertainties concerning the source of losses from terrestrial environments. These exports of C from different terrestrial sources might influence in-stream N transformation rates, but the extent of their effect is also unknown. In order to address these issues, my research plan is developed around the following questions: **(1) What are the sources of water to the stream during baseflow and storm flow? (2) How important is hydrology in controlling DOC concentrations in stream water? (3) How does biological liability of DOC differ among terrestrial sources of water and in stream water during baseflow and storms? And (4) Does biological liability of DOC affect in-stream denitrification rates?**

These questions provide the focus for my proposed research wherein I seek to understand how hydrologic flow paths influence the transport of terrestrially derived C and how terrestrial C influences in-stream denitrification rates. The objective of this research were to develop a hydrologic model that describes the sources of water to White Clay Creek watershed, to determine the importance of hydrology to DOC export, to describe changes in C lability, and to investigate the influence of C quality on in-stream denitrification.

Summary of Research Chapters

Study Site

All research was conducted in stream reaches along the East Branch of White Clay Creek (WCC). WCC is a piedmont stream located in southeastern Pennsylvania (USA) draining a catchment composed of deciduous forests and agricultural land.

Chapter 1

I developed a component flow path model to explain the hydrologic response of WCC during baseflow and storm flow and determined the relative contributions of various terrestrial sources using EMMA. Additionally, I determined how stream water DOC was affected by hydrologic flow and the relative importance of catchment processes versus biological mechanisms of production and depletion by comparing the predicted concentrations from EMMA and the observed concentrations.

Stream water during baseflow and storm flow were sampled as well as terrestrial source waters including soil water in the vadose zone, shallow and deep groundwater in the phreatic zone, overland flow, and spring seep water. Solute concentrations measured in both stream water and terrestrial source waters included a suite of anions (NO_3, NO_2, Cl, PO_4, SO_4) and cations (NH_4, Ca, Mg, K, Na), DOC, and electrical conductivity (EC). Using multiple solute concentrations within a Principal Component Analysis (PCA) framework, I reduced the dimensionality of the stream water datasets and used the principal components to compute the contributions of end-members by solving mass balance equations for both stream water and potential end-members (Hooper et al. 1990, Christopherson and Hooper 1992). Diagnostic tests suggested by Hooper (2003) were used to evaluate and select solutes to use as tracers. The identities of potential end-members were determined by plotting end-member means in the PCA mixing space defined by the stream chemistry and selecting the end-members that bounded the stream data. EMMA estimated source contributions were developed for a long-term dataset (1993-2008) and for five storms. Stream water DOC concentrations were then predicted solely on the basis of variations in end-member source contributions and mean DOC concentrations for terrestrial sources. The ratio of observed to EMMA-predicted DOC concentrations was calculated for each stream datum and transformed to a logarithmic scale.

Stream flow during baseflow was primarily sustained through spring seeps and groundwater discharge, but overland flow paths were activated during storms with sources of stream flow shifting as the storm progressed. The timing of overland flow initiation seemed to depend on pre-storm discharge and antecedent moisture conditions with contributions starting earlier for storms that occurred under wetter conditions. The log transformed ratios of observed to predicted DOC concentrations were more variable at low discharges and during baseflow but converged towards zero for high discharges. Positive ratios observed for low flow suggests DOC production whereas high ratios during storms indicate the possibility of an additional end-member. The findings of this study provide a description of the hydrologic response of the WCC watershed using source areas and contribute to our understanding of how hydrologic flow influences the transport of terrestrially derived DOC to stream ecosystems.

Chapter 2

I determined the BDOC fraction in terrestrial source waters and described the changes in stream water BDOC under different hydrologic conditions. BDOC was measured using plug-flow bioreactors colonized by stream microorganisms and calculated as the difference between inflow and outflow DOC concentrations. The BDOC fraction in sample water was separated into two biological reactivity classes using bioreactors with different retention times since labile DOC molecules are metabolized through shorter bioreactors while semi-labile DOC is metabolized after longer exposures. Terrestrial source water and stream water from 12 storms were analyzed for labile BDOC, and BDOC from three storms was separated into labile and semi-labile components.

The BDOC fraction declined as water percolated through the soil from the unsaturated zone to the shallow groundwater with significant differences in the fraction of labile BDOC due to higher percentages in spring seeps. Both DOC and BDOC concentrations increased during storms. The fraction of labile BDOC in stream water was a function of discharge with increases from low flow to medium flow, but decreases during high flow. Differences between biological reactivity classes were observed indicating differences in the source pool for each class. The activation of different flow paths during storms is illustrated by the dynamic changes in stream water BDOC that occurred over the course of the stream hydrograph. The findings of this study contribute to our understanding of DOC biogeochemistry and the impact of hydrologic flow on the quality of DOC exported from terrestrial environments.

Chapter 3

I determined the effect of source water DOC quality on in-stream denitrification rates. Using laboratory incubations of stream sediments exposed to water with differing BDOC, denitrification rates were determined by adding $^{15}NO_3$ to the source water, enriching the ambient NO_3 pool, and measuring the production of $^{15}N_2$ over time. Baseflow stream water with a labile BDOC concentration between 0.056 and 0.132 mg C/L and a semi-labile BDOC concentration between 0.33 and 0.436 mg C/L was used as a control. Additional treatments including stream water amended with glucose with a labile BDOC concentration of 0.706 mg C/L and overland flow water with a labile BDOC concentration of 1.11 mg C/L and a semi-labile BDOC concentration of 5.59 mg C/L. A continuous-flow isotope ratio mass spectrometer was used to analyze the isotopic composition of N_2 in multiple samples taken over an 8 hour period.

Denitrification rates were calculated using the slope of the regression line between the molar concentration of ^{15}N versus time and the ratio of ^{15}N to ^{14}N.

Denitrification rates were significantly higher for the overland flow treatment, but no significant difference was observed between the control and glucose treatments. These results suggest that large increases in labile BDOC are needed to influence the rates of denitrification or that semi-labile BDOC concentration is more influential in regulating the rate of denitrification. The findings of this study contribute to our understanding of how C from different hydrologic flow paths might influence in-stream N transformation rates. Linking the water cycle to biogeochemical cycling addresses questions regarding the contribution of terrestrial environments to stream ecosystem function and provides information on the downstream impacts of land use activities which is important for public policy decisions concerning upstream and headwater protection and sustainable land use practices.

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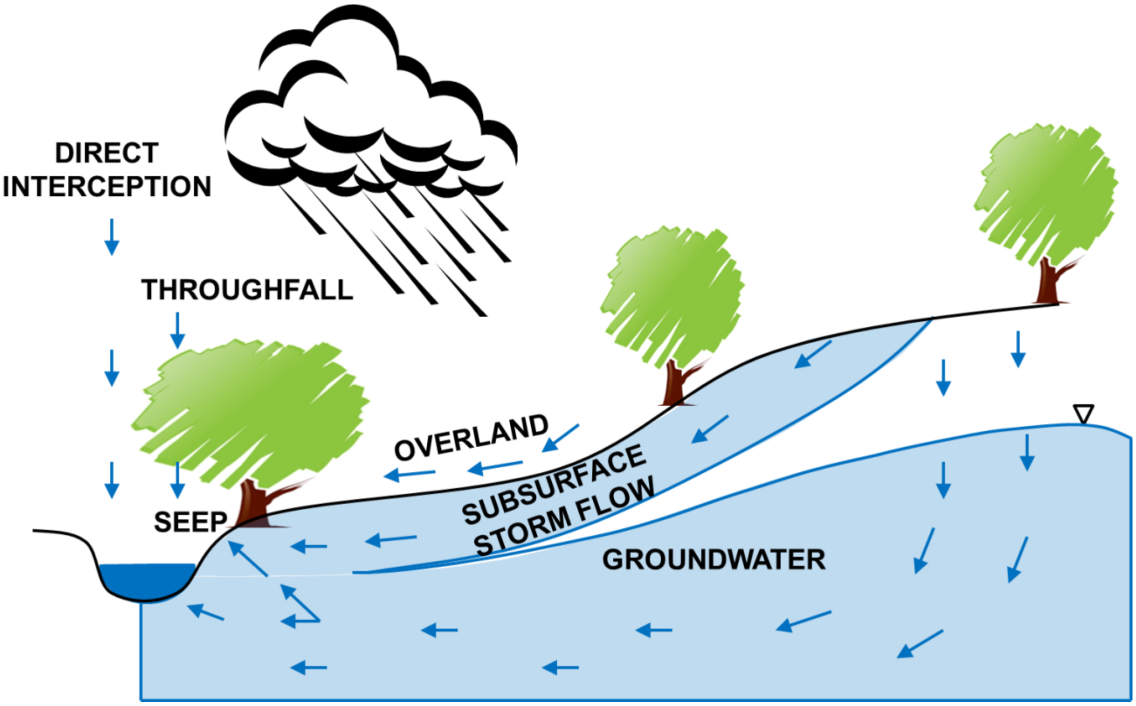
Figure Legends

Figure 1: The main hydrologic flow paths that contribute to annual water yield. Groundwater outflow and spring seeps sustain stream flow during baseflow, while direct interception of precipitation, throughfall, subsurface storm flow, and overland flow are activated during storms.

Figures

Figure 1



CHAPTER 1

Terrestrial source contributions and predicted dissolved organic carbon exports to a piedmont stream, derived from end-member mixing analysis

Abstract

End-member mixing analyses involving concentrations of four geochemical tracers were conducted on baseflow and storm flow chemistry datasets from a small piedmont watershed to identify and quantify the principal hydrologic source components of stream water. Stream DOC concentrations were then predicted based solely on the variations in the derived contributions from each source and DOC concentrations of each source pool. Ratios of observed to predicted DOC concentrations were used to evaluate the relative importance of catchment hydrology compared to in-stream processes where substantially differences between observed and predicted concentrations suggest in-stream processes are important determinants of stream water DOC concentrations. Three sources of groundwater were identified including spring seeps, deep groundwater, and shallow riparian groundwater, and an additional source, overland flow, was activated during storms. Seeps and shallow riparian groundwater contributed 44.6 % and 44.7 % on average to stream baseflow, respectively, and deep groundwater contributed 10.7 % on average. During storms, the end-member contributions progressed from seeps contributing on average 90.4 % before the storm to overland flow contributing on average 63.8 % at peak storm discharge. An analysis of the ratios of observed to EMMA-predicted DOC concentrations suggests substantial in-stream production during baseflow since the majority of the time EMMA under-predicted the stream DOC concentrations. Distinct seasonal patterns observed in the ratios illustrated high values during summer and autumn which is consistent with in-stream DOC sources. High ratios calculated for storms suggest an additional end-member not accounted for since in-stream production is unlikely due to disturbance caused by high discharge. Although the end-members identified as sources to stream flow are consistent with the assumed hydrology of this watershed, the exact calculated contributions could be inaccurate due to uncertainties recognized in EMMA and therefore, the emphasis of this work is on the relative contributions and the sequencing of end-members rather than the reliability of precise end-member proportions. Additional hydrological information should be collected to further differentiate the various flow paths and source contributions of this watershed and test the calculated EMMA predicted contributions.

Keywords

end-member mixing analysis, terrestrial source contributions, dissolved organic carbon, agricultural watershed

Introduction

Identifying terrestrial water sources that contribute to stream flow is central to understanding hydrologic and biogeochemical processes within stream ecosystems. Development of watershed management plans that evaluate the impacts of land use changes on water quality and determine the transport of non-point source pollutants requires quantifying the hydrologic response of watersheds. Because of the potential significance of dissolved organic carbon (DOC) to ecosystem-level processes and to nutrient cycling, characterizing the DOC supply to streams is also important. In forested watersheds, terrestrial sources produce the majority of DOC flowing downstream with distinct flow paths often determining the chemistry of the water discharged into the stream ecosystem (Burns et al. 2003, Weiler et al. 2005). Despite significant advances in hydrologic sciences, there is still only a poor understanding of the specific hydrologic mechanisms acting within terrestrial soils and how terrestrial source water actually gets to the stream channel because complex subsurface flow networks transport water in a dynamic manner such that sources differ between and within catchments (Hinton et al. 1998). However, end-member mixing analysis (EMMA; Christophersen et al. 1990) can be used as an exploratory tool for identifying the principal source components of stream water and for estimating stream DOC concentrations expected due solely to contributions from the identified sources in order to evaluate the relative importance of catchment hydrological processes in controlling stream water DOC concentrations.

EMMA separates geographical source components under the assumption that variations in stream water chemistry can be explained by a dynamic mixture of source solutions (Christophersen et al. 1990). Using natural geochemical variation in end-members representative of distinct flow path pools, such as soil water, groundwater, and overland flow water, mixing equations identify the relevant water reservoirs that contribute to stream flow. EMMA does not require detailed data on the processes controlling soil and groundwater chemistry, and therefore, provides a simple, pragmatic method and an initial assessment of catchment hydrology. EMMA can be applied to datasets of varying spatial and temporal extents and provides a tool for evaluating the linkages between small-scale source measurements and larger-scale catchment hydrology by determining the hydrological aspects of an ecosystem that are important to stream chemistry (Hooper 2001).

The majority of previous EMMA work has focused on identifying hydrologic processes occurring during wet catchment conditions and low soil moisture deficits (Brown et al. 1999). For example, researchers have used EMMA to examine runoff during individual storms to determine the sequence of end-member contributions (Inamdar and Mitchell 2007, McGlynn and McDonnell 2003, Bernal et al 2006), while others have compared multiple storms to determine the influence of antecedent moisture conditions (Katsuyama et al. 2001), and some have evaluated the sources contributing to streams during snow melt periods (Kendall et al. 1999, Liu et al. 2008). Stream flow during storms is sequentially fed by several terrestrial sources, and multiple mechanisms have been hypothesized to control storm flow runoff. For example, throughfall was identified as a controlling source in a forested watershed in New York (Inamdar and Mitchell 2006). Alternatively, the importance of subsurface soil pathways may be important especially when triggered by a perched water table within riparian areas (Whipkey 1962, Dunne and Black 1970 a, b, Weiler 2005). Then again, McDonnell (1990) proposed a sequence of events that included infiltration to deeper soil, expansion of groundwater into the unsaturated hillslope soils, and lateral rapid pipe flow.

Because EMMA work has typically focused on high discharge periods over short time scales, it has often been assumed that baseflow is sustained through groundwater discharge. However, Inamdar and Mitchell (2007) showed that the chemistry of groundwater differs from stream baseflow suggesting that baseflow itself is often a mixture of multiple end-members. Scalon et al. (2001) found that groundwater contributions are underestimated if groundwater were assumed as the only pre-storm baseflow source. Although extensive analysis has been performed on multiyear stream chemistry records from Panola Mountain Research Watershed in Georgia, in which both baseflow and storm flow were evaluated (Christophersen et al 1990, Hooper et al 1990, Christophersen and Hooper 1992, Hooper 2003), much less work has been conducted to determine the mechanisms that control source water routing to the stream during only dry catchment conditions.

EMMA is particularly designed for determining the sources of chemically inert substances since no assumptions are made about processes occurring along flow pathways (Christophersen et al. 1990, Christopohersen and Hooper 1992), but EMMA is also useful to determine if variations in hydrology explain variations in the stream water chemistry of non-conservative substances such as DOC. High DOC export by streams has often been attributed to flow through near-surface pathways where soil organic C levels are elevated (Hagedorn et al. 2000, Hornberger et la. 1994) and flushing of wetland soils (Boyer et al. 1996, 1997, Hinton et al. 1998). Many studies have assumed that terrestrial end-member chemistry controls the variations in stream water DOC concentration and used DOC as a tracer for EMMA (Brown et al. 1999, Inamdar and Mitchell 2006, Morel et al. 2009). Less work has focused on the role of in-stream processes, although Mulholland and Hill (1997) determined that in-stream processes explained much of the seasonality of stream DOC concentrations in an eastern deciduous forested stream, and McKnight and Bencala (1990) illustrated that sorption of DOC substantially changed stream water concentrations over short travel times and short distances within an acidic stream with high metal content. Using the proportional contributions determined by EMMA, DOC concentrations that would be expected if terrestrial sources controlled stream chemistry can be calculated. Subsequent comparisons of this predicted concentration to observed concentrations provides an estimate of how important variations in source contributions are to controlling stream water DOC concentrations.

The objectives of this research were to quantify the hydrologic sources to a small piedmont stream and subsequently determine how hydrologic flow affects stream water DOC by evaluating the relative importance of catchment processes versus biological mechanisms of production and those that result in depletion. I addressed the following questions: (1) What are the sources of water to the stream during baseflow and storm flow? (2) How important is hydrology in controlling DOC concentrations in stream water during baseflow and storm flow? I expected stream flow during baseflow conditions to be sustained primarily through groundwater discharge, but other flow paths to become activated during storms with sources of stream flow shifting as the storm progresses. Here I show that three terrestrial sources can explain the concentrations of conservative solutes in both baseflow and storm flow, but hydrologically-predicted DOC concentrations differ from observed concentrations suggesting other mechanisms influence the delivery and export of DOC.

Methods

Study Site

The East Branch of White Clay Creek (WCC), located in the southeastern Pennsylvania Piedmont (39° 51' 32.18" N, 75° 46' 58.28" W), drains a catchment of 7.25 km^2 composed of deciduous woodlands, meadows, pastures, and agricultural lands that are underlain by metamorphic crystalline rock including gneiss, schist, quartzite, and marble. Soils are 1 to 2 m deep, unglaciated, and primarily *typic hapludults*, except in the riparian zone, where *aqui fagiudults* prevail. The total length of stream channels that run from the headwaters (elevations: 164 m) to the 3rd order stream reach (elevation: 100 m) is 12.9 km.

The 3rd order stream is gauged with a continuously recording automated pressure transducer (MiniTroll) that records stage height at 15-min intervals. Stream stage was converted to stream discharge using a rating curve that was updated weekly and developed from discharge measurements made with the velocity-cross-sectional area method and a handheld Marsh-McBirney flow meter. Mean annual stream flow is 115 L/s (Newbold et al. 1997).

Precipitation data came from the U.S. Climate Reference Network (CRN) station “PA Avondale 2 N Stroud Water Research Center” operated by the National Oceanic and Atmospheric Administration located 250 m northwest of the 3rd order WCC stream site. The CRN station records real time precipitation and air temperature hourly. Annual precipitation averages 1.05 m/yr and is evenly distributed seasonally.

Field sampling

Stream water samples were collected at the 3rd order section of WCC. Stream water grab samples were taken approximately once a month from 1993 to 2008, and baseflow samples (n = 61) were chosen based on discharge at the time of sampling (< 100 L/s) followed by eliminating any samples taken on the rising or falling limb of a storm hydrograph through an inspection of the 15 minute discharge data. Storm water samples (n = 48) were collected hourly during five storms in 2010 and 2011 using an ISCO sampler. During each storm, multiple samples (9-10) were chosen to characterize the storm. Storm event parameters, including precipitation amount and antecedent moisture indexes for 7 and 30 days prior to the storm (API_7 and API_{30}), were tabulated for each storm (Table 1.1).

Terrestrial sources sampled included two deep groundwater wells (depth = 18.9 m, *n* = 7), seven spring seeps (*n* = 22), four shallow groundwater wells (depths between 0.94 and 1.1 m, *n* =55), 10 lysimeters (42 mm ID PVC with round bottom ceramic cup, depths between 0.42 and 0.52 m, *n* = 128) for collecting soil water in the vadose zone, and overland flow (*n* = 17). Spring seeps are permanently flowing sources of groundwater, and grab samples were collected in 2008 and 2011 where they surfaced in broad wetted areas of Worsham silt-loam in the watershed. Shallow wells and tension lysimeters, installed adjacent to the stream channel, were used to sample soil water and shallow groundwater, respectively, at irregular intervals between 2005 and 2011. Shallow riparian wells were purged and then sampled using a hand pump. Lysimeters were put under a vacuum for five to seven days allowing water in the unsaturated soil to travel through the porous cup and collect in the lysimeter for sampling. Overland flow was collected during six storms between January 2010 and August 2011 by either a grab sample or from a collector constructed based on the Low Impact Flow Event sampler design (Sheridan et al. 1996).

All water samples were separated for individual analyses including a suite of anions (NO_3, NO_2, Cl, PO_4, SO_4) and cations ($NH_{4,}$ Ca, Mg, K, Na), DOC, and electrical conductivity (EC). Anion and cation samples were filtered (Sterile Millex® Syringe Filters with a 22 µm pore size) and frozen until analysis. Additionally, cation samples were acidified. Ion concentrations were measured by ion exchange chromatopgraphy using a Dionex DX 3000. DOC samples were filtered (precombusted glass fiber filters, Whatman GF/F) and refrigerated until concentrations were measured using a Sievers 800 or 900 organic carbon analyzer. EC measurements were made at 25° C on unfiltered water using a Mettler Toledo Seven Multi-meter.

Selection of tracers

The selection of solutes as tracers was based their consistency with the conservative mixing hypothesis and their ability to distinguish between end-members (Hooper 2003). Conservative behavior among potential tracers was evaluated by examining the linearity of bivariate solute plots for all pairwise combinations of solutes using the entire stream water chemistry dataset. Linearity suggests but does not prove conservative mixing (Christophersen and Hooper 1992, Hooper 2003). All pairwise combinations of solutes were categorized into non-collinear ($r^2 < 0.2$, $p > 0.05$), weakly collinear ($0.2 < r^2 < 0.5$, $p < 0.05$), and linear ($r^2 > 0.5$, $p < 0.01$). Additionally, Kruskal-Wallis tests were used to evaluate each tracer's ability to distinguish between end-members by determining if concentrations were significantly different among at least one pair of potential end-members.

Eigenvector and residual analysis

Diagnostic tools of mixing models were used to determine the number of end-members mixing to form baseflow and storm flow stream water by examining the appropriate rank of the two stream chemistry datasets and by determining the dimensions that the data span in a Euclidean U-space (Hooper 2003). The correlation matrixes of the multivariate tracer chemistry datasets were used in a principal components analysis (PCA) to determine eigenvectors and eigenvalues. The resulting eigenvectors reduce the dimensionality of the mixing space by transforming the stream chemistry with dimensions equal to the initial number of tracers to a lower dimension with orthogonal axes describing the variance in the stream water chemistry datasets. Tracer concentrations were orthogonally projected by a matrix multiplication of the eigenvectors and standardized stream tracer concentrations.

To determine the dimensionality of the datasets and assess the fit of the stream water chemistry to a lower dimensional mixing subspace, the standardized stream tracer datasets were multiplied with incremental addition of eigenvectors followed by applying a residuals analysis for each solute (Hooper 2003). The minimum number of eigenvectors required to yield a random structure in the residuals indicates the number of eigenvectors needed to definite the mixing subspace and is related to the number of end-members required in the mixing model. The maximum amount of variation in the data that can be explained is limited to that explained by the eigenvectors retained, while the number of end-members needed for the model is one more than the number of eigenvectors retained.

For an individual observation of stream water chemistry, the residual was calculated by subtracting the original value from its orthogonal projection. The orthogonal projection was expressed in terms of the original solutes ($\hat{X}^*$) using the following matrix manipulation:

$$(1) \qquad \hat{X}^* = X^* V^T \, (VV^T)^{-1} \, V$$

where X* is the matrix of standardized stream values with n samples and p solutes, V is the matrix formed by the first m eigenvectors (Christophersen and Hooper 1992). Diagnostic plots were made by plotting the residuals against the observed concentrations, and additional eigenvectors were retained until there was no structure to the residuals. The fit between observed values and their orthogonal projections was further evaluated using the relative root-mean-square error (RRMSE) which provides an indication of the thickness of the data cloud outside the lower dimensional subspace. RRMSE (r_j) was calculated for each solute (j):

$$(2) \qquad r_j = \frac{\sqrt{\sum_{i=1}^{n}(\hat{X}_{ij} - X_{ij})^2}}{n\,\bar{X}_{ij}}$$

where i is the ith sample, $\hat{X}$ is the projected solute concentration, X is the observed solute concentration, and $\bar{X}$ is the mean of the observed solute concentrations.

End-member selection and source contributions

The five terrestrial sources were evaluated as potential contributors to stream water by projecting them into the mixing spaces defined by the stream tracer concentrations and identifying the end-members that best enclose the stream data. The mean tracer concentrations for each source pool were first normalized using the mean and standard deviation of the stream water tracer concentrations. These standardized values were then multiplied by the eigenvectors and graphed in U-space with the stream values.

The proportional contribution of each end-member (P_1, P_2, P_3) was calculated by solving mass balance equations using the orthogonal (U-space) projections of stream water samples ($U1_s$, $U2_s$) and end-member means ($U1_1$, $U2_1$; $U1_2$, $U2_2$; $U1_3$, $U2_3$):

$$P_1 + P_2 + P_3 = 1 \tag{3}$$

$$U1_1P_1 + U1_2P_2 + U1_3P_3 = U1_s \tag{4}$$

$$U2_1P_1 + U2_2P_2 + U2_3P_3 = U2_s \tag{5}$$

EMMA was conducted separately on the baseflow and storm flow datasets.

Analysis of catchment processes vs. in-stream effects

The relative importance of hydrology in controlling stream water DOC concentrations was evaluated by comparing EMMA-predicted stream DOC concentrations with observed concentrations. EMMA-predicted stream DOC concentrations (i.e., the concentrations that are expected if only terrestrial source contributions control stream chemistry) were calculated for each stream sample by multiplying the proportional contribution of each terrestrial source (P_1, P_2, P_3) by the mean DOC concentration determined for each source and summing the products. Ratios of the observed DOC concentration to the EMMA-predicted concentration were then calculated and transformed to a logarithmic scale. If the log transformed ratio of observed to EMMA-predicted DOC concentrations was equal to zero, the variation in hydrologic flow paths controls the stream DOC concentration, whereas a negative ratio suggests depletion occurring and a positive ratio suggests production occurring either along the pathway between the source and the stream or within the stream channel.

Statistical analyses

Statistical analyses were conducted with JMP Statistical Discovery software (v 9.0.2/2010, SAS, Cary, NC). Linear trends among possible tracers were determined based on the correlation coefficient (r^2) and *p*-value for all pairwise combinations of the 11 solutes measured in stream water. Differences in tracer concentrations among end-members were determined using non-parametric Kruskal-Wallis tests. Dimensionality analysis was then conducted on both the baseflow and storm flow datasets by plotting residuals against observed stream concentrations for each solute, and the presence of structure in the residuals was evaluated based on the r^2. Additionally, RRMSE of the stream dataset was used to determine the goodness of fit between observed values and their orthogonal projections.

For the baseflow EMMA, end-member proportions were averaged and reported as mean ± standard error (minimum to maximum), and an analysis of variance (ANOVA) was used to determine differences among seasons. Stream baseflow DOC concentrations were also averaged by season and differences were determined using an ANOVA. Seasonal patterns in the log transformed ratios of observed to EMMA-predicted for the baseflow dataset were determined with an ANOVA followed by Tukey post-hoc analyses.

For the storm flow EMMA, end-member proportions were separated into pre-storm, rising limb, peak, and falling limb based on the timing of precipitation and the stream hydrograph. Pre-storm values were defined as those samples that were taken before the onset of rain. Rising limb, peak, and falling limb samples were based on the timing of the sample compared to the stream discharge for each individual storm.

Results

Tracer selection

All pairwise combinations of the 11 solutes analyzed in stream water were examined for linear trends (Table 1.2). The majority of pairwise combinations were weakly linear ($0.2 < r^2 < 0.5$, $p < 0.05$). Additionally, end-member concentrations of all 11 solutes were at least significantly different among one pair of potential end-members (Figure 1.1, Kruskal-Wallis, df=4, $p < 0.0001$). EC, Ca, and Cl were selected for use in the model because they had the greatest number of linear combinations ($r^2 > 0.5$, $p < 0.01$). Lastly, K was chosen because it was at least weakly linear with all other selected tracers and because it was useful in differentiating the deep well end-member (Figure 1.1).

Eigenvector and residual analysis

A mixing space dimensionality analysis was conducted separately for the baseflow and storm flow stream datasets. An examination of the residuals for each solute in the baseflow stream dataset indicated that two eigenvectors should be retained. The presence of structure in the residuals for all of the solutes ($r^2_{Cl} = 0.52$, $r^2_{Ca} = 0.60$, $r^2_{K} = 0.82$, $r^2_{EC} = 0.56$) when using one eigenvector indicates the need for additional eigenvectors. The retention of an additional eigenvector substantially reduced the linear pattern for K (r^2_K = 0.006), and minimally reduced the linear pattern for the other solutes (r^2_{Cl} = 0.49, r^2_{Ca} = 0.53, r^2_{EC} = 0.53). The addition of a 3rd eigenvector would not substantially change the residual patterns for most of the solutes. The RRMSE was low for all solutes (< 5 %) indicating a good fit between observed values and their orthogonal projections. The first two eigenvectors explain 60.8 % of the variability in the stream chemistry for the baseflow dataset.

An examination of the residuals for each solute in the storm flow stream dataset also indicated that two eigenvectors should be retained. There was high linear structure in the residuals for K (r^2 = 0.69) but a lack of structure for the other three solutes (r^2_{Ca} = 0.13, r^2_{Cl}= 0.17, r^2_{EC} = 0.04) when using one eigenvector. The retention of an additional eigenvector reduced the linear pattern for all of the solutes (r^2_k = 0.01, r^2_{Ca} = 0.12, r^2_{Cl}= 0.05, r^2_{EC} = 0.03) indicating a well-fit model. Again, the RRMSE was low for all solutes (< 5 %) indicating a good fit between observed values and their orthogonal projections. The first two eigenvectors explain 94.7 % of the variability in the stream chemistry for the storm flow dataset.

End-member selection and source contributions

Dimensionality analyses revealed that three end-members were needed to explain the stream chemistry during baseflow and storm flow since the number of end-members is one more than the number of eigenvectors retained. End-members were chosen from the five terrestrial sources pools based on which three enclosed the stream concentrations. The orthogonal projections of the near surface end-members, including shallow groundwater, soil water, and overland flow, were grouped together in the baseflow EMMA mixing space diagram (Figure 1.2). Since overland flow only occurs during precipitation and unsaturated soil water should only move vertically during periods between storms, these end-members were eliminated from consideration as baseflow end-members. The triangle formed by deep groundwater, shallow groundwater, and seep water enclosed all but one of the baseflow data. In the storm flow EMMA mixing space diagram (Figure 1.3), the orthogonal projections of the shallow groundwater and soil water grouped together but plotted in between the overland flow and seep end-members. Therefore, the triangle formed by deep groundwater, seep water, and overland flow enclosed the most stream data.

The smallest end-member contribution to stream baseflow was deep groundwater with a mean proportion of 10.7 % ± 0.9 % and the smallest range (0 % to 52.7 %). Shallow groundwater and seeps contributed equally with means of 44.7 % ± 2.1 % (0 % to 86.2 %) and 44.6 % ± 2.0 (0.8 % to 78.3 %), respectively (Figure 1.4). Mean end-member contributions did not vary significantly across seasons, but seeps were the dominant contributor to stream flow in the summer and shallow groundwater in the winter.

The fractional end-member contributions to stream storm flow were variable, although similar patterns were seen among storms in the evolution of end-member contributions over the course of the storm hydrographs (Figure 1.5). On the rising limb of the storm hydrographs, seep contributed on average

47.0 % ± 6.6 % (0 % to 88.6 %), whereas deep ground water contributed an average of 28.9 % ± 4.8 % (9.0 % to 64.5 %). According to the EMMA predicted contributions, overland flow was not activated immediately but instead was only triggered once discharge increased. Overland flow was predicted sooner for the two spring storms (March 12 and 22, 2010) with contributions occurring once discharge had increased approximately 1.5-fold. These two storms had higher pre-storm discharge (170 and 180 L/s, respectively) as well as higher API_{30} (91 and 103 mm, respectively). During the other two storms in 2010 (September 30 and November 30), substantial overland flow was not predicted until the discharge increased 27 and 2.6-fold, respectively. The mean overland flow contribution on the rising limb was 24.1 % ± 6.4 % (0 % to 90.1 %). Seep contributions decrease as the storms progressed with the lowest mean contribution of 12.4 % ± 5.6 % (0 % to 28.5 %) occurring at peak discharge. Mean contributions from deep groundwater changed only slightly throughout the storm hydrographs with a mean of 23.9 % ± 9.9 % (3.1 % to 53.3 %) at peak discharge and 19.4 % ± 3.0 % (0 % to 58.8 %) during the falling limb. Mean overland flow contributions were greatest at the peak in discharge with a mean contribution of 63.8 % ± 11.4 % (25.9 % to 96.9 %). During the falling limb of the hydrograph, seep contributions increased to 37.7 % ± 5.8 % (0 % to 86.9 %) and overland flow contributions decreased to 42.8 % ± 5.6 % (0 % to 91.1 %).

Analysis of catchment processes vs. in-stream effects

DOC concentrations among source pools were significantly different (Figure 1.1, Kruskal-Wallis, df=4, $p < 0001$) with the highest concentrations measured in overland flow (10.01 ± 0.86 mg C/ L) and soil water (2.30 ± 0.17 mg C/ L). Deep groundwater had the lowest concentration (0.51 ± 0.09 mg C/ L) followed by seeps (0.95 ± 0.11 mg C/ L) and shallow groundwater (1.41 ± 0.13 mg C/ L).

Stream DOC concentrations in the baseflow dataset averaged 1.44 ± 0.04 mg C/ L and ranged from 0.78 to 2.79 mg C/ L. Stream DOC concentrations were significantly different among seasons (ANOVA, $F_{(3,57)}$ = 5.09, $p < 0.01$) with the highest mean concentration in the summer (1.59 ± 0.08 mg C/ L) and lowest mean concentration in the winter (1.20 ± 0.06 mg C/L). The seasonal trend in DOC concentrations in WCC has been previously reported and is unlike the patterns observed for concentrations of the conservative tracers used for EMMA which show no discernible annual variations (Hullar et al. 2006). For the majority of baseflow samples, EMMA under-predicted the stream DOC concentration; the mean EMMA-predicted DOC concentration was 1.11 ± 0.01 mg C/ L with a range of 0.72 to 1.29 mg C/ L. The log transformed ratios of observed to predicted DOC concentrations averaged 0.10 and ranged from -0.16 to 0.41. Distinct seasonal patterns in the observed to predicted ratios were evident with significant differences among seasons (Figure 1.6, ANOVA, $F_{(3,57)}$ = 5.62, $p = 0.002$) driven by differences between the summer and autumn ratios compared to the winter ratios (Tukey's test of summer vs. winter $p = 0.002$, autumn vs. winter $p = 0.009$). Although mean seasonal DOC ratios were greater than zero, positive ratios were observed during summer (0.0 to 0.41), autumn (0.03 to 0.40), and spring (0.05 to 0.15), whereas both positive and negative ratios were observed during winter (-0.16 to 0.28).

Stream DOC concentrations increased on average 6-fold during storms from a mean pre-storm concentration of 1.66 ± 0.27 mg C/L to a mean peak concentration of 9.19 ± 1.07 mg C/L. Again, EMMA under predicted the DOC concentration for all of the samples except three samples around the peak in discharge during the largest storm (September 7, 2011) and two samples at the end of one of the smaller storms (March 12, 2012). However, for two of the five storms, EMMA predictions preserved the pattern in the DOC concentration over time (Figure 1.7). The mean predicted pre-storm concentration (0.91 ± 0.01 mg C/L) was the lowest followed by increases on the rising limb (3.00 ± 0.59 mg C/L) to the highest mean concentration at the peak in discharge (6.62 ± 1.07 mg C/L) and slight decreases on the falling limb (4.75 ± 0.51 mg C/L). Although there was not a significant relationship between discharge and the logarithmic transformation of the ratios between observed and predicted DOC concentrations, the greatest range of ratio values occurred for lower discharges while the ratios decreased towards zero for higher discharges (Figure 1.8).

Discussion

Stream flow sources were quantified for baseflow and storm conditions in this small agricultural watershed, and the relative importance of hydrology versus in-stream processes was evaluated. Determining the temporal persistence of stream flow sources was only possible by assuming that their contributions and mixing in stream flow varied with time. DOC was omitted from consideration as a tracer in EMMA because of the requirement of conservative mixing and time-invariant concentrations in source pools. Although DOC has previously been used to identify flow paths because it represents a solute that typically accumulates in surficial soil layers (Brown et al. 1999, McGlynn and McDonnell 2003, Inamdar and Mitchell 2006, Morel et al 2009), my objectives included determining how stream water DOC is affected by hydrologic flow and required calculating a predicted stream DOC concentration expected if only source contributions controlled stream chemistry. Both the baseflow and storm flow EMMA under-predicted the stream DOC concentrations suggesting some unaccounted for mechanism of production or the possibility of a missing end-member.

During baseflow conditions, EMMA showed that spring seeps and shallow riparian groundwater were the largest contributors to stream flow. Similar results were determined for Point Peter Brook watershed, a forested watershed in western New York, and analyses conducted at different catchment sizes showed seep contributions were more important for small headwater streams (Inamdar and Mitchell 2007). Riparian groundwater has also been shown to dominate baseflow in Panola Mountain Research Watershed, a piedmont watershed in Georgia (Hooper 2001). Although the mean contributions of all the end-members were consistent across seasons, greater variations were seen during winter when shallow groundwater was the dominant source. Analogous results were reported by Mulholland and Hill (1997) with greater contributions from the saturated groundwater zone above the bedrock and the soil vadose zone in transient perched water tables during cooler months. Others have suggested that separating groundwater into two distinct sources is erroneous, and short-term fluctuations in the level of the groundwater table cause variability in the chemical signatures that goes against the time-invariant assumptions of EMMA (Hornberger et al. 1994, Rice and Hornberger 1998, Scalon et al. 2001). The use of pre-storm groundwater concentrations would result in underestimations of groundwater discharge compared to hydrograph separations that account for the time-variant groundwater concentrations (Scalon et al. 2001). However, it is widely accepted that stream baseflow is some mixture of multiple end-members (Scalon et al. 2001, Inamdar and Mitchell 2007, Inamdar 2010).

During storms, additional sources must be activated to account for the geochemical changes that occur in stream water chemistry. Previous EMMA work has identified soil water, hillslope runoff, upland water sources, and shallow soil sources attributed to flow though the O-horizon (Brown et al. 1999, Kendal et al. 1999, Burns et al. 2001, Hooper 2001, McGlynn and McDonnell 2003, Inamdar 2011). The selection of overland flow as an end-member was based on the combination of an a priori assumption due to field observations of saturated surface areas in the watershed during storms and the mean projected overland flow value's ability to enclose the stream concentrations in U-space. Overland flow is typically defined as flow over the land surface as either broad sheet flow or flow in microchannels such as rills (Elsenbeer et al. 1995). The use of overland flow in EMMA does not imply a particular generating mechanism, and could be a mixture of O-horizon soil water and throughfall water depending on the degree of incision of the flow path (Elsenbeer et al. 1995). The majority of EMMA in temperate regions have not documented overland flow, but some have shown the importance of O-horizon sources (Elsenbeer et al. 1994, 1995, Brown et al. 1999, Kendal et al. 1999), which is similar to the overland flow end-member. There is no consensus on whether large or small events favor enhanced contributions from specific catchment sources since results from other EMMA have reported increased runoff from riparian zones (Inamdar and Mitchell 2007), from hillslopes, and from upland sources (Burns et al. 2001, McGlynn and McDonnell 2003).

The mean EMMA derived overland flow contribution at the peak in discharge was 63.8 % ± 11.4 % which is in the range of previously estimated O-horizon and saturated overland flow contributions of 25 % to 80 % (Elsenbeer et al. 1995, Brown et al. 1999). However, my estimates seem relatively high considering field observations of overland flow, and saturated surface areas in the near stream zone were minimal in extent during storms. Additionally, the predicted overland flow contributions continued in some cases up to 20 hours after the flow had returned to near baseflow levels, which is hydrologically unlikely. Although deep groundwater contributions during storms were similar to baseflow contributions, the seep contributions determined for the storm flow EMMA were substantially higher than the contributions determined for the baseflow EMMA. This difference is due to the replacement of the shallow groundwater source with overland flow for the storm flow EMMA instead of increasing the number of end-members due to the activation of precipitation dependent flow paths such as overland flow. Presumably, shallow riparian groundwater continues to contribute to stream flow during storms possibly mixing with soil water in the vadose zone. Since neither shallow riparian groundwater nor soil water were major contributors, these sources were omitted by the storm flow EMMA. Therefore, the seep end-member contributions from the storm flow EMMA may represent a combination of seep water and shallow riparian groundwater and the overland flow contributions a combination of surface runoff, throughfall, and soil water in the surface soil.

Using the log transformed ratio of observed DOC concentrations to EMMA-predicted DOC concentrations to separate the effects of hydrology and biological processing is based on the assumption that EMMA provides a reasonable estimation of stream concentrations expected if only terrestrial flow paths delivered DOC to the stream channel. The greater range in ratio values during low discharge and the convergence of the ratios closer to zero during higher discharge supports this assumption since in-stream processes should be less important during storms due to shorter water residence times and lower surface to volume ratios in the stream channel. During baseflow, hydrologic flow paths which are influenced by geology and land-use, deliver DOC to the stream channel but in-stream processes subsequently alter the amount of DOC exported to downstream reaches. Based on the mean baseflow DOC concentration and the mean EMMA-predicted DOC concentration, in-stream processes increase the DOC by 23 %, on average. Since previous estimates of net DOC flux from the hyporheic zone were between 3.8 % and 10.6 % (Battin et al. 2003), other in-stream sources of DOC would be needed to explain the difference. During storms when in-stream processes become less important, the lower EMMA-predicted DOC concentrations suggest additional sources of high DOC concentration in combination with overland flow, the dominant storm flow source identified by EMMA.

Previous research has largely attributed the temporal and spatial variations in stream DOC to hydrologic shifts in the dominant flow path from deeper to shallower source contributions (Mulholland 1997, McKnight and Bencala 1990, Hornberger et al 1994, Morel et al. 2009). Although DOC is a biologically reactive solute (Aikenhead-Peterson et al. 2003), numerous studies have used DOC as a tracer in EMMA as a result of this assumption that its supply to streams is controlled by the relative importance of different flow paths (Brown et al. 1999, McGlynn and McDonnell 2003, Inamdar and Mitchell 2006, Morel et al 2009). The positive link established between the prevalence of surface and shallow subsurface flow paths through organic rich soils and wetlands is expected due to higher DOC concentrations in these source pools. For example, watershed characteristics such as the proportion of wetlands and the amount of poorly drained soils have been identified as major predictors of DOC concentrations in lakes and streams (Creed et al. 2008, Wilson and Xenopoulos 2008). However, using DOC as a tracer in EMMA not only goes against the requirement of conservative mixing but also creates a bias in the EMMA-predicted DOC concentrations since the analysis uses the DOC concentration to generate source contributions.

Contributions from shallow soil and surface sources obviously play an important role in delivering DOC to the stream during storms as evident from the pattern of the highest storm DOC concentration (14.04 mg C/L, December 1, 2010) corresponding to the highest overland flow DOC concentration (16.19 mg C/L). Although the mean overland flow DOC concentration was not sufficient to explain the increases in DOC during all storms, the extreme values were near or above the peak storm DOC concentration for the majority of storms. This discrepancy in combination with high ratios of observed to predicted concentrations could be due to either an insufficient characterization of the overland flow end-members or may be indicative of a more complex mixing scenario such as the mobilization of soil water or the scouring of the stream bed adding to the DOC pool and contributing the remaining missing DOC during storms. Most likely, shallow riparian groundwater that rises into the vadose zone connects the previously unsaturated soil water source to the stream channel, a mechanism not accounted for by EMMA due to the limitation of using only the three dominant sources.

During baseflow, the distinct seasonal pattern in the log transformed ratios of observed to EMMA-predicted DOC concentrations with highest values during autumn and summer and ratios closer to zero during winter is consistent with results from Walker Branch watershed in eastern Tennessee and the conclusion that in-stream production may be more important than previously thought (Mulholland and Hill 1997). Previous work in WCC has shown that the production of DOC by algae can increase stream DOC concentrations on a diel basis (Kaplan and Bott 1982) which might explain the positive ratio values during spring when algal production should be at its maximum. The high summer ratio values suggest possible algal production in farm ponds located in the headwaters contributing to baseflow DOC. Alternatively, feeding by invertebrate detritivores and physical leaching of leaves in the autumn could explain the higher autumn ratio values (Meyer and O'Hop 1983). The negative winter ratios are most likely a result of the reduced in-stream metabolism that occurs during colder temperatures in combination with the possibility of surface sources freezing.

Uncertainty in EMMA predictions arise from the spatial and temporal variability in end-member tracer concentrations and a lack of compliance with model assumptions (Joerin et al. 2002). For example, tracer concentrations have been shown to vary spatially across some catchments, which directly contradicts the assumption of spatial invariance in EMMA (Katsuyama et al. 2001, Kendall et al. 2001, James and Roulet 2006). James and Roulet (2006) attributed spatial differences in groundwater tracer concentrations to variations in bedrock and differences in residence time of groundwater sources. This is consistent with WCC where the deep groundwater or the spring seep source had the greatest confidence interval and greatest range for all four of the tracers used in EMMA. The lack of a large number of deep wells spread throughout the watershed makes it difficult to assess the spatial variation in the geochemical signature of this end-member. Elsenbeer et al. (1995) showed spatially variability in overland flow concentrations and suggested mixing of overland flow with throughfall which resulted in tracer concentrations being dependent on the patchy distribution of leaf litter. However, the mean tracer concentrations were similar among the three overland flow sites with the exception of DOC which varied within and among sites. Others have noted changes in end-member concentrations over time violating the time-invariant assumption for EMMA (Durand and Torress 1996, Hooper 2001, James and Roulet 2006, Inamdar and Mitchell 2007). For instance, long term monitoring of Panola Mountain Research Watershed revealed decreasing Ca concentrations in shallow soil over a three year period (Hooper 2001, 2003). The use of a six year end-member chemistry dataset allowed for a thorough characterization of tracer concentrations for most of the end-members, and despite variations, no distinct pattern in terrestrial source water chemistry emerged over time. Although the choice of end-members may not be invalid based on the uncertainty attributed to EMMA, the exact calculated contributions could be inaccurate (Inamdar 2011). Therefore, the emphasis must be placed on the relative contributions and sequencing of end-members compared to the reliability of the precise end-member proportions.

While the use of EMMA has provided a component flow path model to explain the hydrologic response of WCC, additional hydrometric information would be useful to further differentiate the hydrology in this watershed. The EMMA results in combination with an expansion of the spatial extent of terrestrial source sampling, the installation of high-frequency, in-situ, water quality sensors, and the continued long term monitoring of this watershed will provide additional insights into the hydrology of small headwater streams. Because of the importance of DOC to in-stream heterotrophic production, the hypothesis that in-stream processes may substantially influence DOC concentrations suggests the need for more watershed scale investigations and identification of additional sources of DOC.

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Table Legends

Table 1.1: Storm event parameters for storm flow dataset of five storms in White Clay Creek watershed during 2010 and 2011.

Table 1.2: Correlation matrix of concentrations for 10 solutes and electrical conductivity (EC) measured in stream water samples (*n* = 121) from White Clay Creek, PA. Highlighted tracers were used for baseflow and storm flow EMMA. Values reported as r^2 (*p*-value).

Tables

Table 1.1

	Date	Total precip (mm)	API$_7$ [a] (mm)	API$_{30}$ [a] (mm)	Pre-storm Q (L/s)	Peak Q (L/s)
1	3/12/2010	55.7	0	90.5	170	1743
2	3/22/2010	26.3	6.7	103.2	183	1373
3	9/30/2010	160.2	19.7	39.6	47	5039
4	11/30/2010	42.3	4.5	51.8	75	1449
5	9/7/2011	80.5	94.8	445.2	- [b]	13780

[a] Antecedent moisture conditions computed using the antecedent precipitation index (API$_X$) which is the summation of the precipitation amounts for X days prior to the storm (Betson et al. 1969, Bowen 1986).

[b] No pre-storm sample was taken for this storm and therefore, no pre-storm Q is listed.

Table 1.2

	SO_4	**Ca**	Mg	Na	**EC**	**Cl**	**K**	NO_2	NH_4	NO_3
PO_4	-0.34 (0<0.01)	-0.36 (< 0.01)	-0.32 (< 0.01)	-0.46 (< 0.01)	-0.39 (< 0.01)	-0.42 (< 0.01)	0.41 (< 0.01)	-0.22 (0.14)	-0.17 (0.08)	-0.27 (< 0.01)
SO_4		0.61 (< 0.01)	0.34 (<0.01)	0.15 (0.11)	0.76 (< 0.01)	0.73 (< 0.01)	-0.38 (0.01)	0.32 (0.03)	-0.31 (0.02)	0.63 (< 0.01)
Ca			0.00 (0.96)	-0.21 (0.03)	0.85 (< 0.01)	0.71 (< 0.01)	-0.55 (< 0.01)	-0.51 (< 0.01)	-0.18 (0.06)	0.71 (< 0.01)
Mg				0.12 (0.22)	0.27 (< 0.01)	0.27 (< 0.01)	0.14 (0.13)	-0.01 (0.93)	-0.38 (< 0.01)	0.21 (0.03)
Na					0.03 (0.73)	0.23 (0.01)	0.13 (0.16)	0.44 (< 0.01)	0.24 (0.01)	-0.23 (0.02)
EC						0.81 (< 0.01)	-0.63 (< 0.01)	0.52 (< 0.01)	-0.35 (< 0.01)	0.86 (<0.01)
Cl							-0.43 (< 0.01)	0.47 (< 0.01)	-0.31 (< 0.01)	0.67 (< 0.01)
K								-0.05 (0.71)	0.02 (0.83)	-0.71 (< 0.01)
NO_2									-0.29 (0.04)	-0.05 (0.74)
NH_4										-0.29 (< 0.01)
NO_3										

Figure Legends

Figure 1.1: Box plots illustrating the chemical signature of potential end-members independently sampled in the White Clay Creek watershed. Each box has lines indicating the 25th percentile, the median, and the 75th percentile, while the whiskers above and below the box indicate the 90th and 10th percentiles.

Figure 1.2: U-space mixing diagram for baseflow stream dataset from White Clay Creek watershed (*n* = 61). The tracers selected for EMMA included Ca, Cl, K, and electrical conductivity (EC), and the selected end-members (deep groundwater, shallow groundwater, and seep water) enclose the stream concentrations as shown by the triangle.

Figure 1.3: U-space mixing diagram for storm flow stream dataset from White Clay Creek watershed that included five storms sampled between 2010 and 2011 (*n* = 48). The tracers selected for EMMA included Ca, Cl, K, and electrical conductivity (EC), and the selected end-members (deep groundwater, seep water, and overland flow) enclose the stream concentrations as shown by the triangle.

Figure 1.4: Box plots illustrating the end-member contributions to stream baseflow in White Clay Creek watershed determined using EMMA. Each box has lines indicating the 25th percentile, the median, and the 75th percentile, while the whiskers above and below the box indicate the 90th and 10th percentiles.

Figure 1.5: End-member contributions to stream storm flow in White Clay Creek watershed. EMMA used on storm flow stream dataset consisted of samples taken during five storms between 2010 and 2011.

Figure 1.6: Comparison of EMMA-predicted DOC concentrations to observed concentrations for baseflow in White Clay Creek watershed. EMMA-predicted DOC concentrations were based on EMMA proportions and average end-member DOC concentrations. Distinct seasonal patterns in the log transformed ratios of observed to predicted are evident with positive values for summer and autumn and both positive and negative values for winter. Line indicates 1:1 line; as such points plotting to the left of the line have a negative log transformed ratio, whereas those right of the line have a positive log transformed ratio.

Figure 1.7: Comparison of EMMA-predicted DOC concentrations to observed DOC concentrations for storm flow in White Clay Creek watershed. EMMA-predicted DOC concentrations were based on EMMA proportions and average end-member DOC concentrations.

Figure 1.8: Relationship between discharge and the log transformed ratio of observed to EMMA-predicted DOC concentrations for baseflow and storm flow in White Clay Creek watershed. EMMA-predicted DOC concentrations were based on EMMA proportions and average end-member DOC concentrations.

Figures

Figure 1.1

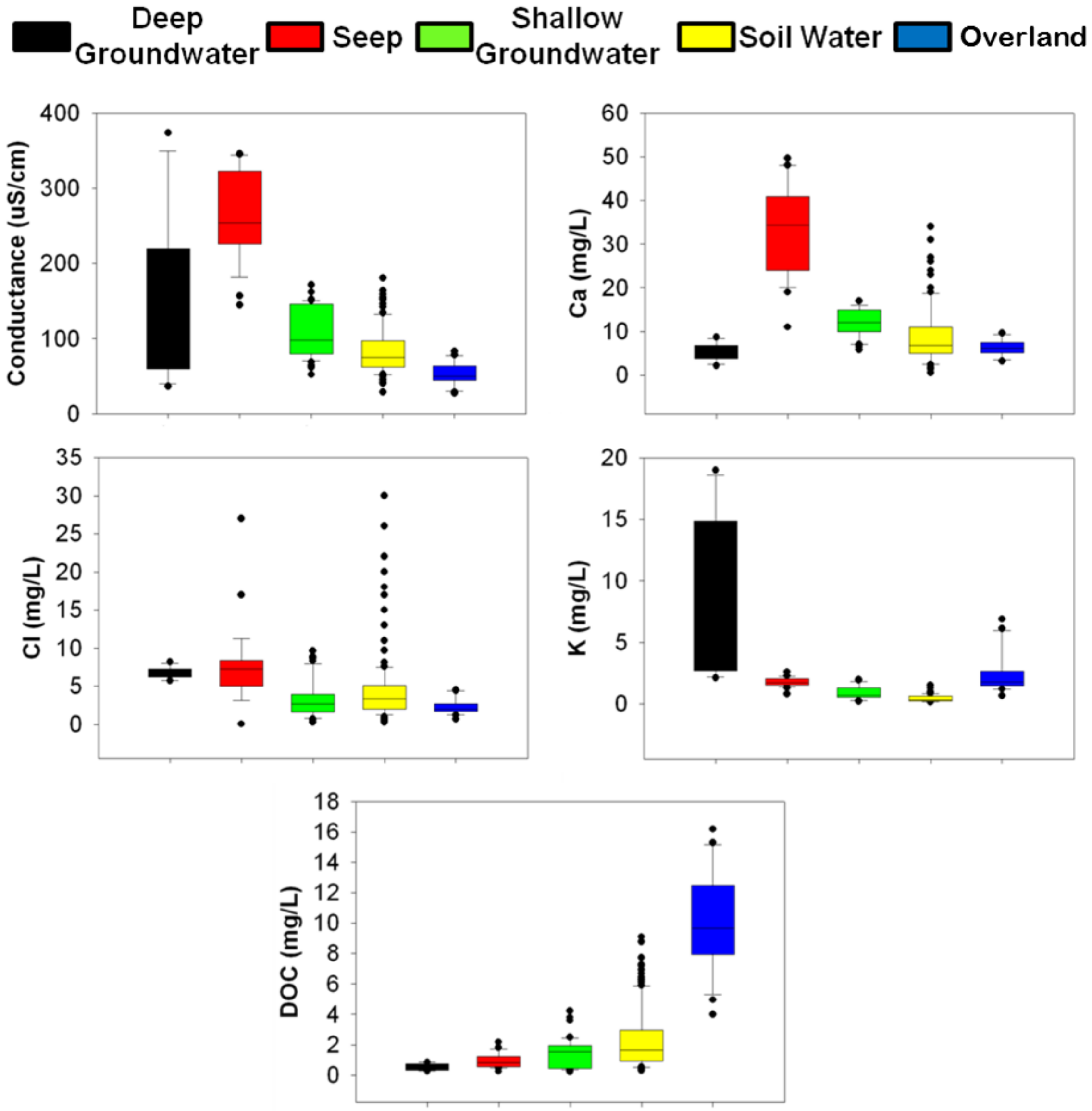


Figure 1.2

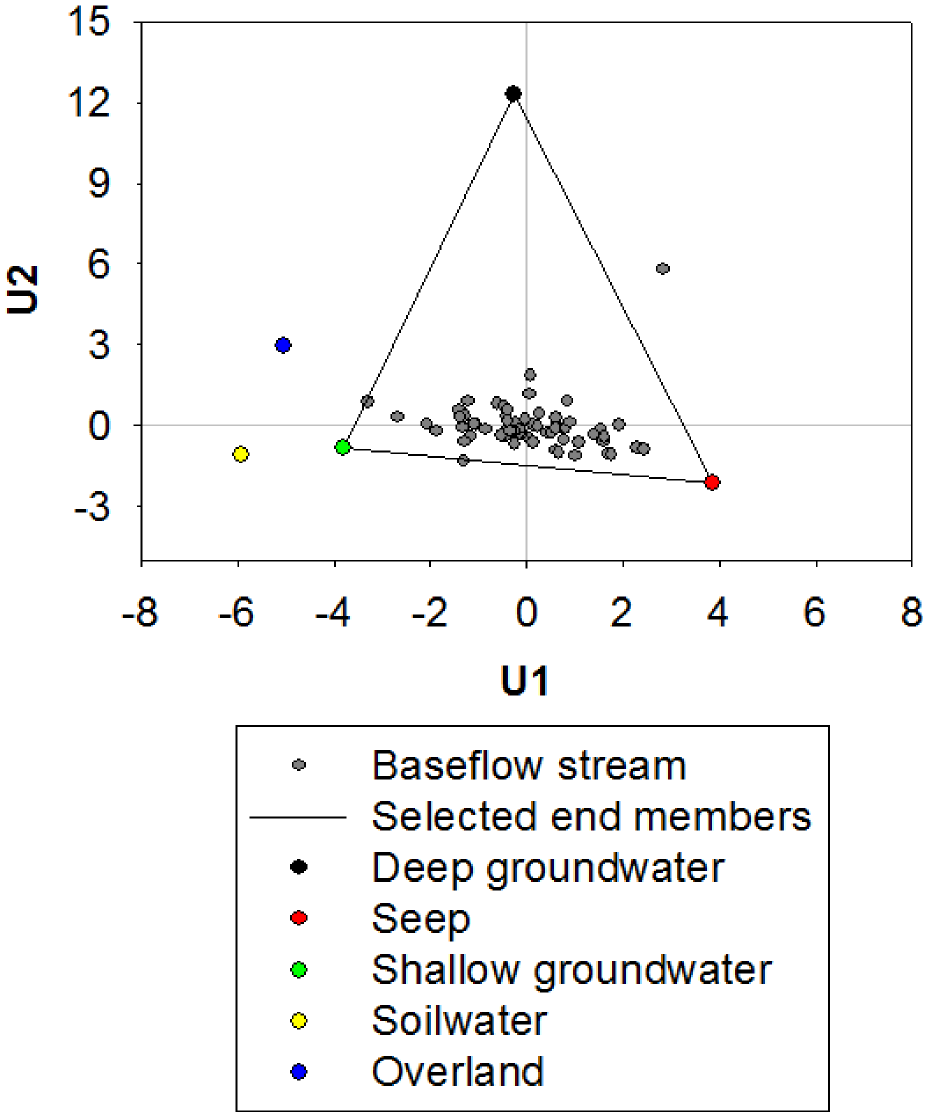

15
12
9
6
3
0
-3
U2
-8
-6
-4
-2
0
2
4
6
8
U1
Baseflow stream
Selected end members
Deep groundwater
Seep
Shallow groundwater
Soilwater
Overland

Figure 1.3

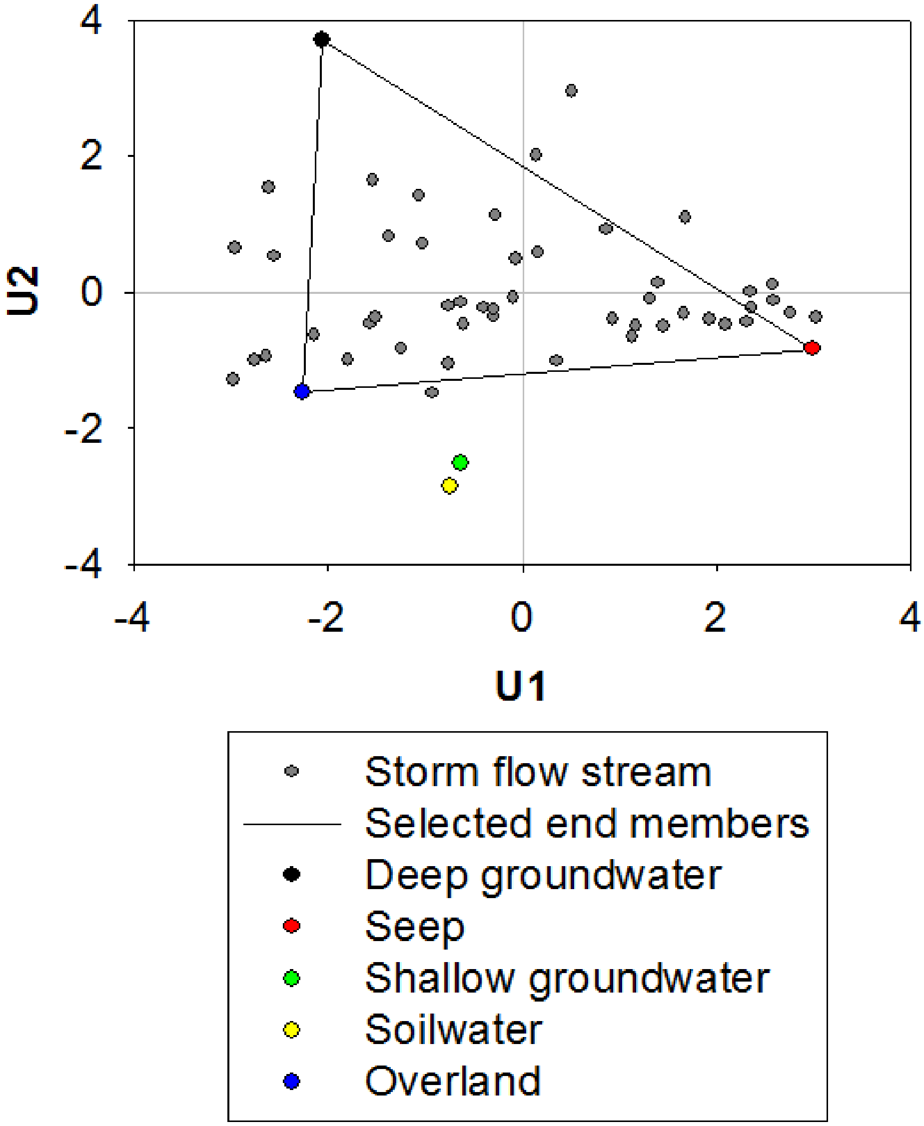

4
2
0
-2
-4
U2
-4
-2
0
2
4
U1
Storm flow stream
Selected end members
Deep groundwater
Seep
Shallow groundwater
Soilwater
Overland

Figure 1.4

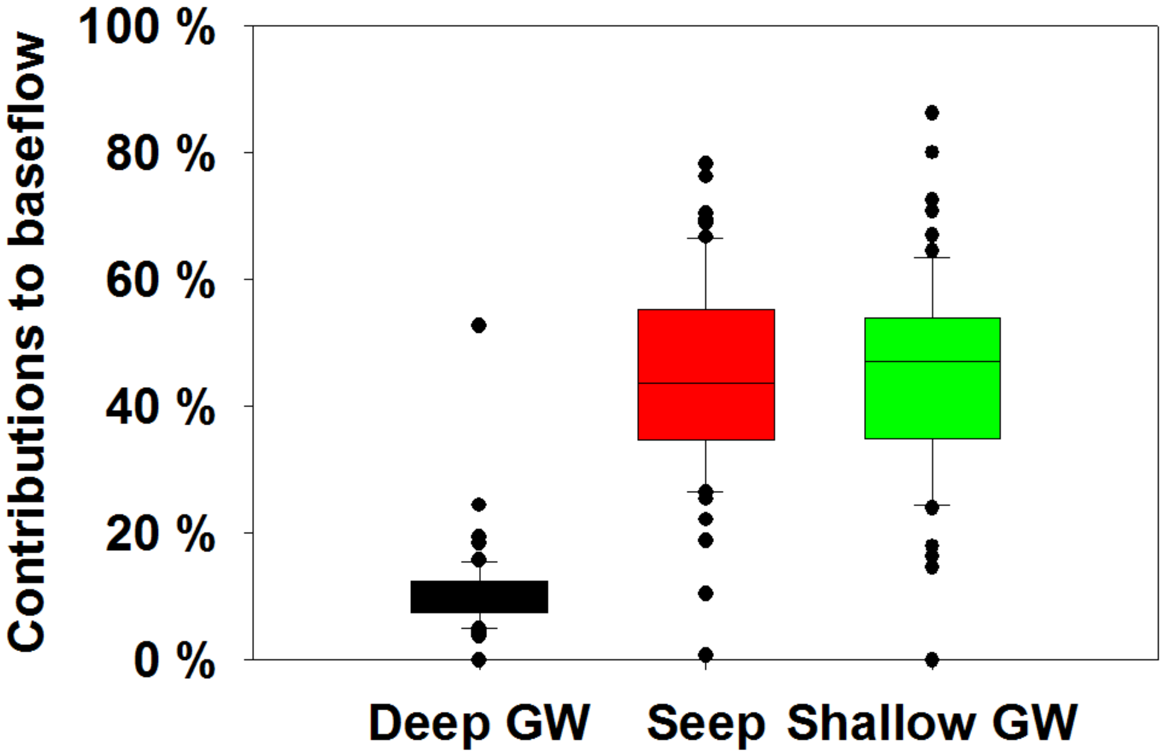

Contributions to baseflow
100 %
80 %
60 %
40 %
20 %
0 %
Deep GW
Seep
Shallow GW

Figure 1.5

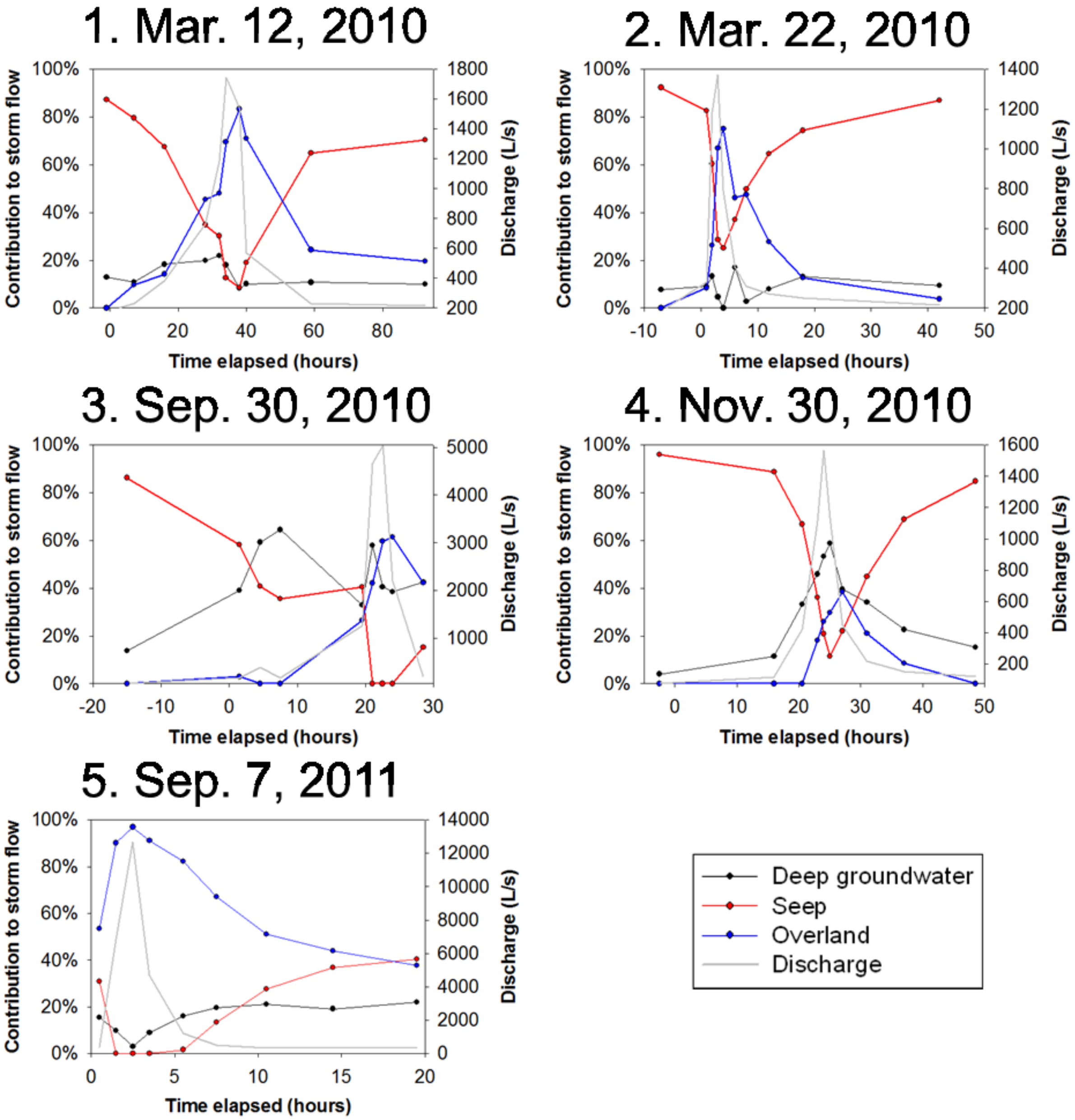

1. Mar. 12, 2010
2. Mar. 22, 2010
3. Sep. 30, 2010
4. Nov. 30, 2010
5. Sep. 7, 2011
Contribution to storm flow
0%
20%
40%
60%
80%
100%
Discharge (L/s)
Time elapsed (hours)
200
400
600
800
1000
1200
1400
1600
1800
0
20
40
60
80
-10
10
30
50
1000
2000
3000
4000
5000
-20
1600
14000
12000
10000
8000
6000
4000
2000
5
15
Deep groundwater
Seep
Overland
Discharge

Figure 1.6

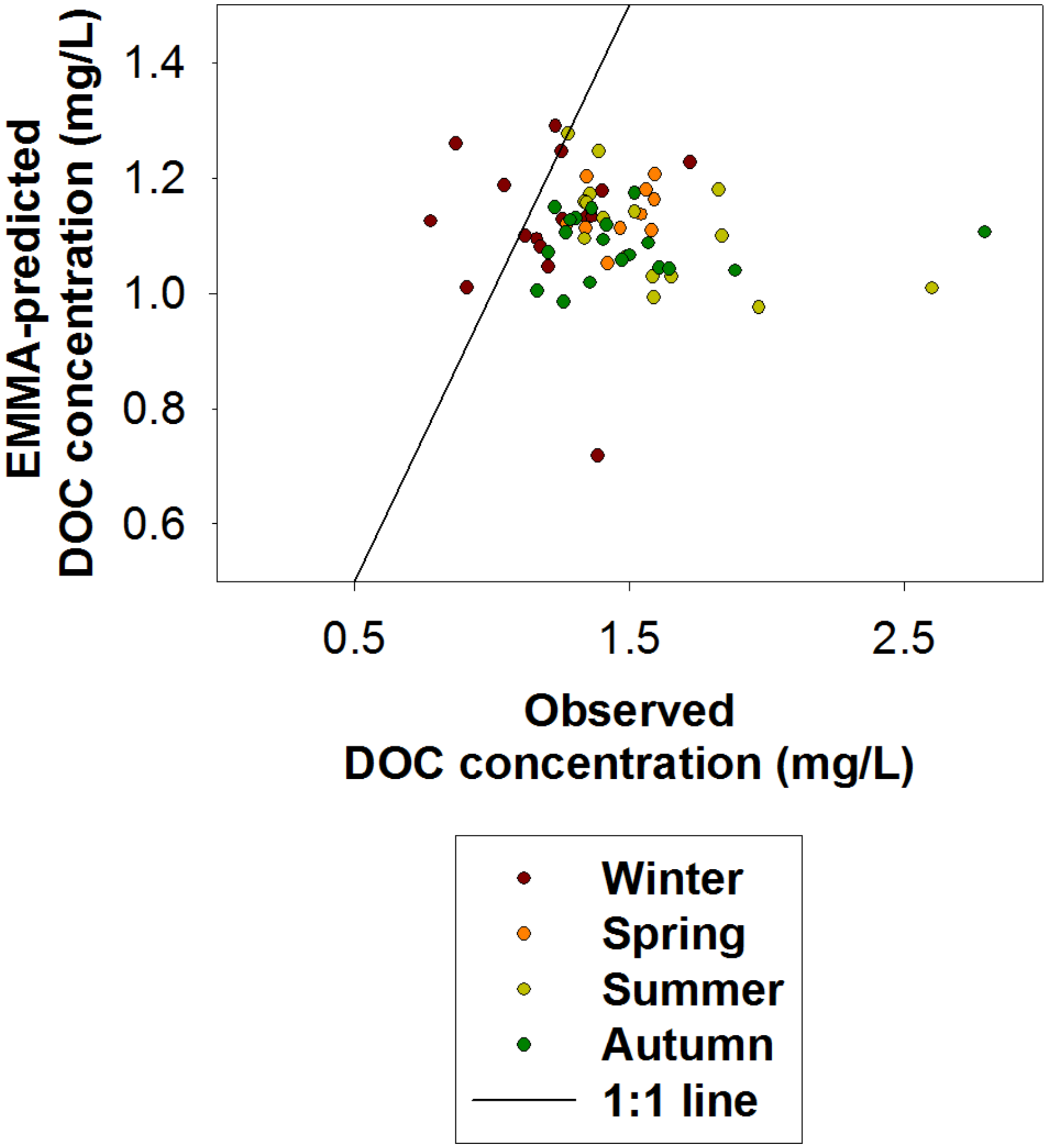

EMMA-predicted
DOC concentration (mg/L)
1.4
1.2
1.0
0.8
0.6
0.5
1.5
2.5
Observed
DOC concentration (mg/L)
Winter
Spring
Summer
Autumn
1:1 line

Figure 1.7

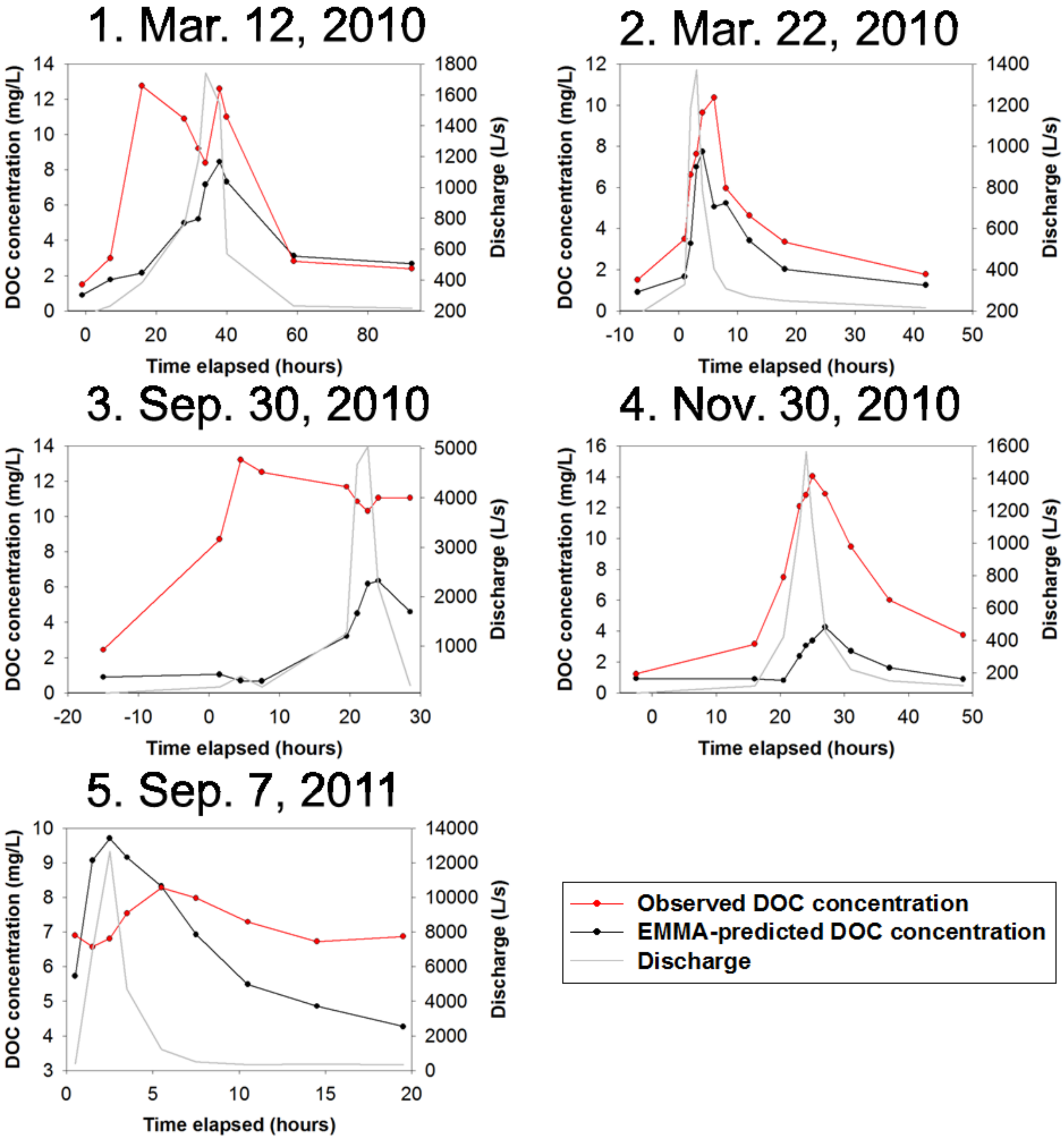
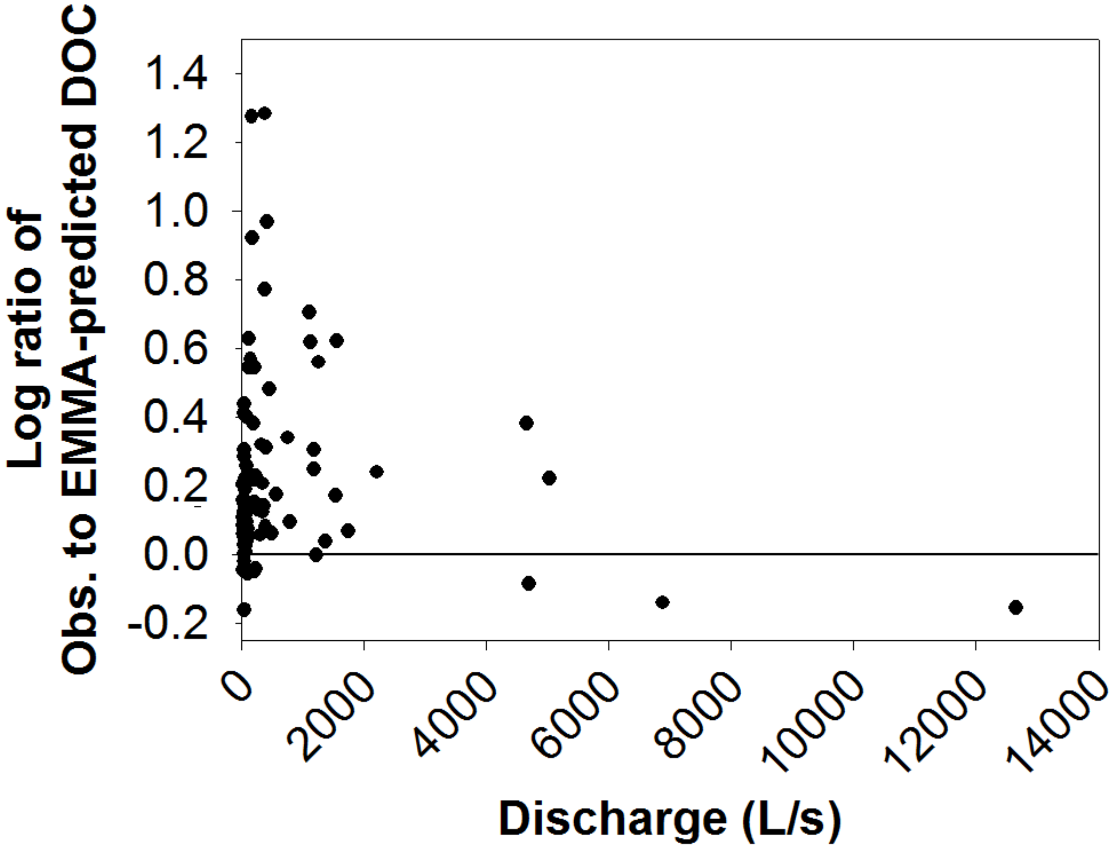

1. Mar. 12, 2010
DOC concentration (mg/L)
0
2
4
6
8
10
12
14
Discharge (L/s)
200
400
600
800
1000
1200
1400
1600
1800
Time elapsed (hours)
0
20
40
60
80
2. Mar. 22, 2010
DOC concentration (mg/L)
0
2
4
6
8
10
12
Discharge (L/s)
200
400
600
800
1000
1200
1400
Time elapsed (hours)
-10
0
10
20
30
40
50
3. Sep. 30, 2010
DOC concentration (mg/L)
0
2
4
6
8
10
12
14
Discharge (L/s)
1000
2000
3000
4000
5000
Time elapsed (hours)
-20
-10
0
10
20
30
4. Nov. 30, 2010
DOC concentration (mg/L)
0
2
4
6
8
10
12
14
16
Discharge (L/s)
200
400
600
800
1000
1200
1400
1600
Time elapsed (hours)
0
10
20
30
40
50
5. Sep. 7, 2011
DOC concentration (mg/L)
3
4
5
6
7
8
9
10
Discharge (L/s)
0
2000
4000
6000
8000
10000
12000
14000
Time elapsed (hours)
0
5
10
15
20
Observed DOC concentration
EMMA-predicted DOC concentration
Discharge

Figure 1.8


Log ratio of
Obs. to EMMA-predicted DOC
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
2000
4000
6000
8000
10000
12000
14000
Discharge (L/s)

CHAPTER 2

Biological lability of dissolved organic carbon in stream water and contributing terrestrial sources

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Abstract

Terrestrial sources supply dissolved organic carbon (DOC) to forested streams and this resource provides energy and C for stream microbial heterotrophs. Hydrologic conditions alter flow paths of terrestrial C to the stream. To quantify the influence of terrestrial inputs and watershed hydrology on stream biodegradable DOC (BDOC) concentrations, we measured BDOC concentrations in water from soils, shallow wells, spring seeps, overland flow, and a 3rd-order stream in the Pennsylvania Piedmont during baseflow and storm flow. Plug-flow bioreactors were used to determine the concentrations of total and labile BDOC with semi-labile BDOC calculated from the difference between the two. With these data, DOC was separated into three biological reactivity classes, labile BDOC, semi-labile BDOC, and a more recalcitrant fraction. From 2009 through 2011, stream DOC concentrations ranged from 0.8 to 15.1 mg C/L and total BDOC ranged from 0.1 to 8.7 mg C/L. Under baseflow conditions, DOC was generally less than 2 mg C/L and BDOC less than 0.7 mg C/L. The BDOC, expressed as a percentage of the DOC, was composed of 8.2% labile BDOC and 29.6% semi-labile BDOC constituents, while the majority of the DOC (66.2%) was a more recalcitrant class of non-biodegradable molecules. During storms, DOC concentrations increased approximately 6- to 12-fold and BDOC concentrations approximately 8- to 27-fold while the percentages DOC in labile and semi-labile biological reactivity classes increased approximately 2-fold to 17.3% and 55.4%, respectively, indicating a selective mobilization of and loading of BDOC. DOC concentrations within terrestrial sources sampled under base flow declined from 2.15 mg C/L in soil water to 1.78 mg C/L in shallow wells to 1.26 mg C/L in spring seeps. The labile BDOC concentrations in these sources also declined from 0.1 mg C/L in the soil water to 0.04 mg C/L in the shallow wells but increased to 0.13 mg C/L in the spring seeps. Overland flow waters during storms had elevated DOC (11.0 mg C/L) and labile BDOC (1.7 mg C/L) concentrations. Storm-associated peak concentrations for the two biological reactivity classes were coincident or nearly so during two storms but divergent during another storm, indicating variation among storms in the hydrologic pathways that deliver these molecules to the stream. Our results demonstrate the role of storms in delivering increased BDOC loads to a headwater stream, and for the first time, reveal the temporal variability of labile and semi-labile BDOC constituents. We argue that storms provide a subsidy for downstream reaches as hydrologic changes in stream depth and velocity increase the uptake lengths for the BDOC constituents approximately 50-fold and export them from the basin.

Keywords

dissolved organic carbon, bioavailability, lability, terrestrial sources, storm flow, labile, semi-labile, deciduous forested watershed

Introduction

In stream ecosystems, dissolved organic carbon (DOC) represents the largest pool of organic matter in transport. The majority of DOC flowing downstream comes from terrestrial sources which enter the stream through multiple flow pathways from the surrounding forested watersheds (Fisher and Likens 1973, Meyer et al. 1998). Storms generate new pathways and alter the dominance of different flow paths and the relative importance of different terrestrial sources. Storms export up to 86 % of the annual DOC flux from small eastern United States forested watersheds, primarily from allochthonous terrestrial organic matter (Royer and David 2005, Raymond and Saiers 2010) due to the activation of shallow pathways through C-rich soil horizons (Kaplan and Newbold 1993, Gremm and Kaplan 1998, Jones et al. 1996, Fellman et al. 2009a). Biotic and abiotic processes that occur as water moves to the stream channel affect the quantity and quality of DOC. Hydrology, therefore, influences a source of energy for heterotrophic bacteria in streams (Aitkenhead-Peterson et al. 2003, Kreutzweiser and Capell 2003, Kaplan et al 2008) and contributes to the regulation of heterotrophic microbial biomass and activity (Kaplan and Bott 1983, Bott et al. 1984) as well as nutrient cycling (Ziegler and Brisco 2004). In the work presented here, we characterized the composition of DOC from different terrestrial sources and described the changes in biodegradability under different hydrologic conditions to advance our understanding DOC biogeochemistry.

DOC in stream water consists of as many as 10,000 individual organic molecules (Kim et al. 2006; Hockaday et al. 2009) that range from defined monomers to more complex, humic substances with a broad spectrum of biological labilities. Stream DOC derived from soil organic matter and leaf litter leachate is often rich in humic substances (Moran and Hodson 1990) which still contribute to stream biodegradable DOC (BDOC) (Qualls and Haines 1992, Volk et al. 1997) and is augmented by autochthonous sources of DOC such as algal exudates, cell lysis, and by-products of grazing that are more readily degraded (Cole 1982, Kaplan and Bott 1982, 1989, Kragh and Sondergaard 2004). Due to the dominance of allochthonous sources in forested streams, our focus is on the contributions to stream water BDOC from terrestrial DOC delivered to the stream and the alterations that occur with changes in hydrology.

To understand the coupling between soil biogeochemical cycling, hydrology, and stream water DOC composition, we measured: (1) changes in the concentrations of stream DOC biological reactivity classes during storms and (2) the biological lability of DOC among terrestrial sources of water. Differences in DOC quality between storm flow and baseflow were expected because hydrologic flow paths change during storms, and terrestrial sources were expected to have high variability in DOC quality, shifting from relatively labile to more recalcitrant sources with soil depth. We define labile BDOC as molecules with turnover times of hours that are metabolized within the stream reach where they originated, semi-labile BDOC as molecules with turnover times of days that travel out of the reach and are transported downstream before being metabolized, and a more recalcitrant class of DOC molecules that have a longer but undetermined turnover time flowing through the river network without being metabolized (Kaplan et al. 2008) and may persist for decades or longer in the oceans (Keil et al. 1994, Carlson et al. 2002).

BDOC was quantified in stream water samples and terrestrial source water using plug-flow laboratory bioreactors of different residence times that were colonized and maintained with stream water to investigate changes in the fractions of labile, semi-labile, and more recalcitrant DOC. We documented dramatic increases in the concentrations and percentages of labile and semi-labile BDOC during storms, suggesting preferential flow through upper soil horizons rich in organic matter. This matched a pattern of declining terrestrial source water DOC and labile BDOC concentrations with increasing soil depth. We show for the first time, changes in the temporal variability of labile and semi-labile BDOC during storms which we attribute to the dynamic mixing of these different terrestrial source pools. Here we present evidence of increased BDOC loading to a headwater stream during storms and use estimates of the increase in uptake lengths associated with storm flows that increase stream depth and velocity to argue that storms provide a subsidy for downstream reaches as DOC and associated BDOC constituents are exported from the basin.

Methods

Study Site

The East Branch of White Clay Creek (WCC), located in the southeastern Pennsylvania Piedmont (39° 51’ 32.18” N, 75° 46’ 58.28” W), drains a catchment of 7.25 km^2 composed of deciduous woodlands, meadows, pastures, and agricultural lands that are underlain by metamorphic crystalline rock including gneiss, schist, quartzite, and marble. Soils are 1 to 2 m deep, unglaciated, and primarily *typic hapludults*, except in the riparian zone, where *aqui fagiudults* prevail. The total length of stream channels that run from the headwaters (elevation: 164 m) to the 3rd order stream reach (elevation: 100 m) is 12.9 km.

The 3rd-order stream is gauged with continuously recording automated pressure transducers (Telog and MiniTroll) that records stage height at 15-min intervals. Stream stage was converted to stream discharge using a rating curve developed from weekly discharge measurements made with the velocity-cross-sectional area method and a handheld Marsh-McBirney flow meter. Mean annual stream flow is 115 L/s, and water temperature averages 10.6°C (Newbold et al. 1997).

Precipitation data came from the U.S. Climate Reference Network (CRN) station “PA Avondale 2 N Stroud Water Research Center” operated by the National Oceanic and Atmospheric Administration located 250 m northwest of the 3rd order WCC stream site. The CRN station records real time precipitation and air temperature hourly. Annual precipitation averages 1.05 m/yr and is evenly distributed seasonally.

Field methods

Between 2009 through 2011, water was collected from the stream during baseflow and storm flow conditions and from terrestrial sources. Samples collected for this investigation included stream water from the 3rd-order section of WCC, unsaturated soil water, groundwater, spring seep water, and overland flow water (Figure 2.1). Baseflow grab samples were taken several times per week, including dates when the terrestrial installations were sampled. Storm water samples were collected hourly using an ISCO sampler during 67 individual storms. Soil water and shallow groundwater were collected within the riparian zone from tension lysimeters (*n* = 10, 42 mm ID PVC with round bottom ceramic cup, depths of 0.42 to 0.52 m) and shallow wells (*n* = 4, 10 cm OD PVC with screening in the bottom 20 cm, depths of 0.94 to 1.1 m). Deeper groundwater samples were collected as grab samples from spring seeps (*n* = 4) where groundwater surfaced in broad wetted areas within Worsham-silt loam on two dates during the summer of 2011. Overland flow was collected during six storms that occurred between January 2010 and August 2011 by either a grab sample or from a collector constructed based on the Low Impact Flow Event sampler designed by Sheridan et al. (1996). All water samples were filtered through precombusted glass fiber filters (Whatman GF/F) and refrigerated until DOC concentrations were measured using a Sievers 800 or 900 organic carbon analyzer.

Biological reactivity methods

BDOC was measured using plug-flow biofilm reactors consisting of chromatography columns filled with sintered glass beads that were kept in the dark in a temperature controlled room (20°C) and continuously fed from reservoirs of filtered WCC water in once through, up-flow mode at 4 mL/min. Filtration of WCC water to supply the reservoirs was performed with a three stage Balston glass fiber cartridge system of nominal 75-, 25-, and 0.3-μm filters in series. These tortuous-path filters remove larger particles, but allow 95% of the bacteria suspended in the stream water to pass into the filtrate (Kaplan and Newbold 1995). The reservoirs of WCC water, cleaned and refilled weekly, served as a continuous source of bacteria and DOC to colonize the bioreactors and maintain bacterial metabolism within the bioreactors.

Biological activity dominates the removal of DOC in colonized bioreactors, and BDOC is calculated as the difference between the DOC concentrations of the inflow and outflow waters. This bioassay method directly measures BDOC concentrations, or the fraction of DOC that can be biodegraded by a natural microbial assemblage under controlled conditions. The most biologically labile DOC molecules that would have fast turnover times and short uptake lengths in the stream are rapidly metabolized over short bioreactor residence times, semi-labile DOC molecules are metabolized after longer exposures, and the more refractory DOC molecules are exported from the bioreactors. Previous work where a ^{13}C-labeled tracer having labile and semi-labile constituents was run through these bioreactors and injected into WCC was used to scale the uptake in the bioreactors to the uptake of similar DOC lability components in the stream (Kaplan et al. 2008). The labile DOC metabolized in bioreactors with residence times of ≤ 3 min had a turnover time of 1.5 h in the stream and an uptake length of 200 m while the semi-labile DOC was metabolized in bioreactors with residence times of > 3 min had an estimated turnover time in the stream of 29 h and an uptake length of 4.5 km (Kaplan et al. 2008). In this work, a bioreactor with residence time of 0.5 min was used to estimate the labile DOC fraction, and a bioreactor with a residence time of 150 min was used to estimate the concentration of the total BDOC fraction; the semi-labile DOC was estimated from the difference between total BDOC and labile BDOC.

BDOC concentrations in stream water under baseflow conditions were determined on stream water that was Balston filtered into reservoirs on a weekly basis and pumped through the bioreactors. Bioreactor effluents were collected after three bioreactor bed volumes had passed to waste. A subsample of the reservoir water was filtered through a GF/F filters for DOC determinations. After collection, bioreactor effluent samples that passed through a 10 *μ*m bed support were analyzed unfiltered.

Samples with elevated DOC concentrations associated with storms and a few of the terrestrial source waters needed to be diluted prior to analysis as pulses of higher DOC concentrations than the bioreactor communities are normally exposed to would result in breakthroughs of DOC and would underestimate BDOC concentrations (Kaplan and Newbold 1995). Therefore, high concentration samples were diluted to approximately baseflow DOC concentration (1.4 mg/L) using the biologically stable effluent from the bioreactors with 150 min residence times that remove all of the BDOC. BDOC of the sample was then calculated based on the ratio of sample to biologically stable effluent in the inflow water.

Sample water from each terrestrial source and samples from 12 storms were analyzed for labile BDOC with a single bioreactor replicate, and stream water from three storms was also analyzed for total BDOC with three bioreactor replicates. Triplicate measurements were taken from each reactor. Inflow and outflow DOC concentrations were measured using a Sievers 800 or 900 organic carbon analyzer. Instantaneous mass fluxes of DOC, labile BDOC, and semi-labile BDOC were calculated as the product of stream discharge and stream water concentration using the data generated for the three storms in which DOC, total BDOC, and labile BDOC were measured.

Statistical analyses

Statistical analyses were conducted with JMP Statistical Discovery software (v 8.0.2/ 2009, SAS, Cary, NC). An analysis of variance (ANOVA) using a linear mixed-effects model with restricted maximum likelihood (REML) estimates was used to determine differences among samples. Tukey post-hoc analysis was then conducted to test all possible pairwise comparisons among means.

The concentration and lability of DOC in stream water samples were quantified and reported as mean ± standard error. Differences between pre-storm, post-storm, and peak storm concentrations of DOC and labile BDOC were examined by an ANOVA followed by Tukey post-hoc analysis. The relationships between the peak percentage of labile BDOC and storm event parameters, including antecedent moisture indexes and precipitation amounts, were examined through regression analyses. The labile BDOC data for the 12 storms were analyzed together to examine the pattern between discharge and DOC bioavailability. Storm data were divided into three categories based on discharge return interval: (1) low flow (< 200 L/s, 1.1 month return interval), (2) medium flow (200 L/s < Q < 1500 L/s), and (3) high flow (> 1500 L/s, 2.7 month return interval). Differences in DOC concentrations and percentages of labile BDOC among discharge categories were determined with an ANOVA followed by Tukey post-hoc analysis.

The within-storm temporal patterns in percentages of labile and semi-labile BDOC were determined from three storms, each representative of a different discharge category, and each storm was analyzed individually. The percentage of semi-labile BDOC was analyzed separately using a mixed-effects model with time as a fixed effect and bioreactor and an interaction term as random effects. To determine if the two biological reactivity classes differed in their response over time, the averages of the triplicate measurements for each of the bioreactors for each sample were analyzed using a fixed-effects model with time, biological reactivity class, and an interaction term.

The concentration and lability of DOC in terrestrial source waters were quantified and reported as mean ± standard error among source type. Baseflow terrestrial sources, including soil water, shallow well water, and seep water, were analyzed using a mixed-effect model with source type as a fixed effect and individual site as a random effect followed by Tukey post-hoc analysis.

Results

Stream water lability

Stream water DOC concentrations ranged from annual winter minima under baseflow concentrations of < 1 mg C/L to peak storm flow concentrations of 5 to 15.5 mg C/L, with baseflow concentrations generally ≤ 2 mg C/L and higher concentrations associated with storms (Figure 2.2). The median total BDOC concentration under baseflow conditions was 0.55 mg C/L and was composed of 0.43 mg C/L (29.6 %) semi-labile and 0.12 mg C/L (8.2 %) labile constituents. The balance of DOC, approximately 65% of the total was in the more recalcitrant class (Figure 2.2).

During storms, DOC and BDOC concentrations increased in stream water. The mean peak DOC concentration of 10.90 ± 0.60 mg C/L (range 6.44 to 12.84 mg C/L) was 3- to 4-fold greater than pre-storm (2.25 ± 0.50 mg C/L) or post-storm (3.98 ± 0.63 mg C/L) mean concentrations (ANOVA, $F_{(2,33)} = 74.34$, $p < 0.0001$) (Table 2.1). Labile BDOC increased approximately 12-fold from mean pre-storm concentration of 0.13 ± 0.02 mg C/L to a mean peak concentration of 1.51 ± 0.31 mg C/L (ANOVA, $F_{(2,33)} = 15.64$, $p < 0.001$), and the percentage of DOC that was labile BDOC increased 2-fold from a mean pre-storm value of 7.53 % ± 1.41 % to a mean peak value of 17.28 % ± 2.58 %. The peak percent labile BDOC values were significantly greater than both pre-storm and post-storm percent labile BDOC (ANOVA, $F_{(2,33)} = 7.05$, $p = 0.0028$). The storms sampled were distributed across all seasons with total precipitation ranging from 21 to 168 mm, peak discharge ranging from 130 L/s to 13,780 L/s, and antecedent moisture conditions ranging from near drought levels with a pre-storm discharge of 50 L/s to near saturated levels with a pre-storm discharge of 250 L/s. The antecedent moisture index for the 7 days prior to the storm (API_7) was inversely proportional to the peak percent labile BDOC ($\beta = 0.0017$, $t_{(10)} = -2.24$, $p = 0.049$) and explained a significant proportion of the variance ($r^2 = 0.33$, $F_{(1,10)} = 5.00$, *p*=0.049). No other storm event parameters were significant. There was a variable relationship of discharge peaks to the peaks in DOC and BDOC concentrations with no consistent hysteresis direction across storms (Table 2.1).

The relationships of stream water DOC and labile BDOC concentrations to discharge exhibited different patterns with DOC increasing with increasing discharge and BDOC concentrations peaking at medium discharges. Mean DOC concentrations at flows < 200 L/s (5.12 ± 0.50 mg C/L) differed from DOC concentrations for flows between 200 and 1500 L/s (7.84 ± 0.52 mg C/L) and flows greater than 1500 L/s, (8.99 ± 0.35 mg C/L) (ANOVA, $F_{(2, 100)} = 10.14$, $p < 0.0001$). Mean labile BDOC concentrations (and percentages of labile BDOC) increased from 0.40 ± 0.05 mg C/L (7.48 % ± 0.65 %) at low discharges to 1.11 ± 0.16 mg C/L (13.40 % ± 1.37 %) at medium discharges and declined to 0.67 ± 0.13 mg C/L (7.14 % ± 1.22 %) at high discharges (Figure 2.3). The percent labile BDOC was significant different among discharge categories (ANOVA, $F_{(2,100)} = 10.14$, $p < 0.0001$) which was driven by the medium discharge mean compared to the low flow mean (Tukey's test, $p = 0.0002$) and to the high discharge mean (Tukey's test, $p = 0.0082$).

The within-storm temporal patterns in labile and semi-labile BDOC concentrations were determined for three storms, each representing a different discharge category. The low flow storm (Figure 2.4, July 25- July 26, 2011) had the lowest peak discharge (130 L/s, 1 month return interval) and occurred under the driest conditions (16 days with no rain). DOC concentration increased 6-fold from 1.77 mg C/L before the storm to 10.54 mg C/ L at the peak of discharge.

The decline in DOC concentrations lagged behind discharge, remaining elevated (5.42 mg C/L) after discharge had declined to 57 L/s, or 22 % above the pre-storm discharge of 47 L/s. The concentrations of labile and semi-labile BDOC increased significantly over time in synchrony with the DOC. Labile BDOC increased 7.6-fold from 0.17 ± 0.00 mg C/L (10.18 % ± 0.30 %) at baseflow to a peak of 1.30 ± 0.04 mg C/L (15.34 % ± 0.43 %), and semi-labile BDOC increased 7.9-fold from 0.52 ± 0.01 mg C/L (31.90 % ± 0.37 %) at baseflow to a peak of 4.03 ± 0.28 mg C/L (58.52 % ± 0.80 %). Percentages of labile and semi-labile BDOC were significantly different over time (ANOVA, $F_{(6,11)} = 3.68$, $p = 0.030$ (labile); REML fixed effects test, $F_{(6,43)} = 74.49$, $p < 0.0001$ (semi-labile)). The REML variance component estimates for the semi-labile percentages showed greater variance among replicate reactors (65.8 %) than among triplicate measurements (34.2 %). A comparison of the percentages for the two biological reactivity classes over time showed significant differences through an interactive effect between biological reactivity class and time (ANOVA, $F_{(6, 13)} = 4.77$, $p = 0.0088$).

The medium flow storm (Figure 2.5, November 29-December 1, 2010) had a peak discharge of 1448 L/s (2.6 month return interval). DOC concentration increased 11.5-fold from 1.22 mg C/L before the storm to 14.04 mg C/L just after the discharge peak. Post storm concentration remained elevated (3.75 mg C/L) nearly 12 hours after the peak discharge when flows had declined to 120 L/s, or 50 % above the pre-storm discharge of 80 L/s. Concentrations of both biological reactivity classes increased, and percentages were significantly different across time (ANOVA, $F_{(9,20)} = 52.72$, $p < 0.0001$ (labile); REML fixed effects test, $F_{(9,16)} = 102.16$, $p < 0.0001$ (semi-labile)). The labile BDOC concentration (and percentage) at baseflow of 0.09 ± 0.00 mg C/L (7.93 % ± 0.22 %) increased 27-fold to a peak concentration of 2.46 ± 0.02 mg C/L (19.30 % ± 0.17 %), and the semi-labile BDOC concentration (and percentage) at baseflow of 0.31 ± 0.01 mg C/L (25.56 % ± 0.57 %) increased 22-fold to a peak concentration of 6.69 ± 0.09 mg C/L (52.24 % ± 0.01 %). The variance for the semi-labile percentages among triplicate measurements (19.30 %) was less than the variance among replicate reactors (39.86 %) and the variance due to differences among the interaction of time and reactor (40.84 %) based on the REML estimates. The patterns of concentration changes for the two biological reactivity classes and DOC were nearly coincident, though semi-labile BDOC peaked first and in synchrony with the discharge peak, while the labile BDOC and DOC peaked just after peak discharge. Semi-labile BDOC concentrations remained nearly constant over a six hour period on the falling limb of the storm hydrograph. These differences were significant over time (ANOVA, $F_{(9,20)} = 10.48$, $p < 0.001$).

The high flow storm (Figure 2.6, September 29 – October 1, 2010) had the highest peak discharge of 5,039 L/s (11 month return interval) and occurred following a very dry summer with a pre-storm baseflow of 40 L/s. The storm was comprised of two separate events that generated two discharge peaks with the larger peak preceded by a smaller 427 L/s peak approximately 18 hours earlier. DOC concentrations increased 6-fold from a pre-storm concentration of 2.44 mg

C/L to a peak of 12.51 mg C/L just after the first discharge peak, remained elevated above 10 mg C/L through the second discharge peak, and declined to 6.80 mg C/L after a further decline in discharge to 100 L/s. Both biological reactivity classes increased and were significantly different across time. The labile BDOC concentration (and percentage) at baseflow of 0.14 ± 0.01 mg C/L (7.66 % ± 0.33 %) increased 12-fold to a peak of 1.68 ± 0.0 mg C/L (17.33 % ± 0.00 %) that coincided with the larger second discharge peak while the semi-labile concentration (and percentage) of 0.73 ± 0.01 mg C/L (29.80 % ± 0.57 %) at baseflow increased 8-fold to a peak concentration of 5.93 ± 0.04 mg C/L (53.67 % ± 0.35 %) that occurred just after the first discharge peak. There were significant differences over time in the percentages of labile BDOC (ANOVA, $F_{(10,20)} = 29.56$, $p < 0.0001$) and the percentages of semi-labile BDOC (REML fixed effects test, $F_{(10,72)} = 74.24$, $p < 0.0001$). The REML variance component estimates for the semi-labile percentages showed greater variance among triplicate measurements (76.9 %) than among replicate reactors (23.1 %). A comparison of the two biological reactivity classes over time showed significant differences in the percentages through an interactive effect between biological reactivity class and time (ANOVA, $F_{(10, 22)} = 21.92$, $p < 0.0001$).

The peak instantaneous mass flux for DOC, labile BDOC, and semi-labile BDOC associated with each of the three storms occurred at or near the peak discharge for the storms. For each constituent, peak mass flux increased with the magnitude of the storm. The peak mass flux of DOC, semi-labile BDOC, and labile BDOC equaled 1,214 mg C/s, 550 mg C/s, and 130 mg C/s, respectively for the low flow storm, 18,560 mg C/s, 9,700 mg C/s, and 2,597 mg C/s, respectively for the medium flow storm, and 50,661 mg C/s, 25,331 mg C/s, and 7,839 mg C/s, respectively for the high flow storm.

Terrestrial source lability

Mean DOC concentrations in potential baseflow terrestrial sources declined with increasing soil depth from 2.15 ± 0.23 mg C/L (*n* = 23, 1.16 to 4.89) in soil waters to 1.78 ± 0.19 mg C/L (*n* = 14, 1.26 to 4.15 mg C/L) in shallow riparian wells to 1.26 ± 0.11 mg C/L (*n* = 8, 0.82 to 1.87 mg C/L) in spring seeps, but were not significantly different (Figure 2.7, ANOVA, $F_{(2, 42)} = 2.94$, $p = 0.064$, Tukey's test of soil vs. seep $p = 0.056$). Water collected as overland flow during storms had the highest DOC concentrations with a mean of 11.01 ± 0.97 mg C/L (*n* = 14, 4.96 to 17.85 mg C/L).

The concentrations (and percentages) of labile BDOC also declined from 0.10 ± 0.02 mg C/L (4.34 % ± 0.50 %) in soil water to 0.04 ± 0.00 mg C/L (2.38 % ± 0.43 %) in shallow groundwater, but were higher in spring seep waters with a mean of 0.13 ± 0.02 mg C/L (10.01% ± 1.46 %) (Figure 2.7). Mean labile BDOC percentages were significantly different among the baseflow terrestrial sources (ANOVA, $F_{(2, 42)} = 23.01$, $p < 0.0001$), and the significance was driven by the higher values for spring seeps (Tukey's test of seep vs. soil $p < 0.0001$, seep vs. well $p < 0.0001$). The fractions of labile BDOC in waters from the lysimeters and wells were not statistically different (Tukey's test of soil vs. well $p = 0.074$). Overland flow waters, the terrestrial source collected during storms, had a mean labile BDOC concentration of 1.70 ± 0.29 mg C/L (15.06 % ± 2.06 %).

Discussion

Stream water lability

The concentration and quality of DOC in stream water has important implications for ecological processes because of its role in regulating stream metabolism and nutrient cycling. Numerous studies have demonstrated that storm flows typically account for the majority of annual DOC export (Jones et al. 1996, Hinton et al. 1997, Fellman et al. 2009a). Much less is known about how the biological lability of DOC changes during storms. Storms alter the dominant runoff pathways, so concentration and compositional changes in stream water DOC occur during periods of high discharge (McDowell and Likens 1988, McClain et al. 2003, Saunders et al. 2006). The effect of storms on BDOC concentration of stream water, however, is variable (Gremm and Kaplan 1998, Neff et al. 2006), with results showing a decrease in BDOC concentrations (Leff and Meyer 1991, Wiegner et al. 2009), increases in labile DOC sources (Sanderman et al. 2009), or no change in the fraction of BDOC compared to baseflow (Buffam et al. 2001).

Changes in both DOC concentration and composition during storm flow are indicative of the hydrological influences on the biogeochemistry of DOC in stream water. DOC concentrations changed quickly during storms with substantial increases on the rising limb of the hydrograph similar to many previous studies (Hinton et al. 1997, Inamdar 2007, 2011, Buffam et al. 2001). The large variations in DOC and % BDOC among storms reflect the high spatial and temporal variability in source DOC in combination with dynamic changes in the relative contribution from each source. The increases in DOC and BDOC of stream water during storms was most likely due to changes in source contributions and flow paths and alterations of the hydrologic connectivity due to differences in storm event parameters.

Dynamic changes in source contributions also cause large variations in the composition of stream water DOC during storms. In this study, labile BDOC increased with discharge between low flow and medium flow conditions further suggesting an increase in runoff contributions from surficial sources, a source pool that has large variations in BDOC. However, our finding that labile BDOC decreased during high flow conditions indicates that terrestrial source contributions during larger storms dilute the surficial sources. Peak concentrations occurring on the rising limb of the hydrograph are indicative of DOC dilution of watershed sources because concentrations begin to taper off despite minimal changes in flow paths (Sanderman et al. 2009). The pattern of peak labile BDOC occurring before the discharge peak during large storms further supports the possibility of dilution of the labile BDOC source. In contrast, semi-labile BDOC did not show the same dilution effects during high flow conditions, and the magnitude of the peak in semi-labile BDOC was similar among the three storms with different discharge magnitudes. The different within-storm temporal patterns observed in the two biological reactivity classes indicate differences in the source pools for each class. These results together suggest that the source pool for labile BDOC is surficial and diluted during larger storms, but surficial and deeper sources contribute to the export of semi-labile BDOC from the terrestrial environment to the stream.

Modeling of storm water sources in a similar forested headwater catchment in the mid-Atlantic Piedmont using chemistry data indicated that contributions from different sources occur in a specific order leading to throughfall and litter contributions impacting the rising limb of the hydrograph while litter and soil water impacted the early part of the recession limb (Inamdar et al. 2011). Therefore, the proportional contribution of water from different terrestrial source pools has a large impact on the composition of DOC delivered to the stream during storms. The increase in DOC transport that occurs during storms likely overwhelms the soil degradation processes resulting in delivery of DOC that has bypassed much of the terrestrial processing.

Storm event parameters and antecedent moisture conditions can also influence the processing of DOC and the connectivity of the terrestrial environment with the aquatic ecosystem. For example, storms that occurred during drier antecedent moisture conditions had higher peak labile BDOC. Additionally, larger variability in the composition of exported DOC was seen during storms with lower API_7 due to the divergent effects of dry antecedent conditions which include either hydrologic fragmentation that requires extensive infiltration before the appearance of source water in stream flow or hydrologic connectivity of isolated terrestrial sources that have accumulated high concentrations of DOC during dry periods prior to the storm (Guggenberger et al. 1998). Long dry periods have been shown to promote the breakdown of organic matter in surficial soils resulting in labile organic matter (Borken and Matzner 2009). However, large amounts of precipitation may be required to reconnect these terrestrial source pools. Once connectivity is reestablished, substantial increases in DOC and BDOC concentrations would be expected as these once dry locations become hydrologically connected. When hydrologic connectivity has been restored, additional rain associated with higher antecedent moisture conditions would result in substantial contributions of storm water yielding a dilution pattern.

Terrestrial source lability

The decrease in DOC concentrations with increasing soil depth of terrestrial source waters has been observed previously and attributed to chemical sorption and bacterial activity as water percolates downwards (McDowell and Likens 1988, Qualls and Haines 1992, Guggenberger and Zech 1993, Guggenberger et al. 1998). Biotic activity and abiotic leaching of plant detritus control the production of DOC in the surface soil (McDowell and Likens 1988) with topsoil acting more as a DOC source than a sink due to negligible sorption (Guggenberger et al. 1998). The slow process of microbial respiration contributes little to the retention of DOC in the subsoil but is critical to maintaining sites for sorption (Jandl and Sollins 1997). The large variation in DOC concentrations among and within terrestrial sources reflects high spatial and temporal variability in DOC mobilization and immobilization within the terrestrial environment.

The variability in biotic and abiotic processes that serve to mobilize or immobilize DOC within the terrestrial environment also influences the composition of DOC and the fraction of different DOC constituents. The high fraction of labile BDOC within the overland flow samples and the decline observed from soil water to shallow ground water mirror the total DOC patterns, but the increased biological lability of spring seep waters does not. There was no significant difference between unsaturated soil water and shallow riparian well water possibly due to the high mobility of DOC in the upper parts of the mineral source resulting in minimal changes in composition (Guggenberger et al. 1998). As water moves down into the mineral soil, selective sorptive removal occurs due to different affinities of DOC constituents to aluminum and iron oxides (Guggenberger et al. 1998). Sorption experiments have shown preferential removal of aromatic C but more mobile sugars passing through the mineral soil to a greater extent than other DOC constituents (Gu et al. 1994, Guggenberger et al. 1998). This may explain the higher lability measured in our seep water. Alternatively, because sample water was collected from the output of the aquifer, the seep water may have mixed with near surface sources of labile carbon before being released into the channel. Collectively, the processes occurring in the terrestrial environment cause high variability in DOC quality and quantity among sources which is then reflected in the water that is delivered to the stream channel.

Baseflow DOC concentrations were between the DOC concentrations measured in the two groundwater sources, shallow riparian wells and seeps, and less than the concentrations in the source waters of precipitation dependent flow paths, overland flow and unsaturated soil water. Autochthonous sources also might contribute to stream water DOC, but the release of DOC from in-stream sources should be minimal due to low primary production in small forested watersheds (Fisher and Likens 1973, Kaplan and Bott 1982). While it is often assumed that baseflow is sustained through groundwater discharge, the chemistry of groundwater can significantly differ from stream baseflow suggesting that a mixture of multiple end-members supply the stream (Inamdar and Mitchell 2007). For example, Scalon et al. (2001) found that groundwater contributions are underestimated if groundwater is assumed as the only source for pre-storm baseflow. However, end-member mixing analyses that use natural geochemical variations in distinct flow path pools to identify relevant water reservoirs that contribute to stream flow (Christophersen et al. 1990) indicate that stream water during baseflow is chemically similar to groundwater sources (Hooper 2001, Inamdar et al 2011).

The biological reactivity of the two groundwater sources also bounded the lability of baseflow further supporting these two terrestrial sources as the main contributors during periods of low flow. Since terrestrial DOC is usually less bioavailable than in-stream sources such as algal exudates (Sun et al. 1997), the low % BDOC of stream water during baseflow further supports limited contributions from autochthonous sources of DOC. However, these small contributions from in-stream sources of labile DOC may play an important role by enhancing decomposition of less labile terrestrial DOC through priming effects

(Bianchi 2011). The lower % BDOC of baseflow and the shallow groundwater source can be attributed to removal of labile carbon by sorption or alteration of DOC by microbial processes while in transit (Qualls and Haines 1992, Guggenberger et al. 1998, Inamdar et al. 2011). The variability in baseflow lability could be due to higher variability in the lability of seep water. The larger variability in seep water DOC composition was expected since flow through karst terrain varies substantially in connectivity and transit time, and previous studies have found large differences in DOC concentration and character at the basin scale (Simon et al. 2010).

The proportional contributions of water from different terrestrial source pools have a large impact on the concentration and composition of DOC delivered to the stream during storms. Increases in DOC concentrations during storms have been attributed to the activation of precipitation dependent flow paths, increases in the hydrologic connectivity between compartments isolated during dry periods, and flushing of shallow soil zones that can accumulate high concentrations of DOC during periods between storms (Hornberger et al. 1994, Creed and Band 1998, McDonnell 2003). Storms with the highest DOC concentrations typically show the largest shift towards surficial source contributions (Inamdar et al. 2011). However, during five of the 12 storms sampled, the peak stream DOC concentration was higher than the average concentration measured in overland flow as well as the other terrestrial sources.

This discrepancy suggests contributions from additional terrestrial sources, although overland flow was collected from a limited number of locations and we do not know how this component varies spatially. Other potential sources including throughfall and stemflow have been identified as a significant source for storm runoff, but concentrations vary among sites and seasons with mean concentrations measured in forested watersheds ranging from 2.2 to 35 mg/L (Hinton et al. 1998, Michalzik et al. 2001, Inamdar and Mitchel 2007, Wu et al 2010, Levia et al. 2011), which could account for the difference between overland flow concentrations and peak storm flow concentrations. It is unlikely that scouring of the stream bed during storms generated much of the storm flow DOC load as the DOC peak concentrations all occurred later than the initiation of bed scour and the area of streambed relative to the drainage area for the stream is small.

The composition of terrestrial DOC delivered to the stream depends on biological transformations (Kaplan and Bott 1983) and abiotic sorption (McDowell and Likens 1988) that occur along the dominant flow pathways. For example, increases in soil biotic demand and higher rates of DOC consumption during passage through the watershed result in lower quality DOC (Michaelson et al. 1998, Fellman et al. 2009b). Furthermore, land use alters the quality of DOC delivered to streams with agriculturally dominated watersheds exporting more labile carbon than wetland dominated (Williams et al. 2010) or forested dominated watersheds (Boyer and Groffman 1996). Residence time and conditions for degradation along flow paths influence the biological reactions that alter the BDOC of terrestrial source waters. The abiotic and geochemical processes which influence the composition of DOC in terrestrial environments result from the large affinity of DOC for sorption to positively charged mineral surfaces in deeper soil layers (McDowell and Likens 1988, Qualls and Haines 1992, Kaplan and Newbold 2003). DOC sorption to soil minerals can increase DOC retention within the soil or result in rapid turnover by biofilms associated with soil surfaces (Sollins et al. 1996, Guggenberger and Kaiser 2003).

In-stream dynamics

Once DOC enters the stream channel, further metabolism and additional abiotic mechanisms can influence the composition of the stream water DOC pool. Microbial communities, especially heterotrophic bacteria, dominate the biological uptake of DOC, which accounts for most of the DOC removal from the water column (Dahm 1981, Tank et al. 2010). Because uptake of nitrogen and phosphorous accompanies the degradation of DOC, nutrient limitations decrease DOC mineralization rates resulting in the accumulation of BDOC with different turnover times (Kragh and Sondergaard 2004). Abiotic sorption also removes a substantial amount of DOC from the water column (McDowell 1985, McKnight et al. 2002), and the preferential sorption of nitrogenous compounds to mineral surfaces in transport can alter the DOC composition (Aufdenkampe et al. 2001). Preservation of labile organic matter due to sorption to mineral surfaces can slow remineralization rates up to five orders of magnitude (Keil et al. 1994). Abiotic sorption occurs very rapidly (Dahm 1981), but biological metabolism results in the uptake of labile DOC near the point of entry to the stream exporting less labile DOC to downstream reaches (Wetzel et al. 1995, Kaplan and Newbold 2003, Kaplan et al. 2006). Photolysis of DOC can result from exposure to UV radiation, but the effects of photolysis on BDOC constituents vary with the chemistry of the source materials. For example, terrestrial DOC exhibited higher photoreactivity than algal derived DOC (Wetzel et al. 1995, Kaiser and Sulzberger 2004), but photolysis of terrestrial DOC increased bioavailability whereas photolysis of autochthonous DOC decreased bioavailability (Kaiser and Sulzberger 2004, Sulzberger and Durisch-Kaiser 2009).

Understanding how the quality of terrestrial DOC influences stream heterotrophic respiration is important for determining global and regional carbon budgets because in addition to transporting DOC to the oceans, stream respiration also releases a significant amount of C to the atmosphere (Richey et al. 2002, Butman and Raymond 2011). Stream water carbon dioxide (CO_2) originates from export of dissolved soil CO_2, acidification of buffered water, precipitation of carbonate minerals, photosynthesis, and oxidation of organic matter including DOC. The source of stream water dissolved CO_2 varies among and within watersheds, but analyses of stable isotopes have indicated that respiration of allochthonous DOC can be a dominate source of CO_2 (Dubois et al. 2010). However, Butman and Raymond (2011) estimated that only 3.6% (3.56 Tg C/ yr) of the annual CO_2 evasion flux from rivers within the conterminous United States was a result of respiration of allochthonous DOC. The recent inclusion of CO_2 efflux from streams in assessments of the global carbon budgets requires an understanding of the relative importance of CO_2 sources, including the metabolism of DOC which based on our results may play a much more significant role in CO_2 efflux from freshwater than commonly appreciated.

Storms may subsidize heterotrophic respiration by overwhelming terrestrial controls on DOC processing, mobilizing previously unavailable sources, and delivering terrestrial DOC to the stream. Increases in bacterial growth rates and abundance have been measured with laboratory bioassays to test the biological reactivity of storm water DOC compared to baseflow DOC (Buffam et al. 2001). Through a combination of increased abundance and growth rates, it was suggested that the assimilation of storm flow DOC could be 10 times faster than baseflow DOC, but no measurements with water column bacteria resuspended by storms were made, so it is not known how scouring impacts the physiology of benthic bacterial populations that are placed into suspension (Buffam et al. 2001). However, there are other reasons why potential increases in bacterial productivity may not be realized in upstream reaches. Increases in suspended bacterial abundances are transient and DOC availability to the native assemblage may be lower despite the increase in DOC quality (Leff and Meyer 1991). Additionally spiraling theory shows that uptake length for a given molecule is directly proportional to stream water depth and velocity (Newbold 1992). In WCC, storms typically increase water velocity from 0.1 m/s to 0.5 m/s and water depth from 0.1m to 1 m. Without factoring in the decrease in mass transfer coefficients associated with the metabolic demands of disturbed benthic communities, this 50-fold increase based solely on hydrology would be expected to increase the uptake length of labile BDOC from 117 m to 2.34 km and the uptake length of semi-labile BDOC from 4.8 km to 240 km, thus transporting the DOC constituents out of the reach, and in some cases into the estuary.

The quality of DOC plays a significant role in determining DOC processing in streams, and therefore, changes in hydrologic flow paths that result in variations in BDOC influence stream ecosystem metabolism. Interactions between water, soil, and microorganisms determine the lability of DOC exported from the terrestrial environment. The balance between production and retention processes results in terrestrial sources functioning as net sinks or net sources of DOC. Subsequent carbon processing within the stream causes losses in terrestrially-derived carbon through microbial respiration and out gassing of carbon dioxide. Integrating stream ecosystem models into a catchment framework requires linking the water cycle to biogeochemical cycles.

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Table Legends

Table 2.1: Storm event parameters, DOC concentration, and percentage of labile BDOC for stream water collected from a 3rd order site of White Clay Creek in southeastern Pennsylvania during 12 storms. Both DOC concentration and the fraction of labile BDOC significantly increased from pre-storm values to peak values with the timing of both peaks differing among storms.

Tables

Table 2.1

	Date	total precip (mm)	$API_{7\,a}$ (mm)	$API_{30\,a}$ (mm)	Peak Q (L/s)	Pre-Storm DOC (mg/L)	Peak DOC (mg/L)	timing of DOC peak	Pre-Storm labile %BDOC	Peak labile %BDOC	timing of labile BDOC peak
1	1/17/2010	21	0	77.8	447	1.22	10.32	with discharge	8.90%	16.62%	before discharge
2	1/25/2010	40.6	21	67.8	1708	1.55	10.04	before discharge	7.10%	7.83%	before discharge
3	3/12/2010	55.7	0	90.5	1743	1.48	12.76	before discharge	12.08%	37.39%	before discharge
4	3/22/2010	26.3	6.7	103.2	1373	1.58	10.36	after discharge	7.59%	30.19%	with discharge
5	4/24/2010	35.4	6.3	95.1	337	1.40	6.44	after discharge	3.64%	20.77%	after discharge
6	7/10/2010	61.4	0	26.6	163	1.39	12.84	after discharge	18.88%	14.11%	after discharge
7	7/19/2010	38.6	56	105.7	797	2.74	10.08	after discharge	3.07%	10.58%	after discharge
8	9/30/2010	160.2	19.7	39.6	5039	2.44	13.22	before discharge	7.66%	17.33%	with discharge
9	11/30/2010	42.3	4.5	51.8	1449	1.22	14.04	after discharge	7.93%	19.30%	after discharge
10	7/25/2011	25.4	0	31.7	130	1.77	10.54	with discharge	10.18%	15.34%	with discharge
11	8/27/2011	167.9	51.1	199.8	7125	2.79	11.09	before discharge	2.37%	9.97%	before discharge
12	9/7/2011	80.5	94.8	445.2	1378 0	7.42	9.06	after discharge	2.62%	7.99%	after discharge

a Antecedent moisture conditions computed using the antecedent precipitation index (API_X) which is the summation of the precipitation amounts for *X* days prior to the event (Betson et al. 1969, Bowen 1986)

Figure Legends

Figure 2.1: White Clay Creek watershed map with stream channel, watershed boundaries, and sampling locations for stream water, unsaturated soil water from lysimeters, groundwater from wells, spring seeps, and overland flow.

Figure 2.2: Annual patterns of stream water DOC, labile BDOC, and semi-labile BDOC concentrations from White Clay Creek during 2009 through 2011.

Figure 2.3: Labile BDOC concentrations in stream water from White Clay Creek in southeastern Pennsylvania as a function of discharge (log scale) during 12 storms that occurred between January 2010 and September 2011. Data are separated into rising limb, peak, and falling limb of the discharge hydrograph illustrating the lack of consistent hysteresis among storms.

Figure 2.4: Temporal variability in DOC, labile BDOC, and semi-labile BDOC concentrations of White Clay Creek stream water during a low flow storm (July 25- July 26, 2010). Labile BDOC is illustrated as the mean (± measurement error) from triplicate measurements taken from one reactor with a residence time of 0.5 min. Semi-labile BDOC was calculated from triplicate measurements of total lability taken from three replicate reactors with residences times of 150 min subtracted by the mean labile BDOC and illustrated as the mean (± SE) for each time point.

Figure 2.5: Temporal variability in DOC, labile BDOC, and semi-labile BDOC concentrations of White Clay Creek stream water during a medium flow storm (November 30 – December 2, 2010). Labile BDOC is illustrated as the mean (± measurement error) from triplicate measurements taken from one reactor with a

residence time of 0.5 min. Semi-labile BDOC was calculated from triplicate measurements of total lability taken from three replicate reactors with residences times of 150 min subtracted by the mean labile BDOC and illustrated as the mean (± SE) for each time point.

Figure 2.6: Temporal variability in DOC, labile BDOC, and semi-labile BDOC concentrations of White Clay Creek stream water during a high flow storm (September 29 – October 1, 2010). Labile BDOC is illustrated as the mean (± measurement error) from triplicate measurements taken from one reactor with a residence time of 0.5 min. Semi-labile BDOC was calculated from triplicate measurements of total lability taken from three replicate reactors with residences times of 150 min subtracted by the mean labile BDOC and illustrated as the mean (± SE) for each time point.

Figure 2.7: Mean (± SE) of DOC and labile BDOC concentrations from baseflow terrestrial sources compared to stream water.

Figures

Figure 2.1

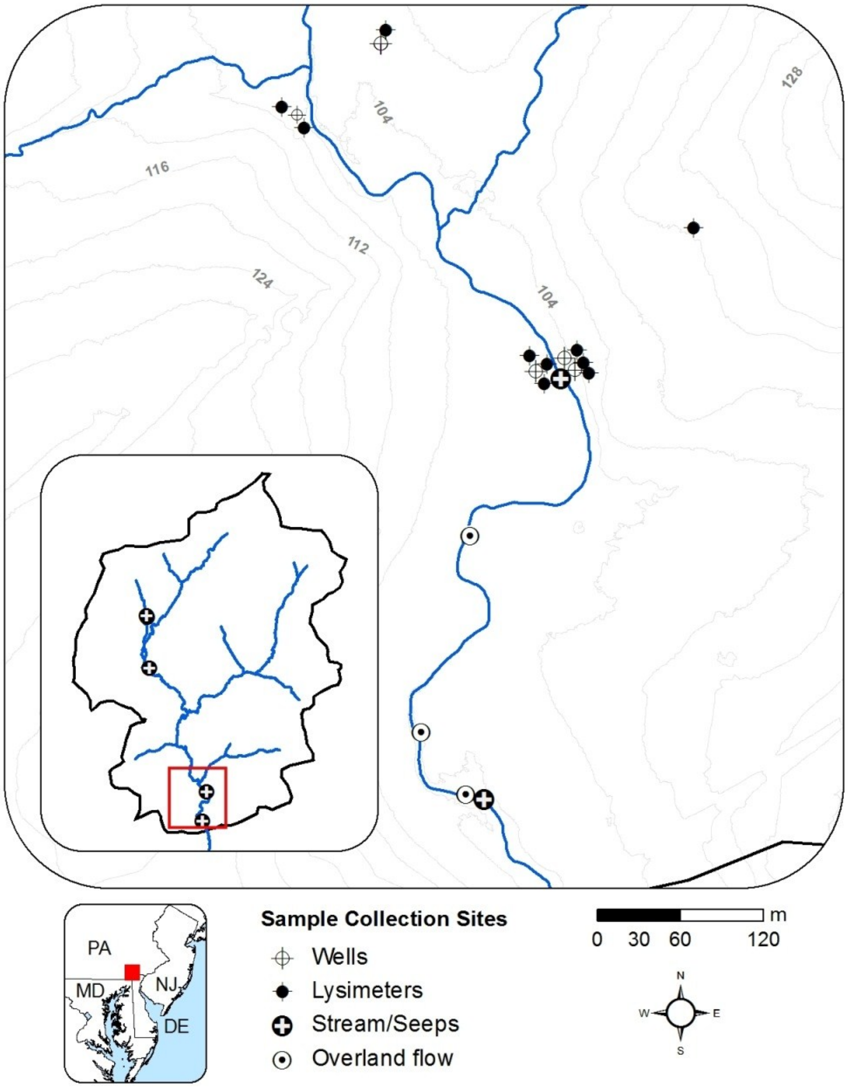
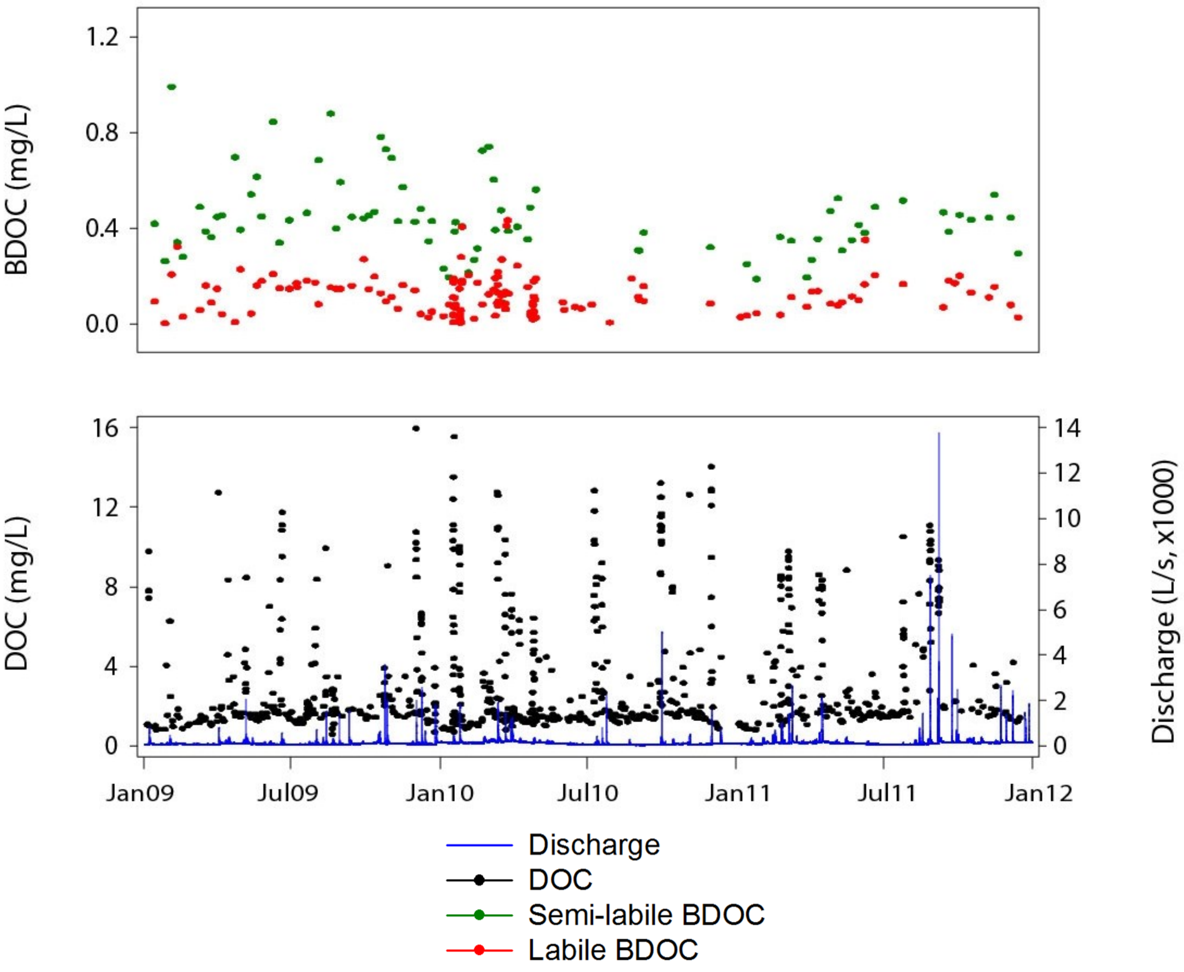


Figure 2.2



BDOC (mg/L)
1.2
0.8
0.4
0.0
DOC (mg/L)
16
12
8
4
0
Discharge (L/s, x1000)
14
12
10
8
6
4
2
0
Jan09
Jul09
Jan10
Jul10
Jan11
Jul11
Jan12
Discharge
DOC
Semi-labile BDOC
Labile BDOC

Figure 2.3

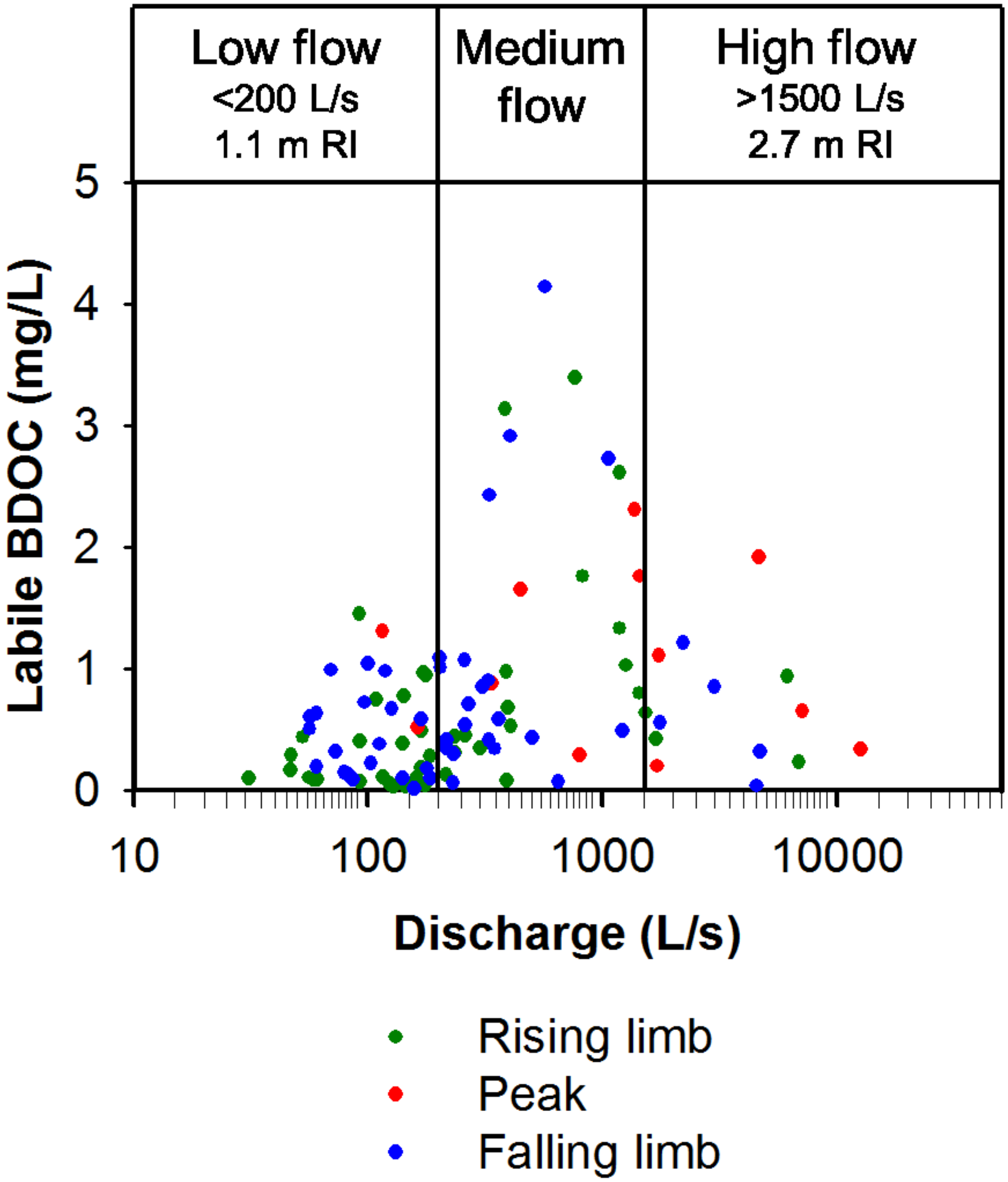

Low flow
<200 L/s
1.1 m RI
Medium flow
High flow
>1500 L/s
2.7 m RI
Labile BDOC (mg/L)
0
1
2
3
4
5
10
100
1000
10000
Discharge (L/s)
Rising limb
Peak
Falling limb

Figure 2.4

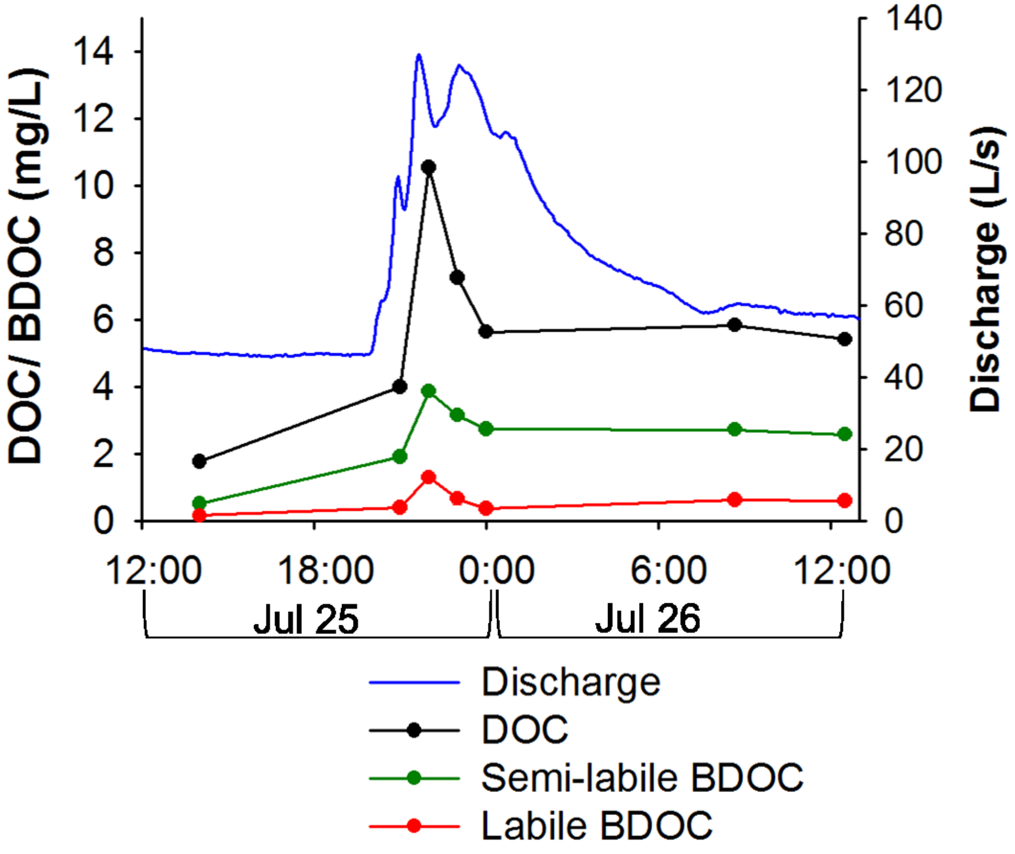

DOC/ BDOC (mg/L)
14
12
10
8
6
4
2
0
Discharge (L/s)
140
120
100
80
60
40
20
0
12:00
18:00
0:00
6:00
12:00
Jul 25
Jul 26
Discharge
DOC
Semi-labile BDOC
Labile BDOC

Figure 2.5

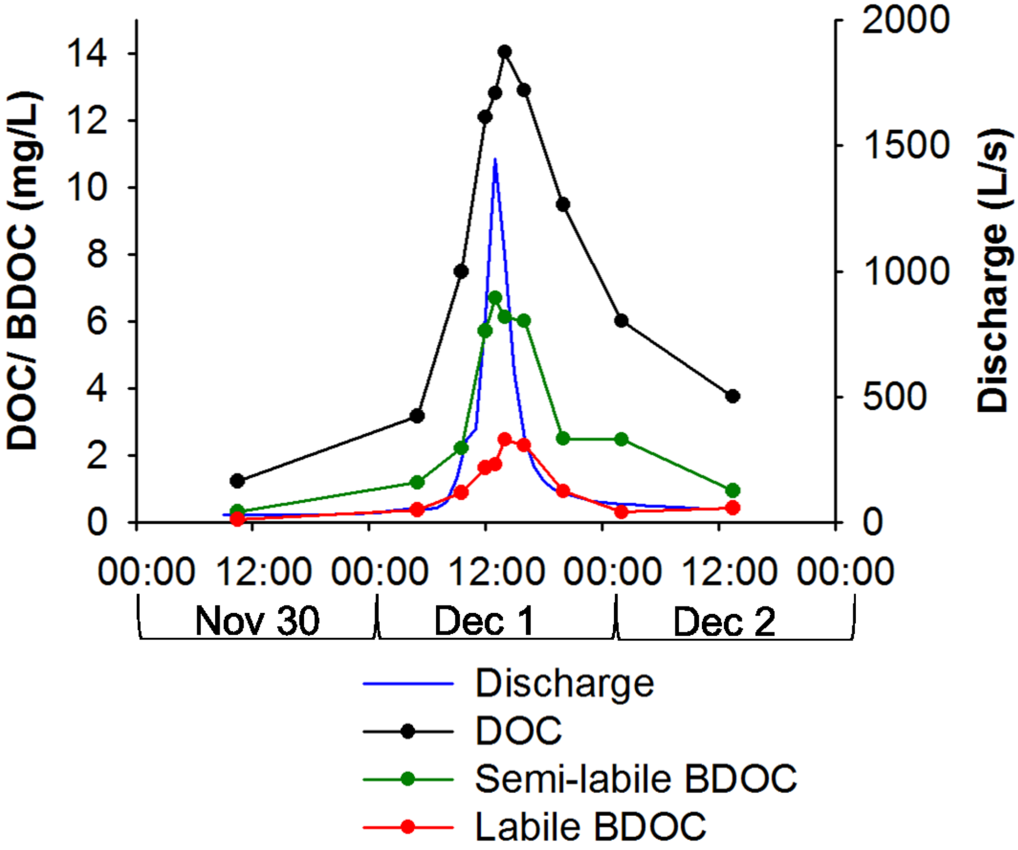
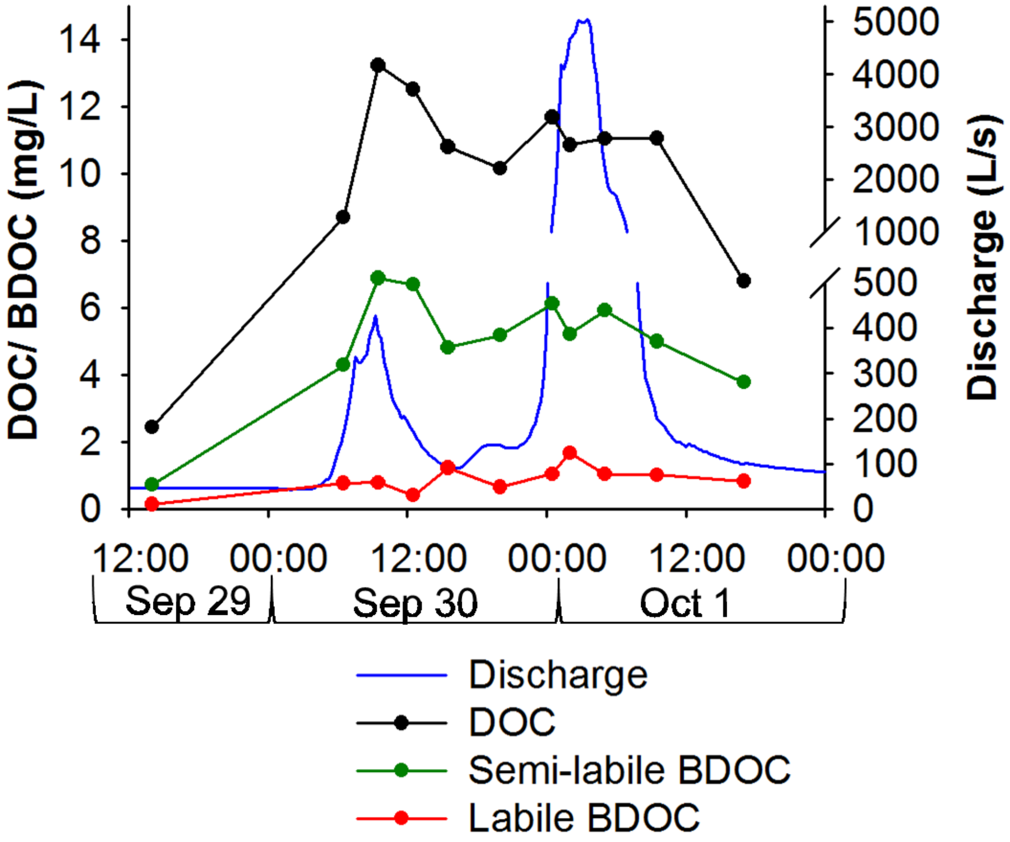
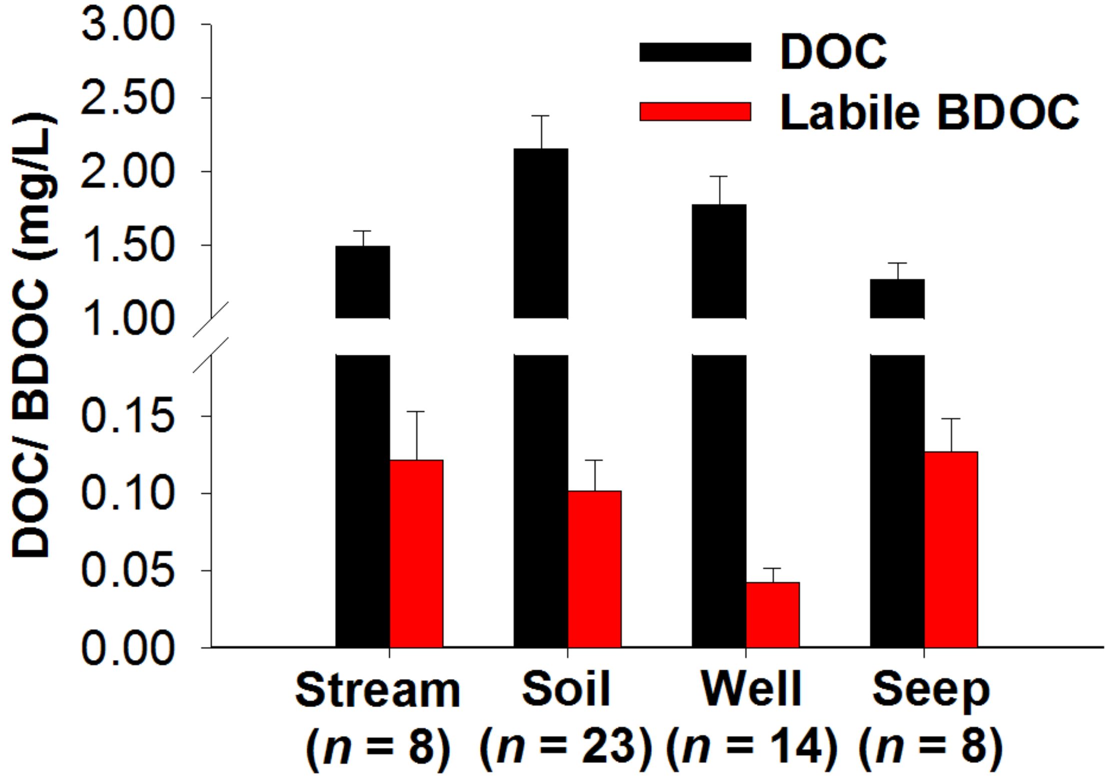

DOC/ BDOC (mg/L)
0
2
4
6
8
10
12
14
Discharge (L/s)
0
500
1000
1500
2000
00:00
12:00
00:00
12:00
00:00
12:00
00:00
Nov 30
Dec 1
Dec 2
Discharge
DOC
Semi-labile BDOC
Labile BDOC

Figure 2.6



DOC/ BDOC (mg/L)
14
12
10
8
6
4
2
0
Discharge (L/s)
5000
4000
3000
2000
1000
500
400
300
200
100
0
12:00
00:00
12:00
00:00
12:00
00:00
Sep 29
Sep 30
Oct 1
Discharge
DOC
Semi-labile BDOC
Labile BDOC

Figure 2.7



DOC
Labile BDOC
DOC/ BDOC (mg/L)
3.00
2.50
2.00
1.50
1.00
0.15
0.10
0.05
0.00
Stream (*n* = 8)
Soil (*n* = 23)
Well (*n* = 14)
Seep (*n* = 8)

CHAPTER 3

Linkages between denitrification in stream sediments and biodegradable dissolved organic carbon

Abstract

Many streams export large amounts of nitrate (NO_3) due to increases in anthropogenic loadings, and these NO_3 fluxes from headwater streams cause eutrophication of downstream aquatic ecosystems. Denitrification, a heterotrophic respiratory process in which microbes use NO_3 as a terminal electron acceptor and organic carbon (C) as an electron donor, is a mechanism that can mitigate the amount of N transported downstream through the permanent removal and reduction of NO_3 to gaseous forms of N. Denitrifiers represent an important yet poorly understood link between C and N-cycling in streams. Experiments were performed to test the influence of dissolved organic carbon (DOC) quality in a terrestrial source water on the removal of N via denitrification in sediment perfusion cores. Biodegradable DOC (BDOC), a measure of DOC quality, was measured with plug-flow bioreactors colonized by stream microorganisms and separated into multiple biological reactivity classes using bioreactors with different residence times. Treatments included stream water amended with glucose, that increased the labile DOC concentration 7 times greater than the stream water control, and overland flow water with order of magnitude greater labile and semi-labile DOC concentrations. Denitrification rates were measured by enrichment with ^{15}N-NO_3, followed by measurements of $^{15}N_2$ production over time. Denitrification rates increased significantly from 44 μmol/ m^2 hr in the stream water control to 1,160 μmol/ m^2 hr in the overland flow treatment, but the glucose addition had no effect. The particulate organic matter content in individual sediment cores also was a significant covariate. The differences in the denitrification response to the glucose treatment and the overland flow treatment suggest either a threshold concentration of labile DOC or that the semi-labile fraction of the DOC pool has a greater influence on denitrification. Based on the concentration of NO_3 in transit during baseflow and storm flow, the measured sediment denitrification potentially removes 3.4 % to 5.1 % of the NO_3 from the water upwelling into the hyporheic sediments and prevents its transport to downstream environments. However, the impact of storms and the concomitant increases in denitrification measured with overland flow depend on changes in the effective residence time of NO_3 at reactive sites that coincide with increases in DOC quality. Since denitrification is potentially an important N sink in stream ecosystems, understanding the influence of organic C and the combined interactive effect of the various controlling factors are necessary for determining management efforts that optimize removal of NO_3.

Keywords

denitrification, dissolved organic carbon, bioavailability, agricultural watershed, stream sediments

Introduction

The availability of reactive nitrogen (N) in the form of nitrate (NO_3) has increased due to high anthropogenic loadings from fertilizer use and fossil fuel combustion (Vitousek et al. 1997, Galloway 1998, Galloway et al. 2008). Consequently, many streams export large amounts of NO_3 to coastal ecosystems. These increases in N availability in aquatic ecosystems cause eutrophication and stimulate extensive algal blooms that ultimately decompose creating large zones of hypoxia (Rabalais et al. 2002). Therefore, increasing the removal of N and reducing stream water N concentrations are common management objectives.

Denitrification is an important mechanism in streams by which heterotrophic microbes reduce NO_3 to gaseous N (N_2 and N_2O) using organic C as an electron donor and mitigate the amount of N transported downstream through the permanent removal of NO_3. In addition to NO_3, denitrifying microbes also require organic carbon (C) making available C an important link between C and N-cycling dynamics in streams (Knowles 1982, Seitzinger 1988, Seitzinger 1994). However, the relative importance of the multiple factors that control denitrification rates is poorly understood because of measurement difficulties and high environmental heterogeneity (Groffman et al. 2006). Additionally, assessing denitrification at the watershed scale is challenging because scaling up from process measurements done in the laboratory to watershed scale models may be inaccurate due to insufficient representation of the true range of variation in biological activity and controlling environmental conditions (Johnes and

Butterfield 2002). Nonetheless, increasing the understanding of the fundamental controls on denitrification enables better predictions on how watersheds will respond to environmental change and can direct more effective management.

Factors that potentially control aquatic sediment denitrification include NO_3 concentration, organic C availability, and dissolved oxygen (DO) concentration (Seitzinger 1988, Martin et al. 2001, Kemp and Dodds 2002, Royer et al. 2004). The prevalence of denitrification studies in low NO_3 streams has emphasized the role of NO_3 supply in controlling denitrification rates (Holmes et al. 1996, Pattinson et al. 1998, Martin et al. 2001). However, excess NO_3 is often present in streams where non-point sources of N from agriculture can exceed point sources as the largest contributors of N (Goolsby et al. 2001).

Many studies have also looked at the effect of DO concentrations on anaerobic denitrification rates in streams (Duff et a. 1984, Christensen et al. 1990, Stepanauskas et al. 1996). Since denitrifiers are facultative anaerobes, certain strains also perform aerobic denitrification, but even so, rates decrease with increasing DO concentrations (Patureau et al. 2000, Chen et al. 2003). Because oxygen levels drop in areas of high microbial activity, which occur in locations where available C is abundant, the regulation of denitrification should be largely controlled by the availability of organic substrates (Hedin et al. 1998, Sobczak et al. 1998, Bernhardt and Likens 2002). Although these factors that theoretically regulate rates of denitrification are known, there is a lack of understanding about which factors have the greatest influence on denitrification activity.

The dependence of denitrification on the availability of oxidizable organic C ultimately connects the removal of NO_3 to C cycling. In forested streams, the predominant form of organic C in transit is dissolved organic carbon (DOC) (Fisher and Likens 1973, Meyer et al. 1998), but the fraction of biodegradable DOC (BDOC) is highly variable (Swank and Caskey 1982, Meyer 1994) and dependent on its source (McKnight et al. 2001). DOC quality rather than actual concentration has been hypothesized to limit sediment denitrification (Baker and Vervier 2004, Royer et al. 2004, Arango et al. 2007, Zarnetske et al. 2011), but some studies did not see any correlations between C quality and denitrification (Inwood et al. 2007). In general, all of these studies measured how denitrification rates responded to predefined and artificial C additions with fewer studies measuring denitrification using in-situ organic matter pools (Baker and Vervier 2004, Barnes et al. 2012). Since the majority of DOC in forested streams is soil or terrestrial derived (Fisher and Likens 1973, Meyer et al. 1998), understanding how different terrestrial source waters influence denitrification rates is vital for elucidating the effect DOC has on regulating in-stream N transformations.

Denitrification studies most commonly measure potential rates that represent optimal conditions for denitrification, destroy sediment profiles, and disrupt microbial aggregates (Rysgaard et al. 1994, Strauss and Lamberti 2000, Arango et al. 2007, Barnes et al. 2012). As such, these incubations can serve in a comparative context but do not provide estimates of in-situ activity. The use of sediment cores results in minimal disturbance to sediment structure, preservation of natural chemical and biological gradients, and maintenance of hydrologic characteristics (Nishio et al. 1982, Steingruber et al. 2001, Sheibley et al. 2003).

In the experiments described herein, a sediment perfusion core technique was developed that simulated groundwater upwelling in the stream bed, and the influence of DOC quality differences in terrestrial source waters on the removal of N via denitrification was tested. I addressed the following questions: (1) Does biological liability of DOC affect denitrification rates in stream sediments? and (2) Does a terrestrial source of labile and semi-labile DOC impact denitrification rates in stream sediments? Denitrification rates were expected to be limited by the availability of labile DOC with increased rates for treatments with higher BDOC concentrations. BDOC was altered by amending stream water with glucose and using overland flow water with high BDOC.

Labile and semi-labile BDOC concentrations were quantified for treatment waters using plug-flow laboratory bioreactors of different residence times that were colonized and maintained with stream water. Treatment water was pumped through stream sediment cores, simulating groundwater upwelling rates. Denitrification rates were measured by enriching the ambient NO_3 pool with ^{15}N and measuring the production of $^{15}N_2$ over time. These measurements allowed me to assess how variations in source water BDOC influence the removal of NO_3 from treatment water, and I found large increases in BDOC are needed to enhance rates of denitrification compared to the stream water control.

Methods

Study Site

The East Branch of White Clay Creek (WCC), located in the southeastern Pennsylvania Piedmont (39° 51' 32.18" N, 75° 46' 58.28" W), drains a catchment of 7.25 km^2 composed of deciduous woodlands, meadows, pastures, and agricultural lands that are underlain by metamorphic crystalline rock including gneiss, schist, quartzite, and marble. Soils are 1 to 2 m deep, unglaciated, and primarily *typic hapludults*, except in the riparian zone, where *aqui fagiudults* prevail. The total length of stream channels that run from the headwaters (elevations: 164 m) to the 3rd order stream reach (elevation: 100 m) is 12.9 km. Mean annual stream flow is 115 L/s, and water temperature averages 10.6°C (Newbold et al. 1997). Annual precipitation averages 1.05 m/yr and is evenly distributed seasonally.

Field sampling

Sediments cores were collected from randomly selected locations at the downstream 3rd order section of WCC on June 20, 2011 (n = 11) for experiment 1 and on October 26, 2011 (n = 6) for experiment 2. Cores were collected by inserting PVC pipe (length = 10 cm, diameter = 3.81 cm) into the streambed until the top was flush with the surface. The top was capped, and the sediment core extracted by digging around the core and gently removing to minimize disturbance. Acrylic plates with holes and Nitex® screening were used for bed supports, and threaded socket couplings were glued over both ends of the core. A rubber stopper was placed within the couplings to fill the void space before threaded caps were screwed on to each socket coupling, and barbed couplings were screwed into each cap providing an inlet and outlet connection (Figure 3.1).

Denitrification experiments

Sediment cores were set up in a water bath continuously fed with stream water to maintain temperatures similar to in-situ conditions. A multi-channel peristaltic pump was used to push water through each core from the bottom upwards in an effort to simulate upwelling. The pump was calibrated to a rate of 2.5 mL/ min which was based on the highest Darcy velocity (3.22 m/ day) previously measured at the 3rd order site (Battin et al. 2003). Cores were run in a once-through mode using a separate reservoir of filtered stream water for seven days to equilibrate the cores before adding treatment water for the denitrification experiments. Temperatures ranged from 18.01 °C to 21.2 °C with a mean of 19.7 °C for experiment 1 and from 9.1 °C to 9.5 °C with a mean of 9.3 °C for experiment 2.

For both experiments, stream water amended with $^{15}NO_3$ (1.43 µmol N-$^{15}NO_3$/ L for experiment 1 and 2.14 µmol N-$^{15}NO_3$/ L) served as a control (n = 5 for experiment 1, n = 2 for experiment 2). The treatment water for experiment 1 consisted of stream water amended with 2.86 µmol N-$^{15}NO_3$/ L and 0.4 mg C/L glucose (n = 5). For experiment 2, overland flow water (collected on August 25, 2011) amended with 0.71 µmol N-$^{15}NO_3$ was used as the treatment water (n = 3). Amendments of $^{15}NO_3$ were tracer level since background NO_3 concentrations ranged from 263 µmol N-NO_3/ L to 273 µmol N-NO_3/ L in stream water and 51 µmol N-NO_3/ L in overland flow water. These experimental waters were pumped through the cores in a once through mode for 100 minutes in order to replace all water in the core. This time interval was determined to be the turnover time of the experimental system by adding a chloride solution to an extra core prior to the experiment and looking at the breakthrough curve (data not shown). The outflow lines were connected to Kynar Gas Sampling Bags with dual valves that were filled with experimental water, and pump lines were attached to the 2nd valve on the sample bags to create a recirculating system. Air bubbles had been removed from the water in the sampling bags to prevent degassing of N_2 during the experiment. The cores were then run in a re-circulating mode for 8 hours, and samples were taken every 100 minutes from a low gas permeable PharMed® BPT tube attached via a tee-connector in the line between the sampling bag and the pump. The sampling bags allowed us to take multiple samples over time without disrupting the experimental system.

Water samples were taken separately for individual analyses including $^{15}N_2$, NO_3, and DOC. Isotope samples were taken in 12.5 mL gas-tight containers (Exetainers, Labco, High Wycombe, UK) with screwcap lids (excluding any air bubbles) by filling from the bottom and overflowing at least two volumes. NO_3 samples were filtered (Sterile Millex® Syringe Filters with a 22 µm pore size) and frozen until analysis by ion chromatography using a Dionex DX 3000. DOC samples were filtered (Whatman GF/F) and refrigerated until concentrations were measured using a Sievers 800 or 900 organic carbon analyzer equipped with an inorganic carbon removal module. For both experiments, an extra core receiving treatment water was used to measure DO concentrations using a WTW Multi 350i DO meter. DO concentrations were also measured in a subset of control cores at the end of the experiments.

Isotope analysis and calculations

To analyze the isotopic composition of N_2 in the water samples, a helium headspace was introduced into the exetainers, and it was assumed that the majority of N_2 diffused into the gas phase after shaking vigorously. The gas was then injected into a gas chromatograph in line with a Thermo-Finnigan DeltaPlus XP Isotope Ratio Mass Spectrometer (IRMS) equipped with a dual inlet system and a Thermo-Finnigan Gasbench II with dual cryogenic traps.

The ratios obtained with mass spectrometry ($[^{15}N_2]/[^{14}\mathrm{N}_2]$) were then used to calculate the concentration of $^{15}\mathrm{N}_2$ in the water:

$$[^{15}\mathrm{N}_2] = \frac{[^{15}\mathrm{N}_2]}{[^{14}\mathrm{N}_2]} \cdot [^{14}\mathrm{N}_2]$$

where $[^{14}\mathrm{N}_2]$ was calculated with Henry's law:

$$K_{\mathrm{H}}(T) = \frac{p_{\mathrm{N}_2}}{[^{14}\mathrm{N}_2]}$$

where $K_H(T)$ is the temperature-dependent Henry constant and p_{N_2} is the partial pressure of N_2 in the atmosphere (0.78 atm). The slope (m_{15}) of the linear regression of $[^{15}\mathrm{N}_2]$ versus time provided a flux in mass per unit time, and the standard error in the slope of the regression was used to estimate the error associated with the denitrification rate for each core. Using this slope, the denitrification rate of $^{15}\mathrm{N}_2$ (D_{15}) was calculated for each core:

$$D_{15} = \frac{m_{15}}{A} \cdot (V_w + \varphi V_s)$$

where A is the surface area of the sediment core, V_w is the volume of water, V_s is the volume of sediment, and φ is the estimated porosity. In order to calculate the denitrification of the $^{14}N/^{15}N$ NO_3 mixture, the isotopic NO_3 enrichment (ε) was calculated using the NO_3 concentrations measured in the experimental water before and after the addition of $^{15}NO_3$:

$$\varepsilon = \frac{[\mathrm{NO_3}]_{after} - [\mathrm{NO_3}]_{before}}{[\mathrm{NO_3}]_{after}}$$

The total denitrification rate (D) for each core was then calculated as:

$$D = \frac{D_{15}}{\varepsilon}$$

BDOC methods

BDOC was measured using plug-flow biofilm reactors consisting of chromatography columns filled with sintered glass beads and colonized in the dark by continuously supplying filtered WCC stream water in a once through mode (Kaplan and Newbold 1995). The stream water served as a continuous source of bacteria to colonize the bioreactors. Once colonized, biological activity dominates the removal of DOC, and BDOC is calculated as the difference between the inflow and outflow DOC concentrations. This bioassay method directly measures the fraction of DOC that can be biodegraded by a natural microbial assemblage under controlled conditions and provides a means for directly comparing BDOC concentrations among samples. The most biologically labile DOC molecules are rapidly metabolized over short bioreactor volumes while more semi-labile DOC is metabolized after longer exposures.

BDOC in treatment water was separated into different biological lability classes using three bioreactors with empty bed contact times (EBCT; equal to the volume of the bioreactor divided by the flow rate) that increased in a geometric series (EBCT = 0.5, 1.5, 3 min) and one larger reactor (EBCT = 150 min). The bioreactor with an EBCT of 0.5 min consumes the most labile DOC fraction in sample water where as the largest reactor (EBCT = 150 min) gives a measurement of total BDOC (Kaplan et al. 2008). The incremental uptake of DOC from each increase in EBCT was calculated by subtracting the BDOC concentration measured in the smaller bioreactor in the series from the BDOC concentration of the next larger bioreactor.

Baseflow stream water taken the same week as the experimental water was used to estimate the labile and semi-labile BDOC of the stream waters used as controls. Assuming that glucose is completely consumed in the smallest bioreactor (EBCT = 0.5 min) (Kaplan et al. 2008), the labile BDOC for the glucose treatment was estimated as the control labile BDOC plus the total concentration of glucose added (0.349 mg C/L). Pulses of higher DOC concentrations than the bioreactor communities are normally exposed to could result in breakthroughs of DOC if run through the bioreactor undiluted. Under these higher DOC concentrations, the bioreactor would not have sufficient time for microbial growth, and results would underestimate BDOC. Therefore, the overland flow sample was diluted to approximately baseflow DOC concentration (1.4 mg/L) using biologically stable effluent from the large reactor (EBCT = 150 min) to normalize for concentration. BDOC of overland flow was then calculated based on the ratio of overland flow water to biologically stable effluent in the inflow water. At a minimum, three bed volumes of sample water were passed through each reactor before sampling the outflow. Inflow and outflow DOC concentrations were measured using a Sievers 800 or 900 organic carbon analyzer.

Sediment organic content methods

The organic content of sediments from each core was determined by taking three sediment subsamples and measuring the ash free dry mass (AFDM). Sediment subsamples were dried overnight at 103 – 105º C, ashed at 500º C for three hours, and reweighed to determine %AFDM as the ratio of organic matter weight to dry weight.

Statistical analyses

Statistical analyses were conducted with JMP Statistical Discovery software (v 8.0.2/ 2009, SAS, Cary, NC). Denitrification rates for individual cores were reported as an area-based flux ± standard error which was estimated by propagating the error in the slope of the regression between the concentration of $^{15}N_2$ in the water and time. Mean treatment denitrification rates were then calculated and reported as the mean ± standard error. Differences in denitrification rates between experiments and between treatments were examined by a two-way analysis of variance (ANOVA) using an interaction term and organic matter content as a covariate. Tukey post-hoc analyses were used to determine what was driving the differences seen in the interaction term. Sediment organic matter content data were analyzed using a non-parametric

Wilcoxon Rank Sum test for determining differences between sediments collected for experiment 1 and experiment 2 and a one-way ANOVA for difference among treatments.

Results

Denitrification experiments

For the control cores, mean denitrification rates were 136 ± 29 μmol/ m^2 hr and 49 ± 5 μmol/ m^2 hr for experiment 1 and 2, respectively (Figure 3.2). For the treatment cores, mean measured denitrification rates were 60 ± 12 μmol/ m^2 hr and 1,000 ± 79 μmol/ m^2 hr for glucose and overland flow, respectively (Figure 3.2). There was a significant interactive effect of treatment and experiment (ANOVA, $F_{(4,10)} = 210$, $p < 0.0001$) driven by a significantly higher rate of denitrification in the overland flow treatment (Tukey's test: experiment 2-treatment vs. experiment 2-control $p < 0.0001$, experiment 2- treatment vs. experiment 1- control $p < 0.0001$, experiment 2- treatment vs. experiment 1- treatment $p < 0.0001$). Additionally, the organic matter content covariate was a significant effect ($F_{(4,10)} = 6.05$, $p = 0.03$), and the inclusion of this covariate reduced the error sum of squares by 40%.

Water chemistry

DOC concentrations in treatment waters ranged from 1.65 mg C/L in the control reservoirs to 2.00 mg C/L in the glucose reservoir to 10.07 mg C/L in the overland reservoir. Stream water used for the controls in experiment 1 and 2 had the lowest amounts of labile and semi-labile BDOC with estimated concentrations of 0.056 and 0.132 mg labile C/L and 0.251 and 0.200 mg semi-labile C/L, respectively (Figure 3.3). The glucose treatment had an additional 0.35 mg labile C/L added to the stream water control used in experiment 1. Overland flow had the highest BDOC concentrations with 1.11 mg labile C/L and 3.21 mg semi-labile C/L.

NO_3 concentrations measured before the experiment in the stream water used as a control for experiment 1 and 2 were 268 and 273 µmol N- NO_3/ L, respectively. Overland flow had a lower concentration of 51 µmol N- NO_3/ L. The NO_3 concentrations decreased during both experiments for all treatments (Figure 3.4), but the overland flow treatment had minuscule changes in concentration with a mean difference of 2.9 ± 5.7 µmol N-NO_3/ L. The greatest decreases in NO_3 were seen during experiment 1 with a final mean concentration of 228 ± 8.0 µmol N-NO_3/ L in the controls and 234 ± 3.0 µmol N-NO_3/ L in the glucose treatment. For experiment 2, the final mean concentration was 262 ± 1.4 µmol N-NO_3/ L for the controls and 51 ± 5.6 µmol N-NO_3/ L for the overland flow treatment.

The DO concentrations of the treatment waters prior to the experiments were 6.0 mg/L (65 percent saturation) and 8.6 mg/L (74 percent saturation) for experiment 1 and 2, respectively. DO concentrations also decreased during both experiments, but the experimental waters never went anoxic (< 0.5 ppm) (Figure 3.5). DO concentrations decreased more in the treatments than in the controls. The greatest decrease in DO concentration was measured in the glucose treatment which had a final DO concentration of 1.9 mg/L (20 percent saturation); whereas, the final DO concentration in the control was 2.3 mg/L (25 percent saturation). During experiment 2, the pattern was similar with a final overland DO concentration of 5.5 mg/L (48 percent saturation) and a final control DO concentration of 6.7 mg/L (58 percent saturation).

Sediment organic content

The mean organic content (%AFDM) of cores collected for experiment 1 was 1.40 % ± 0.13 % ranging from 0.72 % to 2.24 % (Table 3.1). The mean organic content of the cores collected for experiment 2 was 1.66 % ± 0.19 %, ranging from 1.25 % to 3.08 % (Table 3.1). There was no significant difference in organic content between the sediments collected for experiment 1 and those collected for experiment 2 ($W_{(1)} = 49$, $Z = 1.04$, $p = 0.29$). Additionally, there were no significant differences among treatments in the organic content of the cores (ANOVA, $F_{(3, 11)} = 0.58$, $p = 0.64$).

Discussion

Given the significant human alterations of N and C cycling worldwide and the subsequent interest in predicting NO_3 removal potentials of stream networks (Mulholland et al. 2008, Seitzinger et al. 2006), improving our understanding about what factors influence watershed NO_3 removal is critical to accurately predicting future ecosystem effects of climate and land use change. The connection between the availability of labile DOC and N-cycling has been recognized previously (Hedin et al. 1998, Bernhardt and Likens 2002, Thouin et al, 2009); however most of the past work has focused on the response of denitrification to the addition of a labile, homogeneous pool of organic matter (i.e. glucose, acetate). Additionally, amendments of labile DOC have typically increased concentrations over 100% of ambient DOC concentrations (Bernhardt and Likens 2002: ~1000 %, Inwood et al. 2007: ~1200%, Thouin et al. 2009: 125 % to 178 %). The experimental additions of glucose in this study only raised the DOC concentrations by 25%, which is within the realm of natural variations in labile DOC concentration observed in WCC (i.e. during storms) and additions of naturally occurring labile DOC from overland flow collected in the watershed.

Denitrification rates measured by different methods and among different aquatic ecosystems show large variability. Based on a meta-analysis of measured denitrification rates among aquatic environments, lotic ecosystems had the highest mean denitrification rate (289 ± 252 μmol/ m^2 hr) and the highest variability (114 – 404 μmol/ m^2 hr) compared to other aquatic environments (Pina-Ochoa and Alvarez-Cobelas 2006). Measured denitrification rates from the control cores in these experiments (43 to 181 μmol/ m^2 hr) were in the lower range of these previously published measurements from stream sediment cores. The large variability in measured denitrification rates from stream ecosystems is most likely due to variations in environmental and controlling factors. Additionally, the variability in denitrification rates was greater for systems with high NO_3 concentrations (> 50 μmol) and high interstitial DOC concentrations (> 50 mg/g) (Pina-Ochoa and Alvarez-Cobelas 2006). These factors in combination with differences in methodology and experimental design make cross-study comparisons difficult.

The different methods developed for measuring denitrification each have advantages, but also limitations (Steingruber et al. 2001). Slurries and potential denitrification assays, such as the acetylene inhibition technique, allow for controlled experimental variation of parameters and provide information on the controlling factors potentially regulating denitrification under ideal conditions. However, potential rates do not offer realistic estimates of in situ rates preventing scaling up to ecosystem level fluxes. Intact sediment cores minimize disturbance and preserve the natural chemical gradients and biomass distributions (Nishio et al. 1982). Typically, intact sediment core experiments are run as batch-mode assays such that the incubation containers are closed with sediments overlaid with water, and the production of N_2 is measured over time (a time-series experiment) or at the start and the end of the experiment (endpoint experiment) (Steingruber et al. 2001). The destructive sampling required for measurements of N_2 gas in these experiments means that the time-series experiments are based on measurements from different cores, which may vary, representing each time interval and the calculation of one denitrification rate, but the endpoint experiments, which provide one denitrification rate per core, neglects analysis of the linearity in N_2 production.

The use of a sampling bag for the water reservoir combined these methods and avoided the main disadvantages such that a denitrification rate was obtained for each core and sampling over time provided information to verify the linearity of N_2 production. Perfusion cores have the additional advantage over traditional batch-mode assays of simulating hydrological characteristics (Sheibley et al. 2003). Batch-mode assays rely on diffusion of NO_3 from the overlying water into the sediments, but perfusion cores control the delivery of NO_3 to reactive sites at a rate similar to in situ.

Denitrifiers are influenced by NO_3, C, and DO concentrations (Knowles 1982, Seitzinger 1988). The importance of NO_3 concentration has been well established with consistent positive trends in denitrification rates both within and across stream sites (Mulholland et al. 2008), and multiple linear regression analysis showed NO_3 concentration explained 70 % of the variability in measured rates (Pina-Ochoa and Alvarez-Cobelas 2006). However, a threshold NO_3 concentration has been suggested beyond which other factors, such as C or DO concentrations, control denitrification (Inwood et al. 2005, Barnes et al. 2012). Inwood et al. (2005) established 0.03 mmol NO_3-N/L as this threshold NO_3 concentration, and both the control and treatment waters exceeded this threshold concentration suggesting NO_3 saturation. Therefore, if no NO_3 limitation exists because N is in excess, WCC is an ideal system to study the effect of either DO or C concentrations on denitrification.

The influence of DO concentrations on denitrification depends on the interaction of multiple factors including oxygen consumption rates, oxygen diffusion, and the denitrifying community structure. For example, anaerobic microsites can occur in areas of localized high oxygen consumption which might be associated with the breakdown of organic matter and decreases in the thickness of the oxic zone (Goering 1985, Seitzinger 1988). Stream sediments are highly heterogeneous and could also have anaerobic microsites within otherwise aerobic environments due to decreased oxygen diffusion into aggregates of sediment particles (Duff and Triska 1990, Kemp and Dodds 2002). Furthermore, some groups of denitrifiers are able to use both oxygen and NO_3 as electron acceptors via aerobic denitrification (Gao et al. 2010, Bougon et al. 2009).

The experimental water from all of the perfusion core treatments remained oxic throughout the experiments (1.9 to 8.6 mg/L). The lower DO concentrations in the treatments compared to the controls suggests that the addition of labile DOC did in fact increase the rate of oxygen consumption. However, the experimental water with the lowest DO concentration (glucose) did not have the highest denitrification rates suggesting that DO concentration is not entirely controlling denitrification. Although denitrification is not completely inhibited by oxygen, aerobic denitrification, if occurring in the perfusion cores, would most likely be minimal. Aerobic denitrification rates are between 0.3 % and 3 % of anaerobic denitrification rates and decrease significantly with increasing oxygen concentrations (Parkin and Tiedje 1984, Chen et al. 2003). Although aerobic denitrifiers have been isolated from a variety of environments including freshwater sediments, seawater sediments, peat samples, pond water, and soils (Carter et al. 1995, Patureau et al. 2000, Gao et al. 2010, Bougon et al. 2011), only about 10% of denitrifiers have been found to be capable of aerobic denitrification (Pastureau et al. 2000). Because I did not purge the experimental systems to make them anoxic, the perfusion cores were designed to provide a quasi-realistic simulation of natural stream hyporheic conditions and measure actual denitrification (including both anaerobic and aerobic denitrification) rather than potential. Denitrification assays performed in other streams previously established a positive relationship between denitrification and DOC concentration under conditions of high NO_3 concentrations (Inwood et al. 2007, Zarnetske et al. 2011, Barnes et al. 2012). However, the use of total DOC in denitrification models is misleading because the entire DOC pool is not available for microbial processes, and previous studies have shown that DOC concentration is not related to measurements of C quality (Jaffe et al. 2008). For example, Barnes et al. (2012) showed a relationship between potential denitrification rates and the chemical characteristics of the DOC pool as determined by 3-dimensional fluorescence suggesting that the rate of denitrification could be governed by DOC quality.

The measurement of lability profiles and the quantification of the fraction of different biological reactivity classes in the treatment waters allowed for a determination of the influence of DOC lability on denitrification in sediments from WCC. The concentration of labile DOC was 7 times greater in the glucose treatment compared to the control with equal concentrations of semi-labile DOC and no difference in denitrification. On the other hand, the concentration of both labile and semi-labile DOC in the overland flow treatment was close to an order of magnitude greater than the control, and the rate of denitrification was 2 orders of magnitude greater. The lack of increase in denitrification after the addition of glucose in combination with the large increase in denitrification in response to increases in semi-labile DOC in the overland flow treatment suggest that there is either a threshold concentration of labile DOC needed to stimulate denitrification or that the semi-labile fraction of the DOC pool has a greater influence. Since most denitrification experiments with DOC amendments have increased concentrations 1 to 12 times the ambient DOC concentration, it is difficult to determine if a threshold DOC concentration exists. However, the glucose results are in contrast to results from the Upper Bann River, an agricultural stream in Ireland, where glucose additions in stream sediment incubations at concentrations 2.5-fold greater than those added in this work (final concentration of 1.0 mg C/L) were efficient in stimulating denitrification with a 2-fold increase in NO_3 reduction rates (Kelso et al. 1999). Alternatively, the 16-fold increase in semi-labile BDOC associated with the overland flow treatment could have stimulated denitrification (1,000 μmol N/ m^2 hr). Comparable additions of acetate to sediment cores draining agricultural land in the upper Mississippi River basin resulted in similarly high measurements of denitrification with rates up to 1,100 μmol/ m^2 hr (Smith et al. 2006). It is difficult to separate out which fraction of the DOC pool had the greatest control over the rate of denitrification because of the large increases in total DOC that accompanied the increased labile and semi-labile DOC in the overland flow treatment.

Although denitrifiers are limited to using dissolved substances that can be transported across cell membranes, it has been suggested that particulate organic carbon (POC) could also positively influence denitrification (Arango et al. 2007). Abiotic leaching and exoenzyme activity can extract DOC from POC (Smith et al. 1992, Seitziner 1988), and decomposition of POC can also reduce DO concentrations expanding the anaerobic habitat enhancing denitrification.

Research in marine environments has shown that enzymes released by bacteria attached to aggregates render aggregates soluble, transferring organic matter from the particulate to the dissolved phase, and due to the low C demand of the attached bacteria most of the DOC formed diffuses into the surrounding waters (Smith et al. 1992). This mechanism of uncoupled solubilization where bacteria mediate the transition of POM to DOC may produce a confounding variable in the denitrification experiments. Although %AFDM did not differ significantly among treatments, there was a significant effect of organic content as a covariate in the denitrification experiment ANOVA, and the highest denitrification rate (1,160 µmol NO_3-N/ m^2 hr, core #25, overland flow treatment) was measured in the core with the highest %AFDM (3.08%). Arango et al. (2007) hypothesized that substratum % organic matter was more important in regulating stream denitrification than DOC, and showed a positive relationship between denitrification rates and organic matter content in sediment incubations from agricultural streams in Michigan with a wide range of substrate characteristics (0.7 % to 72.7 %). Additionally, sediment organic matter content has been shown to be the best predictor of sediment community respiration in streams suggesting that community respiration is not significantly supported by water column DOC (Hedin 1990). The small range of %AFDM (0.72 % to 3.08 %) among the experimental cores in these experiments makes it difficult to determine the influence of sediment organic matter content, and more research on the importance of POC to denitrification would be required to determine if its influence is via direct or indirect mechanisms.

Denitrification rates should also be influenced by temperature due to temperature effects on microbial growth and metabolism. In general, increases in denitrification occur with increasing temperature (Seitzinger 1988, Rysgaard et al. 2004). Because the stream temperature differed between the two experiments, a temperature coefficient (Q_{10}) was calculated based on the denitrification rates (D) measured in the controls for experiment 1 and 2 and the mean water bath temperature (T) during experiment 1 and 2:

$$Q_{10} = \frac{D_1}{D_2}^{\frac{10}{T_1 - T_2}}$$

Although the mean control denitrification rates for experiment 1 and 2 were not significantly different (Tukey's test: experiment 2-control vs. experiment 1-control *p* = 0.345), a Q_{10} of 2.56 calculated indicates a positive effect of temperature on denitrification. The Q_{10} for denitrification typically ranges between 1.5 and 3.5 (Seitzinger 1988, Knowles 1982, Rysgaard et al. 2004). Using this Q_{10}, temperature corrected denitrification rates for experiment 2 are higher than those measured with a mean control temperature-corrected rate of 129 ± 14 μmol/m^2 hr and a mean overland flow temperature-corrected rate of 2,664 ± 211 μmol/m^2 hr. Using these corrected rates does not change the ultimate result of the statistical analysis which still shows a significant interactive effect of treatment and experiment driven by a significantly higher rate of denitrification in the overland flow treatment and organic matter covariate as a significant effect.

Since other factors such as nitrification rate and oxygen concentrations are also influenced by temperature, it is difficult to separate the effect of temperature alone. Temperature changes may also influence the rate of POC hydrolysis via extracellular enzymatic activity and dissolution to DOC (Mayer et al. 2006, Conant et al. 2011, Shank et al. 2011). The rate of enzyme-mediated reactions such as the depolymerization of POC to DOC increases at higher temperatures, but aquatic research has shown microbial communities have a range of isoezymes with different temperature optimas (Hahn and Pockl 2005, Grzymski et al. 2008). Additionally, any temperature effect was overwhelmed by the influence of the overland flow treatment since the denitrification rates for the overland flow treatment were an order of magnitude greater than the rates measured in the controls and glucose treatments and temperature correction only increased this difference.

The importance of denitrification to stream ecosystem N retention depends on the rate relative to the concentration of NO_3 moving downstream. NO_3 concentrations are at a maximum during baseflow (mean = 0.27 ± 0.004 mmol NO_3-N/L, monthly samples from 1993 to 2001, n = 61) when stream flow ranged between 20 L/s and 100 L/s. The concentration of NO_3 removed via denitrification in the hyporheic zone during baseflow can be calculated by dividing the average control treatment denitrification rate measured of 2.57 ± 0.51 mmol NO_3-N/ m^2 day by the estimated upwelling groundwater Darcy velocity of 0.27 m/ day (Battin et al. 2003). At this upwelling rate (equivalent to a baseflow of 75 L/s), an average of 0.010 mmol NO_3-N/L is removed from the upwelling groundwater with about half of the ultimately removed NO_3 passing through the water column then exchanging back down into the hyporheic zone before being removed. In the absence of sediment denitrification, baseflow exports of NO_3 would be 3.4 % higher.

Additionally, reach-scale N removal can be calculated by applying stream nutrient spiraling metrics (Newbold et al. 1981) and using calculations from Royer et al. (2004). An uptake velocity of NO_3 due to denitrification ($V_{f,dn}$ in m/s) can be calculated by dividing the denitrification rate by the NO_3 concentration of the influent groundwater (inferred to be 3.4 % higher than the stream water concentration) in the stream. A loss rate (-k in percentage per day) can then be calculated by dividing $V_{f,dn}$ by the average stream depth (0.15 m, Battin et al. 2003), and a denitrification uptake length ($S_{w,dn}$) can be calculated by dividing the specific discharge (discharge/ stream width) by $V_{f,dn}$. The width of the 3rd order reach at baseflow was 4.78 m, averaged from 35 transects. Using the control treatment denitrification rates (1.06 to 4.34 mmol NO_3-N/ m^2 day), the baseflow $V_{f,dn}$ ranges between 0.003 mm/min and 0.011 mm/min which is within the range reported for other agricultural headwater streams (0.001 mm/min to 0.238 mm/min, Royer et al. 2004, 0.06 mm/min, Mulholland et al. 2008). These $V_{f,dn}$ correspond to a loss rate between 2.52 % per day and 10.36 % per day. Using the range of baseflow discharges observed (20 to 100 L/s), denitrification uptake lengths were calculated between 23 km and 478 km. Denitrification uptake lengths measured in other agriculturally influenced streams ranged from 1 km to 320 km, and results greater than 200 km were also reported (Royer et al. 2004, Mulholland et al. 2009, Roley et al. 2012). These are also consistent with the low percentage (3.4 %) of influent NO_3 that is removed within the stream sediments.

Long uptake lengths (> 200 km) indicate that denitrification has minimal influence on the export of NO_3. However, the high values calculated should not be used as exact estimates of the actual transport distances because of changes in morphology, hydrology, and uptake rates along the length of WCC, and therefore, these calculations serve only as a relative index of the importance of denitrification.

During storms, NO_3 concentrations decrease (mean = 0.05 ± 0.01 mmol NO_3-N/L, peak discharge concentrations measured during storms in 2010 and 2011), but the downstream flux of NO_3 increases (mean = 132 ± 45 mmol NO_3-N/s) due to the increase in discharge (discharge between 790 and 12,660 L/s). Although multiple sources contribute to stream flow during storms, the overland flow treatment is a relatively good correlate to storm flow since the average peak concentration of labile BDOC measured during 12 storms was 1.51 ± 0.31 mg C/L and the average peak concentration of semi-labile BDOC measured during three storms was 5.12 ± 1.21 mg C/L, both of which are similar to the BDOC concentrations measured in the overland flow treatment. Based on the downstream storm flux (132 mmol NO_3-N/s) and a stream surface area of 24,000 m^2 (Newbold et al. 1997), the areal loading rate during storms is approximately 475 mmol NO_3-N/ m^2 day (although not sustained for the entire day). The average denitrification rate for the overland flow treatment (24 ± 1.9 mmol NO_3-N / m^2 day) would therefore remove 5.1 % (0.0025 mmoles/ L) of the influent storm flow. However, the impact of scouring and suspension during storms on the physiology of benthic bacterial populations is unknown, but the metabolic demands of disturbed benthic communities may decrease (Newbold 1992). Also, enhanced flow both from upwelling groundwater and enhanced downwelling that potentially occurs during high flows (Battin et al. 2003), may alter denitrification rates relative to those measured in the perfusion cores. For example, denitrification was highest at downwelling zones in Sycamore Creek, Arizona, suggesting a dependency on surface-derived organic matter since upwelling of groundwater only supplies low concentration DOC and BDOC waters to reactive sites (Holmes et al. 1996). Therefore, it is unknown how the NO_3 removal rate during storms changes, and the increased fluxes might illustrate subsidies of NO_3 being exported to downstream reaches.

Reach-scale N removal dynamics also change drastically during storms. Using the mean overland denitrification rate and the mean storm NO_3 concentration at peak discharge, the calculated $V_{f,dn}$ of 0.33 mm/min is up to 2 orders of magnitude greater than the baseflow $V_{f,dn}$, which is expected since the uptake efficiency relative to availability should increase with decreasing NO_3 concentration (Mulholland et al. 2008). Using a mean bankfull depth of 0.45 m (Cianfrani et al. 2006), a loss rate of 107 % per day was calculated for storm flow, which is consistent with high denitrification rates coinciding with low NO_3 concentrations (Royer et al. 2004). At peak flows, however, the residence time of water in the basin is less than one hour, so this removal rate is consistent with the 5% removal estimated above. The $S_{w,dn}$, calculated based on a mean bankfull width of 6.91 m (Cianfrani et al. 2006), was between 21 km and 330 km, indicating that the importance of denitrification in removing NO_3 during storms is similar to baseflow suggesting that the large increases in areal denitrification rates due to higher concentrations and quality of carbon compensates for the increases in the downstream NO_3 flux.

Removal of NO_3 via in-stream denitrification is important in reducing the export to downstream environments, but riparian zones also function as a control point for the fluxes of terrestrial N into the stream channel. Riparian zones have been identified as hot spots of N removal (Peterjohn and Correll 1984, Hill 1996, Newbold et al. 2010), and denitrification occurring in riparian forest buffers can reduce or buffer the loss of N from terrestrial environments. Measurements of riparian zone denitrification rates indicate variability within and among sites resulting in variations in N exports to streams (Hanson et al. 1994, Johnston et al. 2001). For example, denitrification rates measured from a riparian forest buffer on a 4th order stream in France varied between 9 μmol NO_3-N/ m^2 hr and 232 μmol NO_3-N/ m^2 hr (Pinay et al. 1993). Measurements from 1st order streams ranged from 1.4 μmol NO_3-N/ m^2 hr to 478 μmol NO_3-N/ m^2 hr for an agricultural stream in the Midwest (Roley et al. 2012) and from 8 μmol NO_3-N/ m^2 hr to 32 μmol NO_3-N/ m^2 hr for a southeastern coastal plain stream (Lowrance et al. 1995). Riparian denitrification rates are lower than in-stream denitrification rates (Roley et al. 2012), and subsurface nitrate budgets for a similar agricultural watershed showed riparian forest buffers removing only 26% of the subsurface inputs of NO_3 (Newbold et al. 2010). Including riparian zone denitrification rates in aquatic N flux models is important due to the soil-stream interface acting as a control point for fluxes of terrestrial N (Boyer et al. 2006), and management practices that enhance both in-stream denitrification coupled with efforts to reduce N inputs from the adjacent terrestrial environment would be most effective.

Because denitrification is potentially an important N sink in stream ecosystems, understanding the influence of organic C, among other factors, has profound implications for managing streams when attempting to reduce NO_3 load to downstream environments. Removal of NO_3 is especially important in agricultural streams with chronically high NO_3 concentrations, and permanent removal by processes such as denitrification is the most desirable. Management of agricultural landscapes in order to promote drainage typically involves removal of riparian vegetation and woody debris from streams (Allan and Flecker 1993); however, these practices remove C which could possibly limit stream benthic denitrification and accentuating already high NO_3 export to downstream ecosystems. In contrast, engineers constructing treatment wetlands and remediating contaminated groundwater have manipulated C levels to optimize NO_3 removal (Bickers and van Oostrom 1995, Schipper and Vojvodic-Vukovic 2000), and the same principles could be applied to managing stream ecosystems. Results from this study show that the availability of labile C can exert some control on the rate of NO_3 removal, and benthic processes have the potential to reduce or eliminate the export of N from WCC headwater streams. Although strategic additions of labile DOC could be difficult and associated with negative downstream effects, management efforts to optimize removal of NO_3 by denitrification could stimulate natural inputs of labile C through the promotion of wetlands or organic rich soils within the watershed (Hedin et al. 1998). The biogeochemical implications of this apparent linkage between C and NO_3 removal emphasize the importance of understanding the various factors controlling denitrification. Understanding the environmental conditions and the combined interactive effects of the various controlling factors that reinforce NO_3 uptake is necessary to assess the impact of stream systems on the export of NO_3 from watershed with high NO_3 inputs.

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Table Legends

Table 3.1: The organic content (%AFDM) of sediments used to measure denitrification in stream sediment perfusion cores and the total measured denitrification rates (D) for each core expressed as an area-based flux ± SE which was estimated by propagating the error in the slope of the regression between the concentration of $^{15}N_2$ in the water and time. There was no significant difference between the organic content of the sediments collected for experiment 1 and those collected for experiment 2, as well as no significant differences in organic content among treatments. However, organic content was a significant covariate in the two-way ANOVA conducted to test the differences in denitrification rates between experiments and between treatments.

Tables

Table 3.1

Core	Treatment	%AFDM	Denitrification μmol/m^2 hr
1	Control 1	1.11%	167.6 ± 0.07
2	Control 1	1.78%	187.4 ± 0.05
3	Control 1	1.11%	118.1 ± 0.07
4	Control 1	1.09%	29.5 ± 0.01
8	Control 1	1.96%	179.3 ± 0.05
5	Glucose	0.72%	86.6 ± 0.04
6	Glucose	2.24%	33.5 ± 0.02
7	Glucose	1.41%	73.9 ± 0.02
9	Glucose	1.19%	76.3 ± 0.04
10	Glucose	1.37%	29.3 ± 0.01
22	Control 2	1.33%	53.7 ± 0.02
23	Control 2	1.51%	43.5 ± 0.01
24	Overland	1.41%	894.3 ± 0.10
25	Overland	3.08%	1155.2 ± 0.08
26	Overland	1.25%	952.1 ± 0.26

Figure Legends

Figure 3.1: Diagram of perfusion core setup. Stream sediment cores were collected from White Clay Creek, and denitrification experiments were run with cores set up in a re-circulating mode. Experimental waters were pumped through from the bottom up to stimulate upwelling. Kynar Gas Sampling Bags with dual valves were used so that multiple samples could be taken over time without introducing air into the closed system.

Figure 3.2: Mean (± SE) of denitrification rates (D) measured during two experiments using stream sediment perfusion cores exposed to water with different concentrations of biodegradable dissolved organic carbon (BDOC). Differences among treatments were determined using a two-way analysis of variance (ANOVA) with an interaction term and organic matter content as a covariate. Significant interaction term determined using Tukey's test illustrated by *.

Figure 3.3: Biological lability profiles of biological reactivity classes of dissolved organic carbon (DOC) from experimental waters used during two denitrification experiments as a function of empty bed contact time (EBCT) of stream water fed bioreactors.

Figure 3.4: Mean (± SE) of NO_3 concentration over time during two different denitrification experiments.

Figure 3.5: DO concentrations measured over time during two different denitrification experiments.

Figures

Figure 3.1

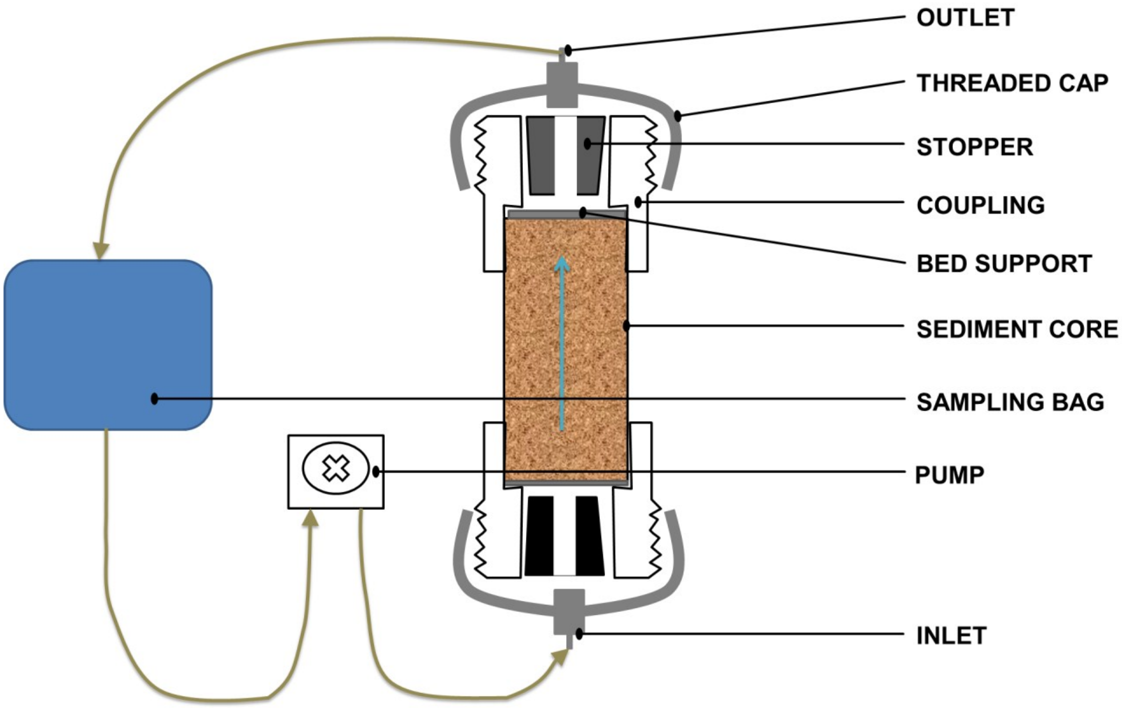


Figure 3.2

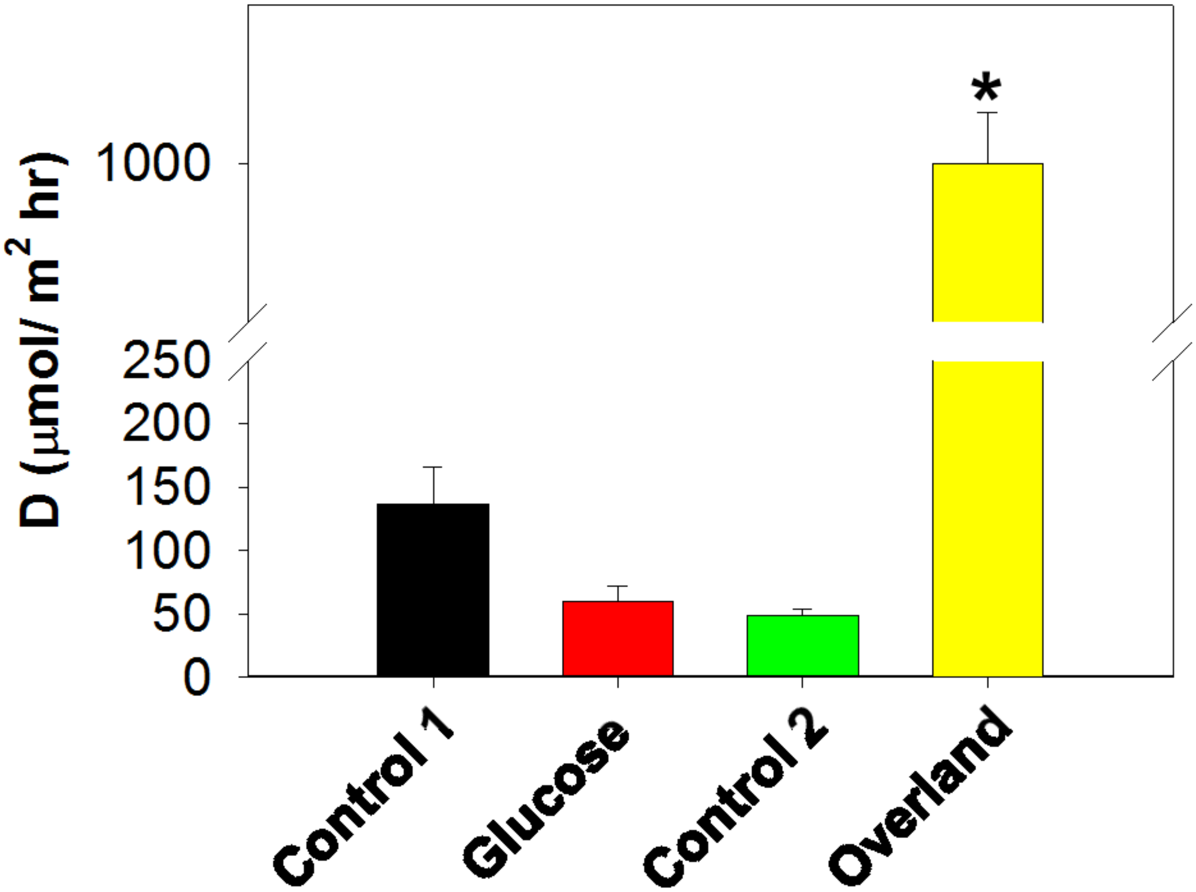
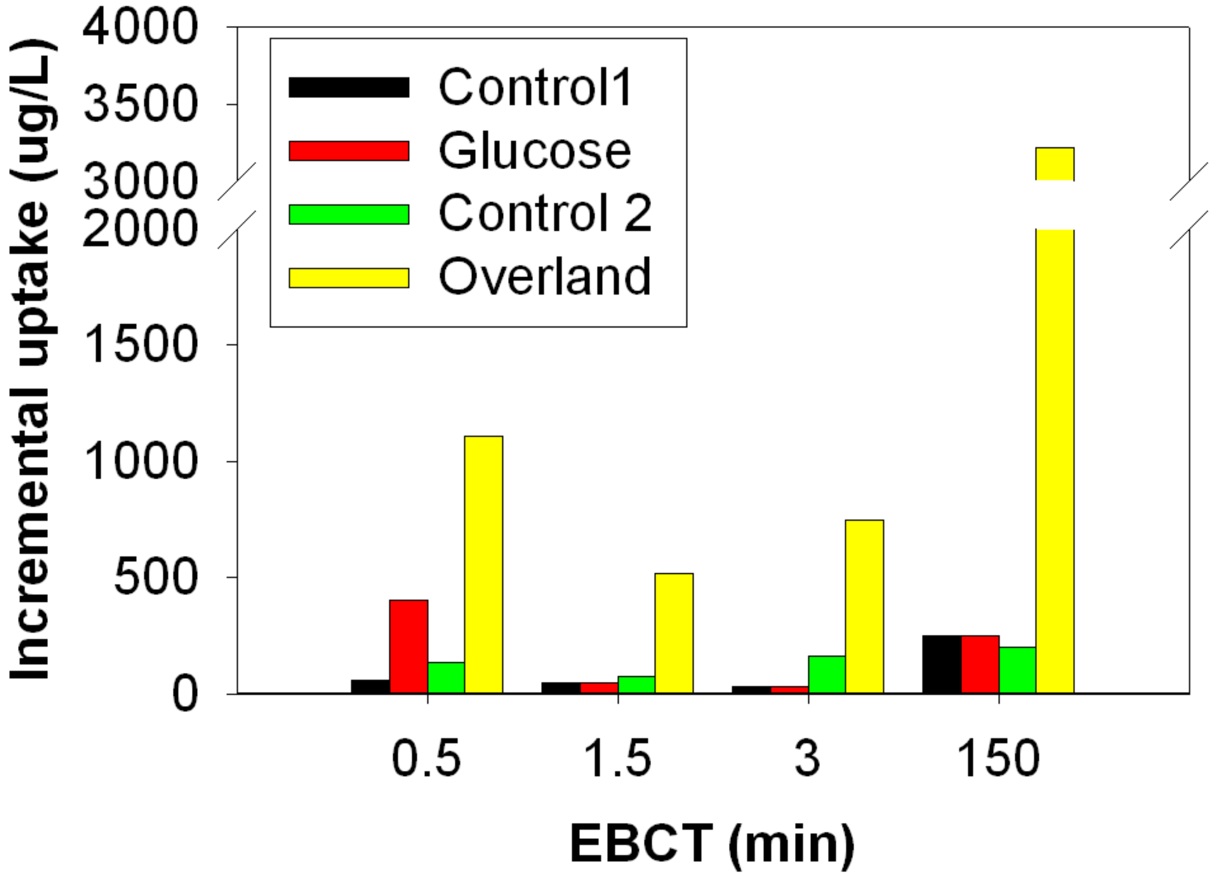

D (μmol/ m² hr)
1000
250
200
150
100
50
0
*
Control 1
Glucose
Control 2
Overland

Figure 3.3



Control1
Glucose
Control 2
Overland
Incremental uptake (ug/L)
4000
3500
3000
2000
1500
1000
500
0
0.5
1.5
3
150
EBCT (min)

Figure 3.4

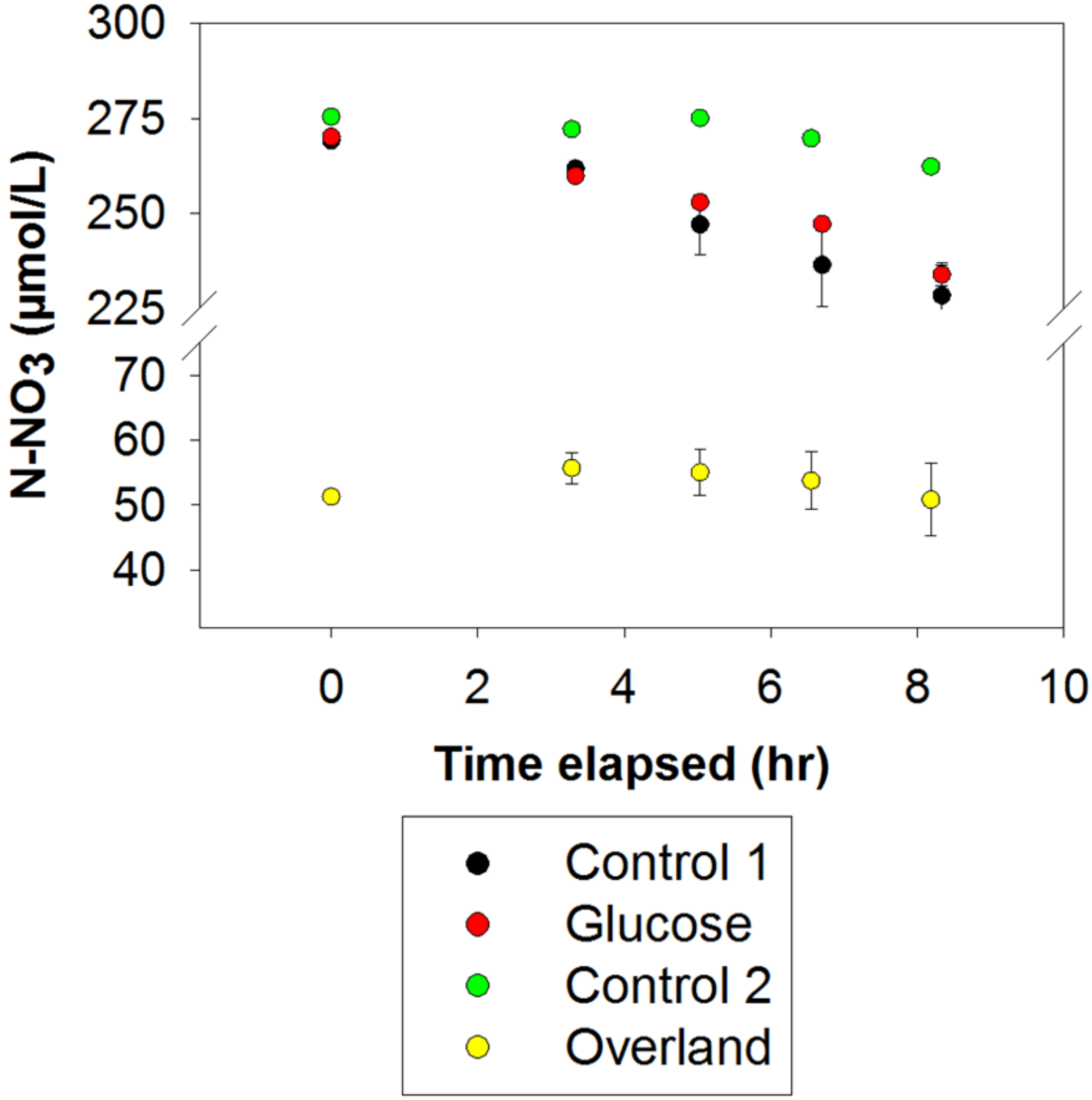
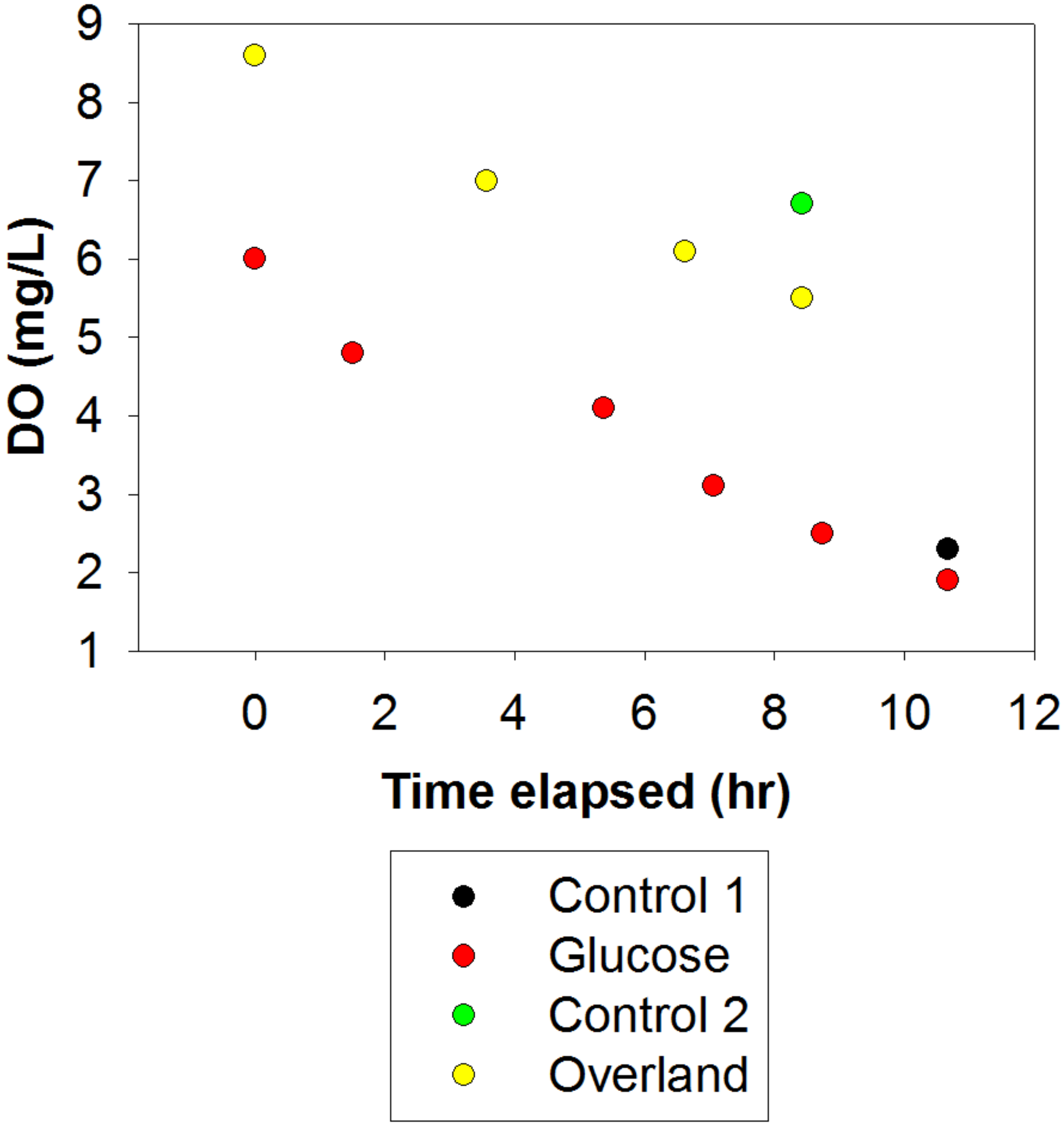

300
275
250
225
70
60
50
40
N-NO$_3$ (µmol/L)
0
2
4
6
8
10
Time elapsed (hr)
● Control 1
● Glucose
● Control 2
● Overland

Figure 3.5


9
8
7
6
5
4
3
2
1
DO (mg/L)
0
2
4
6
8
10
12
Time elapsed (hr)
Control 1
Glucose
Control 2
Overland

SUMMARY OF RESULTS

AND

CONCLUSIONS

Dissolved organic carbon (DOC) is the main energy source for heterotrophic stream microbes (Findlay and Sinsabaugh 1999), and C availability is also linked to in-stream N-cycling (Ziegler and Brisco 2004). The majority of stream water DOC comes from the terrestrial environment via hydrological flow through the subsurface, and therefore the concentration and composition of stream water DOC should depend on the proportional contributions of terrestrial sources and the relative importance of hydrology versus biological mechanisms of production and retention. The objectives of this dissertation were to quantify terrestrial source water contributions to a headwater stream, to characterize the carbon (C) in the source waters, and to determine the impact of terrestrial C on linkages between C and nitrogen (N) cycling. The export of terrestrially-derived DOC was evaluated by quantifying terrestrial source water contributions and characterizing source water DOC. The influence of terrestrial source DOC on in-stream N-cycling was evaluated by testing the influence of DOC quality on rates of denitrification, an important N removal mechanism.

Summary of Results

The main connection between terrestrial environments and aquatic ecosystems occurs via hydrologic transport. The hydrologic response of White Clay Creek (WCC) watershed was evaluated by determining water contributions from terrestrial sources using an end-member mixing analysis (EMMA). Source water contributions varied substantially between baseflow and storm flow, and these results support the assertion that complex subsurface flow networks transport water and dissolved solutes in a dynamic manner (Hinton et al. 1998).

As expected, EMMA demonstrated that groundwater sources sustain baseflow, but overland flow is activated during storms with contributions starting earlier for storms with higher antecedent moisture conditions. The selection of an overland flow end-member for the storm flow EMMA indicates the potential importance of a surface or very shallow subsurface source to streams that has typically been overlooked. These EMMA-derived contributions provide a first approximation of the sources to WCC. Using the EMMA-derived source contributions, the EMMA-predicted stream water DOC concentrations were calculated and provided an estimate of the concentration expected due solely to contributions from the identified terrestrial sources. The ratio of observed to EMMA-predicted DOC concentrations permitted an evaluation of the relative importance of hydrology in controlling stream water chemistry. The high ratios of observed to EMMA-predicted DOC concentrations calculated for the majority of samples suggest some mechanism of production occurring along flow paths or within the stream channel during low flow and the potential presence of additional sources of DOC during storms.

The influence of watershed hydrology on the composition of stream water DOC was evaluated by measuring the biodegradable DOC (BDOC) in terrestrial source waters and stream water under different hydrologic conditions that alter source contributions. The concentrations of DOC in terrestrial source waters decline with soil depth. The labile BDOC concentrations in source waters also declined from overland flow to soil water to shallow well water, but BDOC in seep water was greater than that of both unsaturated soil water and shallow riparian well water suggesting that the processes occurring in the terrestrial environment cause high variability in DOC quality. Both DOC and BDOC concentrations increased during storms, but there were significant differences in the responses of the labile and semi-labile biological reactivity classes over time. The dynamic patterns in stream water BDOC observed through this study contribute to our understanding of DOC biogeochemistry and reveal that storms provide subsidies to downstream reaches and export labile BDOC from the watershed.

Based on the evidence of high variability in BDOC among terrestrial sources, the effect of DOC quality on in-stream denitrification rates was tested using both glucose amended stream water and overland flow water. No significant increase in denitrification was observed for the glucose treatment, but rates were significantly higher for the overland flow treatment. These results suggest either a threshold value of labile BDOC is needed to stimulate denitrification or that the concentration of semi-labile BDOC is more influential in regulating denitrification. Additionally, sediment organic matter content was a significant covariant suggesting the possibility of a positive influence of particulate organic C on denitrification.

Conclusions

The evidence presented in this dissertation demonstrates the importance of considering both transport and biogeochemical processes in concert rather than independently. During baseflow, exchange rates are slow and residence times of water in the hyporheic zone are long. Both of these conditions are conducive for higher DOC uptake and greater NO_3 loss potential via denitrification. However, higher amounts of recalcitrant DOC and lower denitrification rates during baseflow result in longer uptake lengths and export to downstream reaches. Alternatively, higher exchange during storms and microbial community disturbance due to storm flow result in lower biogeochemical processing potential despite higher denitrification rates associated with greater concentrations of labile and semi-labile BDOC in storm flow.

During baseflow, groundwater sources (spring seeps, deep groundwater, and shallow riparian groundwater) were determined to be the only contributors to stream flow. Seep water had higher labile BDOC compared to other groundwater sources, and therefore seeps may be an important source of C to WCC. Previous work has shown dramatic increases in DOC concentrations with distance from spring seeps, but the amount of carbohydrate-C as a percentage of the total DOC concentration decreased (Kaplan et al. 1980). Within the stream channel, spring seep water presumably mixes with shallow and deep groundwaters, which are sources with lower labile BDOC concentrations but higher total DOC concentrations. The high ratios of observed to EMMA-predicted DOC concentrations suggest additional mechanisms of production further explaining the increases in DOC observed downstream of spring seeps. The large distance between the spring seep locations and the 3rd order stream site make it unlikely that seep water plays such an important role in controlling the stream chemistry, as the EMMA results would suggest. Previous work in nested headwater catchments have shown similar terrestrial sources contributing to stream water at different scales but changes in the relative contributions with groundwater contributions increasing downstream (Brown et al. 1999, Soulsby et al. 2003, James and Roulet 2006). The impact of catchment size on source contributions in WCC was not assessed. In order to evaluate whether the relative source contributions change significantly with catchment scale, future work applying EMMA methods to stream water samples from the 2nd order tributaries could be compared to the results from the 3rd order stream reach.

Stream water DOC concentrations increased during storms as additional terrestrial storage zones discharged into the channel, and antecedent moisture conditions seemed to be a significant factor controlling the influence of storms on terrestrial DOC export. For example, EMMA-derived contributions of overland flow, the source with the greatest DOC and BDOC concentrations, were greatest for storms occurring during wetter conditions, but storms during drier conditions had higher peak labile BDOC. These results together suggest that either overland flow is not the only source of labile BDOC or that overland flow waters vary in concentration over time. High ratios of observed to EMMA-predicted DOC concentrations during storms suggest additional sources contributing to stream flow which might include a rising groundwater table that connects the previously unsaturated soil water source to the stream. Moreover, DOC in the upper soil profile during storms has been shown to be an infinite source (Jardine et al. 1990, McGlynn and McDonnell 2003, Sanderman et al. 2009), and therefore, indicates the possibility of dilution of high DOC and BDOC sources such as overland flow during high discharge and wetter conditions.

The evaluation of possible dilution effects is difficult due to the dynamic changes in source contributions that occur during storms and the high variability in storm event parameters. However, certain patterns are evident among storms in WCC suggesting dilution of DOC and labile BDOC during larger storms. For example, the ratio of observed to EMMA-predicated DOC concentrations were less than 1 during the most extreme high discharge and under the highest antecedent moisture index values, both conditions that would favor dilution effects and make uptake unlikely due to decreases in the mass transfer coefficients associated with the metabolic demands of disturbed benthic communities (Newbold 1992). The pattern of decreasing labile % BDOC at high flows despite increases in BDOC flux further supports the possibility of dilution. Others have attributed the dilution of DOC during storms to higher groundwater contributions with low DOC concentrations during the falling limb of the hydrograph compared to the rising limb (Van Verseveld et al. 2008), but high nitrate concentrations in groundwater of WCC suggests this is not the case.

Alternatively, DOC concentrations in throughfall can decline during storms (Van Verseveld et al. 2008) resulting in dilution of overland flow if saturated surface areas intercept throughfall contributions and rapidly deliver them to the stream due to high connectivity (Michalzik et al. 2001, Inamdar and Mitchell 2006). The pattern of higher ratios on the rising limb of the hydrographs followed by decreases in the ratio as the storm progresses supports this hypothesis that throughfall subsidizes overland flow contributions by adding high concentrations of DOC at the beginning of the storm and causes dilution of DOC at the end of the storm. However, without detailed temporal measurements of DOC in terrestrial source waters, especially overland flow, it is difficult to quantify whether terrestrial source pool DOC is variable in concentration or an infinite source that is being diluted by precipitation water.

The impact of terrestrial DOC on stream ecosystem function depends on both the rate of supply of DOC to reactive sites as well as the effect of C on rates of biogeochemical processing. The dependence of denitrification on the availability of oxidizable organic C ultimately connects the removal of NO_3 to the dynamic changes in BDOC delivery from terrestrial sources. The objectives of the denitrification experiments were to determine the influence of BDOC on denitrification. These experiments did not take into account hydrologic variations that happen in conjunction with changes in BDOC, and therefore it makes relating laboratory measurements to in situ process rates difficult. For example, the chemistry of overland flow is similar to storm flow, but the experiments did not stimulate the change in transport that occurs during storms.

The laboratory results did suggest a threshold value of labile BDOC needed to significantly influence the rate of NO_3 removal via denitrification and demonstrate the importance of semi-labile BDOC, a larger pool of available C than labile BDOC. Understanding the environmental conditions that control denitrification is necessary before the combined interactive effects can be assessed. Future work should be aimed at further defining the influence of different biological reactivity classes of DOC on denitrification by using BDOC in multiple concentration levels. Additionally, the influence of increased DOC quality in conjunction with decreases in residence time should be evaluated to determine the influence of storms due to the disproportionately large role storms play on DOC export to downstream environments.

Broader impacts

This dissertation combines the fields of ecosystem ecology, hydrology, and biogeochemistry in an effort to increase our understanding of the role of terrestrial environments in delivering C to stream ecosystems. Broadening the boundaries of stream ecology to encompass the surrounding terrestrial environment stresses the dynamic aquatic-terrestrial linkages, puts streams within the context of the broader ecosystem, and allows for greater understanding of export processes that influence stream biogeochemical cycles (Hynes 1975).

Biological processes cannot be considered independently of hydrologic processes since interactions between water, soil, and microorganisms determine the concentration and lability of DOC that is associated with different flow paths. Understanding changes in hydrology that accompany land-use change are particularly critical for managing watersheds. Because this research addressed questions regarding the contribution of terrestrial environments to stream ecosystem function, it provides data on the downstream impacts of land-use activities which are important for public policy decisions regarding upstream and headwater protection and sustainable land-use practices. Additionally, the seasonal variations observed highlight the importance of temporal changes that should be considered in combination with predicted climate fluctuations.

Insufficiency in linking the water cycle to biogeochemical cycles needs to be addressed through additional research.

My goal was to better understand how terrestrial DOC influences stream ecosystem function by explicitly considering how streams connect with the surrounding land-based environment. The results presented by this dissertation highlight the importance of multidisciplinary research and demonstrate the need for greater considerations of the interactive effects of transport and biogeochemical processing in stream ecosystems.

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