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Flow Regime Influences on Stream and Riparian Soil Carbon Dynamics in the Ozark Highlands and Boston Mountains of Arkansas

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Flow Regime Influences on Stream and Riparian Soil Carbon Dynamics in the Ozark Highlands and Boston Mountains of Arkansas

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by

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ABSTRACT

The natural flow regime exerts primacy over lotic ecosystem patterns and processes. However, little work has examined the influence of flow regime on instream and riparian carbon (C) dynamics in minimally-impacted, temperate forested systems. To date, most research efforts have focused on characterizing C movement and transformations across biomes and land use categories; however, flow regime represents an overlooked, finer level of detail that may drive differences in ecosystem function. My dissertation objective was to measure C fixation and movement within and across multiple environmental spheres (e.g. within stream channels, between stream surfaces and the atmosphere, and from riparian soils to the atmosphere) to determine whether estimated differences in hydrologic characteristics may give rise to natural variation in ecosystem function. I measured stream C gross primary production (GPP) and ecosystem respiration (ER), stream carbon dioxide (CO_2) and methane (CH_4) flux rates and sources to the atmosphere, and riparian soil C respiration for study sites classified into the two dominant natural flow regimes in northern Arkansas: Groundwater Flashy (i.e. *Groundwater*) and Runoff Flashy (i.e. *Runoff*). Groundwater streams tended to exhibit greater and more variable GPP, ER, and CO_2 -evasion and CH_4 -evasion across sites than *Runoff* streams, though no differences between flow categories were statistically significant. Soil respiration differed across seasons and differed between flow regimes during spring ($p= 0.01$). Runoff sites tended to have greater rates of soil respiration over the year. Hydrologic variables explained up to 88% variation in stream processes even though categorical comparisons between natural flow regimes often did not differ. Field-estimated annual GPP was negatively impacted by the number of no-flow days across flow regimes ($\rho= -0.88$, $p= 0.02$), and annual net metabolism became more heterotrophic with increasing average annual discharge across sites ($\rho= -0.74$, $p= 0.002$). In addition, source composition of C fluxes to the atmosphere depended upon hydrology; isotopic data revealed both biogenic and thermogenic CH_4 in *Groundwater* streams while *Runoff* streams only evaded thermogenic CH_4.   My efforts provide evidence that instream and riparian soil processes, such as GPP, ER, and C gas fluxes, are influenced by variation in hydrology within a forested biome.

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He loved fishing and being near water- I think of him every time I step into a stream.

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INTRODUCTION

Anthropogenic climate change presents a pressing problem that requires a detailed and nuanced understanding of the carbon (C) cycle to address current problems and mitigate future impacts. Human impacts such as the conversion of forest to agricultural and urban land decrease the size of terrestrial biomass and soil organic matter, which serve as important sinks of C, while the extraction and combustion of fossil fuels release C into the atmospheric sink (Bala et al. 2007, Gurney et al. 2009). These activities have led to a shift in global C dynamics characterized by movement of C from historic pools, such as burial in deep ocean and terrestrial sediments, to the atmosphere. Coupled models of atmospheric C gas concentrations and global temperature clearly illustrate that increasing greenhouse gas emissions from human activities are raising the average global temperature. Future climate scenarios predict a suite of environmental consequences of continued rapid movement of C to the atmospheric gas pool and warmer average global temperatures: changes in precipitation patterns, sea level rise, ocean warming, melting glaciers and decreased snowpack, and changes in community structure and function of plants and animals (Parmesan and Yohe 2003, IPCC 2013, McNutt 2013). Further, changing precipitation patterns have the distinct possibility of altering C dynamics in streams and rivers, as C is a primary nutrient in these systems that spirals down the river continuum, is taken up by organisms, and moves into the floodplain at various points depending upon streamflow (Newbold et al. 1982, Hall et al. 2016).

Carbon enters streams through various sources in both organic and inorganic forms. Lateral inputs of organic matter from the landscape via abscission and flooding represent an important subsidy of particulate and dissolved organic C as well as microbially-derived inorganic C in forested streams (Nakano and Murakami 2001, Marcarelli et al. 2011). Instream gross primary production (GPP) provides another component of the stream C budget as light energy is fixed into algal biomass and later can be respired as CO_2 (Odum 1956). Inorganic C also enters streams by groundwater intrusion and diffusion of atmospheric CO_2 directly into the stream or via precipitation (Hall and Tank 2005, Doctor et al. 2007). Carbon is ultimately transported downstream or evaded to the atmosphere as CO_2 or CH_4 (Cole et al. 2007, Battin et al. 2009). These inputs and exports of C can be largely dependent on a number of flow-related variables, such as water velocity, flow magnitude, groundwater intrusion, and air-water gas exchange (Finlay 2003).

The natural flow regime is considered "the master variable" in streams and rivers, controlling ecosystem processes and functions at hierarchical temporal and spatial scales (Power et al. 1995, Allan and Castillo 2007). Flow regime is comprised of the magnitude, timing, duration, frequency (or predictability), and rate of change of water moving through a channel (Poff et al. 1997). The dynamic movement of water and sediment within a channel as a function of a system's typical flow characteristics organizes the habitat in a way that provides a template upon which life history strategies and morphological characteristics are forged. Indeed, flow regime dictates the habitat template (Southwood 1977) and, in turn, the species that reside there. Further, natural flow variability through the river continuum over time provides a mosaic of habitat patches at differing stages of succession following disturbances such as flooding and drying (Pringle et al. 1988, Townsend 1989, Winemiller et al. 2010). Hence, flow regime can exert strong controls on species distributions and abundances and, in turn, ecosystem function and services (Poff et al. 1997, Hart and Finelli 1999).

Human-altered flow regimes can influence both the ecology and biogeochemistry of lotic ecosystems. The science of environmental flows investigates the quantity, timing, and magnitude of water needed to preserve freshwater ecosystems and the human well-being that depends on these ecosystems. Aquatic systems rely on environmental flows to sustain ecosystem functions and services (IPCC 2013, Stocker et al. 2013). Disruption of or long-term shifts in natural flow characteristics from land use alteration or changes in precipitation patterns can result in a system taking decades to even centuries to reach a new dynamic equilibrium as the physical environment and biota adjust to the new flow regime (Poff et al. 1997). Clear management guidance is critical to preserving flow-ecology relationships under the continued pressures of climate change and conversion of land to urban or agricultural uses to accommodate the needs of the expanding human population. Altered weather patterns marked by more intense droughts punctuated by more frequent, intense storm events are expected to impact aquatic communities, making it necessary to quantify current flow-ecology relationships as soon as possible to provide data to inform management and policy decisions (Gleick 1989, Vörösmarty et al. 2000).

One framework for determining and implementing environmental flow standards is the ecological limits of hydrologic alteration (ELOHA). ELOHA is a synthesis of methods and techniques for assessing environmental flows and hydrologic characteristics. The goal of the framework is to provide quantitative information to create ecologically sound and socially agreeable policies and management decisions. ELOHA consists of four steps that can be undertaken to develop regional flow standards. First, long-term hydrologic data can be used to build a "hydrologic foundation" consisting of hydrographs through time and across various high or low flow events. Then, streams are classified into distinct flow regimes using ecologically-relevant flow variables. Third, hydrologic alteration based on comparisons between historic baseline and current flows is determined. Finally, flow-response relationships are quantified using the literature and field studies. ELOHA can be used to inform the public, stakeholders, and policymakers of the ecological consequences of altering streamflow while providing a tangible path by which to implement sound, ecologically-relevant environmental flow standards (Arthington 2006, Poff et al. 2010).

There is currently a paucity of research directly linking ecosystem-level metrics, and specifically C fixation, release, and evasion, to flow regime. This lack of data is surprising given the urgency of climate change and the need for flow-ecology relationships at multiple levels of ecological organization (Poff et al. 2010, Poff and Zimmerman 2010). In this dissertation, the natural flow regime paradigm was utilized to investigate how carbon dynamics may vary between two modeled flow regimes with regard to instream C fixation and release (i.e. gross primary production + ecosystem respiration), stream surface CO_2 and CH_4 evasion to the atmosphere, and the return of CO_2 from soil and terrestrial organic matter to the atmosphere via riparian soil respiration. Respective flow sources predicted to dominate two of the seven natural flow regimes modeled by Leasure et al. (2016) were also determined to test and validate flow classifications soil respiration. This work represents a characterization of C movement across three environmental spheres within streams nested in a temperate forested biome. Here, I present evidence that factors related to C movement as well as certain aspects of C transport may differ by flow regime.

Flow classification using long-term hydrologic data is an important step toward developing environmental flow standards that fully account for the flow-ecology relationships present in a system, and marks the second step within the ELOHA framework. Flow regime classification ultimately provides a context for determining flow-ecology relationships within different stream types that can be used to inform management and policy (Arthington et al. 2006, Poff et al. 2010). Natural flow regimes of the Ozark and Ouachita Interior Highlands were recently modeled by Leasure et al. (2016). The two dominant flow regimes in the Ozark

Highlands and Boston Mountains ecoregions of northern Arkansas consist of Groundwater Flashy and Runoff Flashy systems (hereafter *Groundwater* and *Runoff*, respectively), and characterized by differences in drying, flooding, and flow sources.

Whole-stream metabolism is an ecosystem-level metric that may be susceptible to changes in flow (Acuña et al. 2004). Flow extremes, such as drought or large floods, depress primary production and respiration while lack of flow variation due to upstream dams also hinders metabolism by reducing the sloughing of senescent algal cells (Uehlinger et al. 2003, Chester and Norris 2006). Flow regime-related differences in the number of dry days or flood events may, in turn, give rise to flow type-specific differences in ecosystem function within a biome and land-use category. Additionally, understanding how differences in streamflow influence GPP and respiration in minimally-impacted systems can provide a baseline for future work evaluating potential interactions between land use, flow regime, and stream C dynamics. Chapter 1 consists of a 422-day field experiment to test the effect of modeled flow regime on stream gross primary production and ecosystem respiration, the net balance of which may affect net C emissions, in Groundwater and Runoff streams within temperate deciduous forests. A simulated dataset was also constructed from field measurements to evaluate potential outcomes with increased sample size.

Carbon budgets have only recently begun to include greenhouse gas emissions from streams (Cole et al. 2007). Streams were historically cast as "pipes", transporting C and nutrients down the river continuum and ultimately to the ocean. However, recent efforts have revealed that streams are also active reactors of carbon (Battin et al. 2009, Casas-Ruiz et al. 2017). Carbon in the stream is not only transformed between organic and inorganic forms within the water column, but is also actively evaded to the atmosphere in the form of CO_2 and CH_4. Other studies have elucidated patterns and controls on stream C fluxes, such as stream size, instream processes, landscape position, and biome (Dawson et al. 2001, Guerin et al. 2006, Hotchkiss et al. 2015). However, no studies to date have quantified CO_2 and CH_4 fluxes within the context of flow classification within a biome and land use category. In Chapter 2, I employed a floating chamber method in a summer field study in which I sampled gas evading from the stream surface over a range of discharges to investigate the influence of flow regime on gas evasion rates, sources, and factors related to C gas evasion rates.

Soil respiration represents the primary pathway by which CO_2 once held in terrestrial biomass returns to the atmosphere (Schlesinger and Andrews 2000). The primary drivers of respiration are soil temperature and moisture (Raich and Tufekciogul 2000, Raich and Schlesinger 2002), and agricultural and urban land use increase C flux rates from soil (Schlesinger and Andrews 2000, Lohila et al. 2003, Kaye et al. 2005). Additionally, greater respiration rates have been found in the riparian areas of forested streams than adjacent hillslope soils (Pacific et al. 2008, Pacific et al. 2011). However, no work has investigated whether stream flow regime may give rise to differences in riparian soil respiration rates. Further, relationships between soil C flux, temperature, and moisture may also differ between flow regimes. In Chapter 3, I report findings from a field study in the riparian zones of *Runoff* and *Groundwater* flow regimes in the same six streams within the Ozark Highlands and Boston Mountains utilized in Chapters 1 and 2. This study provides insight into potential flow classification influences on riparian CO_2 flux rates as well as how factors related to soil CO_2 flux rates may vary between due to adjacent stream flow regimes.

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CHAPTER ONE

The influence of flow regime on Ozark stream metabolism

ABSTRACT

The natural flow regime is considered the "master variable" in lotic systems, controlling every aspect of structure and function at organism, population, community, and ecosystem levels. however, few studies have compared ecosystem-level metrics across multiple flow regimes within a biome or land use category. I sought to estimate forested stream metabolism across the two dominant flow regimes in northern Arkansas, Runoff Flashy (*Runoff*) and Groundwater Flashy (*Groundwater*) streams. Flow regimes differed in intermittency, turbidity, and water temperature, which I expected to result in differences in annual production and respiration. I tested these hypotheses using three main approaches: 1) I measured annual metabolism (i.e. gross primary production, ecosystem respiration, and net ecosystem production) as well as a suite of biological, physical, and chemical variables in three runoff and three groundwater-dominated primarily deciduous-forested stream reaches, 2) I evaluated potential relationships between metabolism metrics and environmental characteristics, including flow-ecology relationships, and 3) I used field data to create a larger, simulated stream-hydrologic and -metabolism dataset (n=15 per stream type) to estimate the statistical power needed to ascertain potential differences in future studies. Annual gross primary production (GPP) and annual ecosystem respiration (ER) estimated from continuous field data collected from June 2015-16 did not differ over the study period, though both tended to be numerically greater in *Groundwater* streams ($p = 0.25$, $p = 0.22$, respectively). GPP averaged 327.8 (standard error= $\pm$ 131.1) g O_2 m^{-2} y^{-1} in *Runoff* streams and 533.1 (standard error= $\pm$ 57.9) g O_2 m^{-2} y^{-1} in *Groundwater* streams, while ER averaged 556.7 (standard error= $\pm$ 261.0) g O_2 m^{-2} y^{-1}in *Runoff* streams and 1,402.1 (standard error= $\pm$ 480.0) in *Groundwater* streams. Field-estimated GPP was negatively impacted by the number of no flow days across flow regimes ($\rho = -0.88$, $p = 0.02$), and net metabolism became more heterotrophic with increasing average annual discharge across sites ($\rho = -0.74$, $p = 0.002$). Simulated *Groundwater* streams exhibited 44% greater primary production ($p = 0.04$) and 124% greater respiration rates ($p = 0.007$) than simulated *Runoff* streams. Computer-simulated stream data revealed that *Groundwater* streams may be more autotrophic with increasing numbers of high-flow days, while *Runoff* stream annual GPP was suppressed by high-flow days (GPP: $\rho = -0.57$, $p = 0.03$), suggesting climate-change or human-driven changes in stream flow could change annual processes. Characterizing ecosystem-level responses to differences in flow can reveal mechanisms governing stream metabolism and, in turn, provide information regarding trophic state and energy inputs. This work highlights the importance of variation in flow within a biome and land use category to ecosystem function.

INTRODUCTION

The natural flow regime exerts primacy over water quality and quantity, habitat structure, disturbance regime, and, in turn, ecological processes and functions in lotic systems. Flow regime is characterized by the timing, duration, magnitude, frequency, and rate of change of water flowing through a channel over various temporal scales (Poff et al. 1997), arranging habitat space and thereby creating a unique template for life history strategies and community interactions (Southwood 1977, Poff and Ward 1990). Natural disturbances, such as flooding and drought, serve as life cycle prompts for many fishes and macroinvertebrates, whose reproductive cues are intimately linked with predictable, seasonal changes in flow (Poff and Ward 1989, Huryn and Wallace 2000, Humphries and Baldwin 2003, Lytle and Poff 2004). Flow regime is ultimately a byproduct of landscape-level processes and variation, as climate, topography, geology, vegetation, and soils interact to determine primary water sources (e.g. groundwater vs. runoff), quantity of within-channel flow, and geomorphology. Indeed, flow regime is typically a region and land cover-specific phenomenon; streams reflect the diverse biomes that generate and sustain their flows as well as the relative contributions of groundwater, surface water, soil water, and precipitation (Hynes 1975).

Even though the importance of naturally variable flow has become widely accepted by freshwater scientists over recent decades, water resource management has been slow to implement this model into policy goals (Poff et al. 1997, Bunn and Arthington 2002, Biggs et al. 2005, Poff et al. 2010). This natural variation in quantity, quality, and timing of flows that is necessary to support a system's physical, chemical, and biological integrity while also providing ecosystem services to humans defines a stream's environmental flows (Dyson et al. 2003, Poff and Zimmerman 2010). Static measures of hydrology, primarily minimum daily flows, are still the norm across thousands of river miles, and environmental flows remain threatened by overuse of water for human uses such as agriculture and industrial ends (Richter 2003). Environmental flow protection is critical to preserve many of the services streams provide to humans, such as natural regular floodplain inundation that supplies water, nutrients, and organic matter to agricultural fields and wildlife as well as variable instream flows that preserve water quality, habitat heterogeneity, and biodiversity (Postel and Richter 2003, Richter 2003). The natural flow regime requires greater attention from management to develop benchmarks that are tailored to an area's distinct environmental flows, as biota in differing flow regimes are likely adapted to different habitat and flow templates. This has remained largely untouched even with existing literature that provides suggestions and frameworks for the synthesis of environmental flows and current flow assessment techniques (Richter and Thomas 2007, Poff et al. 2010, Merritt et al. 2010, Olden and Naiman 2010).

Humans continue to alter flow regimes directly and indirectly with devastating consequences to biodiversity and supplies of potable water. Direct amendments to channel flow, such as channelization and dredging, reduce habitat heterogeneity and biodiversity. Damming also negatively impacts biotic interactions and ecosystem function by preventing immigration and emigration through the river continuum (Ligon et al. 1995, Lajoie et al. 2007, Dugan et al. 2010). Landscape changes such as construction of impervious surfaces, cultivation of fields for row crop or pastoral agriculture, and removal of native vegetation indirectly alter the physical and chemical characteristics of streams as water moving over the landscape is not sequestered by vegetation and thus enters streams in increased amounts at higher velocities, resulting in flash flooding that introduces sediments, nutrients, or other pollutants into the channel (Jones et al. 2000, Paul and Meyer 2001, Walsh et al. 2005). In addition to increased flow flashiness, the magnitude of flooding is often more severe adjacent to anthropogenic activities. Urban and agricultural land use modify natural disturbance regimes (e.g. flooding and drying), disrupting critical cues in the life cycles of many aquatic biota (Allan 2004, Paul and Meyer 2001, Poff et al. 2006). Further, climate change is also altering weather patterns, increasing storm intensity, and contributing to increased stream intermittency as average global temperatures continue to rise (Stocker et al. 2013). Given these widespread hydrologic impacts and the potential for flow regimes to continue to change drastically as the human exerts increasing pressure on water resources and global climate, recent efforts have focused on characterizing ecological flow-response relationships to ascertain environmental flows necessary to maintain biodiversity and ecosystem function that can, in turn, inform management decisions.

The ecological limits of hydrologic alteration (ELOHA) framework synthesizes existing methods and provides a clear, stepwise outline for determining environmental flow standards.

The goal of ELOHA is to catalyze development of regional flow standards based on flow-ecology relationships within minimally-impacted as well as altered systems to establish thresholds past which biodiversity and ecosystem function decline. The framework consists of scientific and social processes that integrate empirical findings with societal needs and values, with an ultimate objective of maintaining ecological function while providing for human needs. The scientific process consists of establishing a hydrologic foundation utilizing hydrograph data from reference and impacted streams, classifying stream flow types based on hydrology and geomorphology, assessing flow alteration, and establishing flow-ecology relationships from biological data, which provide a scientific basis for environmental flow standards that can then be developed based on management needs and social objectives (Poff et al. 2010).

Flow-ecology relationships are needed to determine whether flow alteration may have differing consequences across multiple flow regimes (Poff et al. 2010, Arthington et al 2012). Several natural flow categories exist for streams within the Ozark and Ouachita Interior Highlands in Arkansas, eastern Oklahoma, and southern Missouri (Leasure et al. 2016), but efforts to characterize these systems based on their unique hydrologies in the field have only recently begun. Leasure et al. (2016) revealed distinct geographic areas demarcated by dominant flow types which are likely functionally unique. Key differences between flow regimes are frequency and duration of low flow days and floods as well as dominant water sources. Detailed field measurements are needed to confirm modeled flow classifications, especially in headwater systems where the resolution of data used to construct flow class models is low. Additionally, recent efforts in Arkansas have begun to establish ecological flow-response relationships at the population and community levels (Bruckerhoff and Magoulick 2017, Yarra and Magoulick 2017, Lynch et al. 2018) but to date no ecosystem-level metrics have been employed to establish relationships between flow regime and aquatic primary producers, which are foundational players in stream food webs. Further, it is imperative to ascertain the extent of variation in ecosystem function explained by flow classification within reference forested streams before examining anthropogenic alteration of water quality and quantity.

Stream metabolism is an indicator of nutrient cycling, carbon (C) dynamics, and trophic status that is sensitive to natural and anthropogenic disturbances, revealing ecosystem-level responses to changes in hydrology and geomorphology. Net ecosystem metabolism (NEM) is defined as the difference between gross primary production (GPP) and ecosystem respiration (ER) (e.g. NEM= GPP-ER) (Izagirre et al. 2008, Williamson et al. 2008). Metabolism can reveal whole-stream responses to landscape changes as well as predict potential bottom up effects on higher trophic levels.

Ecosystem metabolism is driven by proximal factors such as light and nutrients, which are influenced by distal influences from the surrounding watershed (Bernot et al. 2010). The indirect and direct susceptibility of primary production and respiration to landscape-level variation makes it a good metric for assessing impacts at the ecosystem level. Additionally, daily metabolism can vary temporally due to changes in light levels, organic matter inputs, algal biomass, and hydrology. Annual metabolism integrates this variability and estimates are greatly dependent upon the frequency of daily measurements; less frequent measurements can result in erroneous annual metabolism budgets for a given stream (Roberts et al. 2007). While others have examined daily metabolism in Ozark streams, these studies were short in duration, likely missing patterns or variation in metabolism that would be useful in characterizing natural Ozark forested stream function. I sought to remedy the issue of low temporal resolution by collecting continuous measurements through an annual time step.

Previous work assessing annual metabolism across multiple streams has focused primarily on the effects of biome and land use (Bott et al. 1985, Mulholland 2001, Bernot et al. 2010). The large dependence of other annual metabolism budgets on flow timing and magnitude (e.g. Roberts et al. 2007) suggests they will vary significantly across differing flow regimes within the same biome. Further, comparing variation in function within and among flow classes provides greater insight into processes and variables controlling metabolism. A stark paucity of work has addressed whether streams of comparable size but distinct hydrologic regimes exhibit similar metabolism and associated mechanisms such as light and nutrient regimes. Addressing this lack of data will reveal how flow-ecology relationships vary over the landscape. Further, such efforts yield a more complete portrait of natural ecosystem production in the absence of anthropogenic pressures that will reveal to what extent landscape alteration is affecting regional C cycles.

Primary production and respiration provide an ecosystem-level portrait of organic matter movement and transformation, biomass at multiple trophic levels, trophic structure, and potential drivers of other ecosystem processes. Primary production consists of the amount of C fixed into autotrophic biomass, which represents the energetic foundation for consumers across trophic levels. Respiration represents the release of inorganic C from organisms, revealing a complete picture of instream organic matter consumption supported by both allocthonous and autochthonous production (Mulholland et al. 2001). Importantly, metabolism reveals C movement through a whole system; given that C cycling is coupled with other nutrient cycles and forms the basis of both autochthonous and allochthonous production, drivers of stream metabolism likely control other ecosystem-level rates such as nutrient uptake and processing and secondary production (Meyer et al. 2007, Izagirre et al. 2008, Bernot et al. 2009)

Existing conceptual models of headwater stream metabolism have posited that factors controlling metabolism differ by biome (Mulholland et al. 2001), land use category (Bernot et al. 2009), and season (Roberts et al. 2007) (Figure 1a). In reference systems, biome and season are considered the primary drivers of differences across streams. However, others have shown distinct hydroecological regions at hierarchical spatial scales characterized by significant variation in flow dynamics within a biome (Poff et al. 2006, Leasure et al. 2016). This intra-biome variation arises from changes in climate and geology down the stream continuum and across sub-basins (Thoms and Parsons 2003). Differences in metabolism within a biome would represent a departure from existing models, which have until now not explored finer resolution in annual estimates of C production and release based solely on flow regime.

Flow variability within a stream can be a determinant of annual metabolism, as flow extremes can exert a strong influence on organic matter movement through the system (Acuna et al. 2004, Roberts et al. 2007). High flows can depress primary production during while increasing production rates in autumn by removing leaves shading the stream. Storms can also influence respiration rates by reducing respiration initially due to loss of autotrophic biomass, then increasing rates as the autotrophic community recovers from scouring (Roberts et al. 2007, Izagirre et al. 2008). Consistently higher discharge, or higher discharge in one year compared to another, can depress metabolism rates by preventing regrowth of algal biomass. Further, others have found clear relationships between hydrologic regime and benthic organic matter that supports respiration (Acuna et al. 2007). Hot, dry summers that increase water temperature but reduce depth can support extensive algal production may lead to an overall reduction in metabolic rates over summer in the absence of scouring floods (Izagirre et al. 2008).

I propose a conceptual model of ecosystem metabolism informed by the natural flow regime paradigm (Poff et al. 1997) (Fig. 1b). Similar to existing models, climate and geology give rise to differences across biomes. However, I suggest that changes in geology and benthic substrate, dominant water source, and local weather across a landscape can also result in differences in flow within a biome. In turn, flow regime differences may interact with hydrologic alteration from land use change as well as season to control trends in annual production and respiration. This model not only refines and strengthens the predictive power of existing knowledge of factors controlling stream metabolism, but also provides a framework for developing flow-ecosystem relationships that are still rare in the literature.

Leasure et al. (2016) utilized daily records from 64 reference streams and mixture model cluster analysis to identify seven natural flow regimes in the Ozark and Ouachita Interior Highlands region of Arkansas, Missouri, and Oklahoma: Groundwater Stable, Groundwater, Groundwater Flashy, Perennial Runoff, Runoff Flashy, Intermittent Runoff and Intermittent Flashy. Two flow classifications, Groundwater Flashy and Runoff Flashy, represent the dominant flow types in northern Arkansas and southern Missouri. These flow regimes were demarcated by ecoregion, representing distinct hydroecological regions that may give rise to differences in stream ecosystem function.

Several key variables that define the natural flow regime of an area are likely related to annual metabolism. Drying and flooding can both temporarily depress primary production and respiration, while the weeks following these disturbances are typically marked by high rates of production and respiration as algae recolonize the benthos (Uehlinger 2000, Uehlinger 2006). Specifically, the number of dry days, number of days experiencing high flows (defined as >75% average daily flow), and number of flood events affect production and respiration; the strength of this effect would be dependent upon on the magnitude, frequency, and duration of the disturbance. My primary objective was to determine whether differences exist in stream total annual gross primary production, total annual ecosystem respiration, and total annual net ecosystem production between two flow regimes in northern Arkansas to characterize natural variation in ecological-flow responses. These flow regimes, Runoff Flashy and Groundwater Flashy (hereafter *Runoff* and *Groundwater*), comprise the majority of stream length in the Ozark and Boston Mountains ecoregions. I also sought to determine whether differences exist in variables such as stream discharge and geomorphology, light, periphyton biomass, physicochemical variables (e.g. total nitrogen, total phosphorus, turbidity), and flow metrics. I predicted that both stream types would be net heterotrophic, with ecosystem respiration outpacing primary production, given that all streams in the proposed study are forested and thus receive annual subsidies of leaf litter every autumn. I expected total annual gross primary production to be higher in *Groundwater* streams, as these streams tend to exhibit perennial flow, have relatively stable hydrology over the year (albeit greater numbers of floods, which may encourage algal growth by reducing competition), and exhibit low turbidity. Further, *Runoff* streams dry several days to weeks of the year, leading to the demise of the algal community in areas of no flow. I expected ecosystem respiration to be greater in *Groundwater* systems as well given that more primary production would in turn result in higher rates of respiration, and predicted that algal biomass would be higher in *Groundwater* streams. I expected streams to yield similar concentrations of total nitrogen (TN), total phosphorus (TP), and turbidity. I expected streams to differ in number of no flow days, number of high flow days (defined as the number of days above 75% of maximum annual flow), and number of flood events owing to the spatial separation of flow regimes by ecoregion.

METHODS

This study was conducted in six temperate, deciduous-forested, minimally-impacted headwater streams [forested land cover ranging from 84 to 97% of total watershed area (CAST 2007)] in Arkansas (Figure 2). I chose three *Groundwater* streams and three *Runoff* streams categorized based on an existing model (Leasure et al. 2016).

Streams were of similar size and discharge, and four were located upstream of USGS gauging stations. Two streams, Roasting Ear and Spring, were not located upstream of USGS gauging stations, but were located near streams of the same order and similar size with gauges. There were significant relationships between measurements made in those two stream reaches and two "proxy gauges" within the same watershed, allowing for quantification of flow metrics for all six streams over the year. I quantified high flow days, number of floods, and number of days with no flow by establishing upstream-downstream discharge relationships between study reaches and downstream gauges. High flow days were defined as exceeding the 75th percentile of mean annual discharge. Floods were defined as discrete hydrograph peaks exceeding the 100th percentile of mean annual flow.

I calculated reach-scale metabolism for a 200-meter length of each stream using data collected via the open-channel single-station method (Odum 1956, Riley and Dodds 2012). Others have compared the single- and dual-station methods and have shown similar estimates in streams with high air-water gas exchange velocities (Roberts et al. 2007, Bernot et al. 2010). Dissolved oxygen (DO) and temperature were measured every 15 minutes by Hydrolab DS5X multiparameter sondes (Hach Company, Loveland, CO) from May 2015 to June 2016 in a well-mixed area at the bottom of each study reach. Sondes were calibrated monthly and data were corrected when necessary by comparing with DO concentrations determined via Winkler titrations (Dodds et al. 2018). Reaeration coefficients as estimates of air-water gas exchange were determined via propane release in five out of six streams, while nighttime regression was utilized in one *Runoff* stream, Murray Creek. Propane release was necessary in five streams because nighttime regressions yielded significant relationships between ER and K_{600}, whereas no such relationship was present at Murray Creek. Corrections for groundwater contributions to reaches receiving appreciable inputs were made according to Hall and Tank (2005) by measuring DO in water at discernible upwellings as well as discharge down the reach to determine groundwater gains and losses down the study reach. Photosynethetically-active radiation (PAR) measurements were logged concurrently with metabolism parameters using an Odyssey light meter positioned in an area near the stream with open canopy. Stream metabolism was estimated based on diel changes in DO, temperature, depth, and light measurements; I used R package StreamMetabolizer to solve for GPP and ER utilizing a general single-station metabolism maximum likelihood model:

$$O_{2(t)} = O_{2(t-\Delta t)} + \left(\frac{GPP_{Total}}{\underline{z}} \times \frac{PAR_{(t)}}{\sum PAR_{24}}\right) + \left(\frac{ER_{Total}}{\underline{z}} \times \Delta t\right) + K_{(t)}\left(O_{2sat(t)} - O_{2(t)}\right)\Delta t \quad [1]$$

where t is time and Δt is the time step between measurements (15 minutes), $\underline{z}$ is mean reach depth, $\sum PAR24$ is daily photosynthetically-active radiation, and K(t) is air-water gas exchange corrected for temperature. Solving this equation for GPP_{Total} and ER_{Total} yielded daily metabolism estimates for every day that a sonde was deployed at each stream (from 158 to 215 days). Data were not collected every day of the 422-day study due to flash floods, drying, and/or equipment failure. I estimated daily metabolism on days no measurements were available to ensure comparability among sites and flow regimes. This was accomplished by calculating daily GPP using an exponential model based on significant relationships between measured daily GPP and daily temperature at all *Runoff* streams and one *Groundwater* stream, Sylamore Creek. Daily GPP was modeled based on relationships between daily discharge and metabolism at the two

Groundwater sites due to the absence of a significant link between daily GPP and temperature at the other two *Groundwater* sites (Spring and Roasting Ear creeks). Daily ER was modeled based on significant linear relationships between GPP and ER on days for which data were available at each site. I assumed GPP and ER values of 0 g O_2 m^{-2} d^{-1} on days in which streams dried as well as when sites experienced flash flooding and for seven days after peak flows when flows were still elevated (Fisher et al. 1982, Austin 2015). Seasonal metabolism was calculated by averaging daily rates from the beginning of the respective season (e.g. equinox or solstice). This approached was used due to there being fewer dates sampled in Spring 2015 (the study began in mid-spring) and Summer 2016 (the study ended in mid-summer) compared to other seasons. Summing daily rates by season would hinder comparisons, so averages of daily rates over the season were used. However, annual metabolism was always estimated by addition of daily estimates since the number of days across streams was equal and, thus, comparable.

Persulfate digests of unfiltered water samples were followed by colorimetric analyses to determine nutrient concentrations. Total nitrogen (TN) was measured monthly by automated cadmium reduction on a Lachat Quikchem 8500 (Hach Company, Loveland, CO). Total phosphorus (TP) was measured monthly using the ascorbic acid method (APHA 2005). For algal biomass, I collected six cobbles per reach at six equidistant transects down the stream reach. Monthly algal biomass was calculated based on chlorophyll *a* concentration per unit rock area as well as ash-free dry mass (Steinman et al. 2006). Canopy cover was determined for each stream channel once in summer and once following abscission using a densiometer to calculate percent coverage. Discharge was measured monthly using the mid-section method along with stream dimension measurements and dominant benthic substrate (Gore 2006). I conducted chloride drips to validate the accuracy of depth measurements in *Groundwater* streams due to the gravel- and pebble-dominated benthos at those sites.

To double statistical power and explore flow-metabolism relationships within each flow regime, I simulated a dataset with 15 streams from each flow class (N=30) sample from a random distribution using each variable in the field data set from three streams to set mean and standard deviation parameters for each flow class. Homogeneity of variances was tested using an F-test. Student's t-tests were utilized to determine differences in monthly measurements of geomorphology, nutrients, turbidity, chlorophyll a, and ash-free dry mass, as well as daily discharge and other flow metrics, daily primary production, respiration, and metabolism between flow classes in experimental and simulated streams. Spearman's correlations were employed to examine relationships between daily metabolism variables (GPP, ER, and NEP) and monthly physicochemical parameters (e.g. total nitrogen and phosphorus, temperature), monthly biological metrics (e.g. chlorophyll *a*, algal ash-free dry mass), and daily flow metrics (e.g. discharge, number of low flow days, number of high flow days) in experimental and simulated streams. All statistical analyses were performed in R version 3.4.3. The statistical significance threshold was $p \leq 0.05$. Variation in all metrics is reported in $\pm$ 1 standard error.

RESULTS

METABOLISM

Daily GPP in *Runoff* streams averaged 0.81 ($\pm$ 0.34) g O_2 m^{-2} d^{-1}, while *Groundwater* streams averaged 1.3 ($\pm$ 0.14) g O_2 m^{-2} d^{-1}. Daily ER in *Runoff* streams averaged -1.4 ($\pm$ 0.66) g O_2 m^{-2} d^{-1} and -3.3 ($\pm$ 1.1) g O_2 m^{-2} d^{-1} in *Groundwater* streams. Variances in both average daily GPP was six times greater in *Runoff* streams, while and average daily ER tended to be three times numerically greater in *Groundwater* sites, though all variances were statistically similar

(GPP: p= 0.28, ER: p= 0.50). Daily rates of GPP and ER for each stream are shown in Figure 5. Floods denoted in Figure 5 only correspond to events that were large enough to cause more than one to two-day gaps in the dataset; thus, the number of flood events counted in the study (defined as a discrete hydrograph peak exceeding the 100th percentile of average annual flow) is greater than the number of flood events shown.

Average daily GPP was largest in summer 2016 in *Groundwater* streams (0.94 ± 0.15 g O_2 m^{-2} d^{-1}), while *Runoff* streams exhibited the greatest average daily GPP in summer 2015 (1.80 ± 0.97 g O_2 m^{-2} d^{-1}) (Figure 4). Average daily GPP tended to be greater in *Groundwater* streams throughout the year. Average daily ER was greatest in spring 2016 across streams. Ecosystem respiration also tended to be numerically greater in *Groundwater* streams throughout the year. Variation in GPP across both flow classes was largest in summer 2015. *Groundwater* stream ER varied most in summer 2016 at ± 2.32 g O_2 m^{-2} d^{-1}, while spring 2016 yielded the greatest measure of variation across *Runoff* streams over the study period at ± 1.53 g O_2 m^{-2} d^{-1}.

Annual GPP ranged from 122.8 to 571.8 g O_2 m^{-2} y^{-1} in *Runoff* streams, and 446.3 to 643.0 g O_2 m^{-2} y^{-1} in *Groundwater* streams. Annual ER in *Runoff* streams ranged from -225.8 to -1071.9 g O_2 m^{-2} y^{-1} and -494.0 to -2,125.9 g O_2 m^{-2} y^{-1} in *Groundwater* streams. Annual GPP and ER did not differ over the study period, though both tended to be numerically greater in *Groundwater* streams (p= 0.26, p= 0.22 (Figure 3). Variance in annual GPP was five times higher across *Runoff* streams, while variance for annual ER estimates was three times greater in *Groundwater* streams; however, variances in annual metabolism metrics were not statistically different (GPP: p= 0.33, ER: p=0.46).

Streams tended to become more heterotrophic with increasing annual GPP (ρ= -0.49, p= 0.36), while annual ER tended to vary positively with annual net ecosystem metabolism (NEM)

(ρ= 0.77, p= 0.10). Annual GPP tended to exhibit an inverse relationship with annual respiration (ρ = -0.83, p= 0.06). Daily GPP and ER were negatively related in each stream; these relationships were more significant and exhibited more linear correlations in *Groundwater* streams compared to *Runoff* streams (Table 1). Daily GPP was positively correlated with daily NEP in all *Runoff* streams as well as one *Groundwater* stream, but was negatively correlated with NEP in Roasting Ear Creek and Sylamore Creek. Daily ER was positively correlated to daily NEP across all streams; these relationships were most linear and most significant in Roasting Ear Creek and Sylamore Creek.

METABOLISM AND LIGHT

Mean daily PAR ranged from 149 to 1,284 umol m^{-2} s^{-1} at *Groundwater* sites and 105 to 1,300 umol m^{-2} s^{-1} at *Runoff* sites. Maximum daily PAR was greatest in summer, decreasing gradually across sites beginning in late August 2015. Daily PAR gradually declined through autumn, stabilizing in early November. Light availability began to slowly increase in mid-January 2016, though minima were not observed until February at both *Groundwater* and *Runoff* sites. However, PAR tended to increase overall, exhibiting greatest variation in daily measurements in spring 2016. Light availability varied up to 922 umol m^{-2} s^{-1} at *Groundwater* sites between March and April; PAR varied up to 928 umol m^{-2} s^{-1} at *Runoff* sites during the same period. Daily PAR stabilized once again in late April at *Runoff* sites and early May at *Groundwater* sites. While minimum daily PAR measurements were recorded for similar dates, maxima occurred at differing times of year. Maximum daily PAR at *Groundwater* sites was recorded in July 2015, while maximum PAR at *Runoff* sites was recorded in April 2016.

Average annual GPP over the year was marginally negatively correlated with average annual PAR across streams (ρ= -0.83, p= 0.06). Daily GPP exhibited a positive correlation with daily PAR at all *Runoff* sites and one *Groundwater* site, Sylamore Creek. The two *Groundwater* streams that did not yield significant correlations between daily GPP and daily PAR did exhibit clear positive trends. I found a saturating trend between daily PAR and daily GPP in Roasting Ear Creek, with GPP dropping off at approximately 490 umol m^{-2} s^{-1}.

Canopy cover averaged over the two dates when coverage was measured (i.e. summer and following abscission) varied across *Runoff* sites from 42 to 57%; *Groundwater* streams varied from 38% to 90% cover. Average canopy cover after leaf-out was 52% at *Runoff* sites and 65% at *Groundwater* sites. Cover was similar between flow regimes (p= 0.75). Canopy cover was not related to annual GPP (ρ= 0.09, p=0.92), annual ER (ρ=-0.26, p=0.66), or annual NEM (ρ= 0.60, p=0.24).

Annual GPP showed no clear relationship with annual discharge across sites (ρ=0.20, p= 0.71) (Figure 6). Two *Runoff* streams tended to exhibit lower discharge and GPP compared to *Groundwater* streams, while I found Little Piney Creek to have greater GPP compared to *Groundwater* sites with similar annual discharge. Greater discharge tended to depress respiration (ρ= -0.50, p= 0.37) and drive streams to be more heterotrophic over the year (ρ= -0.74, p= 0.002) (Figure 7). Annual GPP was not correlated with the number of no flow days across streams (ρ= -0.15 , p= 0.77). As with GPP and ER, data points were not grouped by flow regime, as sites experienced perennial flow in one *Runoff* stream (Murray Creek) and two *Groundwater* streams (Sylamore and Spring creeks).

METABOLISM AND FLOW METRICS

Daily discharge was negatively correlated to daily GPP in three streams that spanned both flow regimes (Table 1). Daily discharge in one *Groundwater* stream, Sylamore Creek, was positively related to daily GPP. Daily ER was related to discharge in all Groundwater streams, though the direction of the relationship differed by stream; two sites displayed negative relationships between daily ER and discharge, while a positive relationship between these two metrics was shown at Sylamore Creek. Respiration in two out of three *Runoff* streams was not related to discharge, though a positive relationship was shown between ER and discharge at Murray Creek.

Runoff streams experienced two moderate storm events during summer 2015, while *Groundwater* streams experienced three smaller events during the same period. Variation in precipitation across flow types is due to the location of study sites, as *Runoff* streams are positioned southwest of *Groundwater* sites. Little to no rain fell across Arkansas from early August to November 7, 2015, causing two *Runoff* streams and one *Groundwater* stream to dry and leaving two *Groundwater* streams and one Runoff stream with markedly reduced flows. However, *Runoff* streams that dried experienced longer drought periods, as Little Piney and Big Piney dried for 47 and 33 days, respectively, whereas Roasting Ear dried for 9 days. *Runoff* streams dried around the end of September and beginning of October while Roasting Ear dried only at the end of October. Regular rain events returned on November 5th, 2015, with four significant rain events depositing 21.89 centimeters across Groundwater sites and 27.99 centimeters at Runoff sites through the end of the month. A large flood event in late December 2015 required approximately 10 days for sites to return to near winter base-flow conditions. Between March and June 2016, regular rains briefly increased flows every one to three weeks. *Groundwater* streams experienced one to two more high-flow events than *Runoff* streams during this time. Flow metrics at each site are listed in Table 2.

Annual GPP showed no clear relationship with mean annual discharge across sites ($\rho=0.20$, $p= 0.71$) (Figure 6). Two *Runoff* streams tended to exhibit lower discharge and GPP compared to *Groundwater* streams, while Little Piney Creek was shown to have greater GPP compared to *Groundwater* sites with similar annual discharge. Greater discharge tended to numerically depress respiration (ρ= -0.50, p= 0.37) and drive streams to be more heterotrophic over the year (ρ= -0.74, p= 0.002) (Figure 7). Annual GPP was negatively correlated with the number of no flow days across streams (ρ= -0.88 , p=0.02) (Figure 8). As with GPP and ER, data points were not grouped by flow regime, as sites experienced perennial flow in one *Runoff* stream (Murray) and two *Groundwater* streams (Spring and Sylamore).

BIOLOGICAL DRIVERS OF METABOLISM

Neither annual GPP nor annual ER were significantly correlated to chlorophyll *a* or ash-free dry mass (AFDM) across sites during the study (Figure 9). Annual metabolism metrics were also not related to annual averages of TN or TP over the year. However, both chlorophyll *a* and AFDM were greater in *Groundwater* streams (chl *a*: p= 0.001, AFDM: p= 0.02), even with similarly low nutrient concentrations (Total N: p= 0.2, Total P: p= 0.21). Annual TN was low in all streams, averaging 0.10 ± 0.03 mg/L in *Runoff* streams and 0.56 ± 0.26 mg/L in *Groundwater* streams. Total phosphorus averaged 6.21 ± 0.63 µg/L annually in *Runoff* streams and 8.70 ± 1.38 µg/L in *Groundwater* streams.

PHYSICAL AND CHEMICAL PARAMETERS

Annual GPP was not related to turbidity (ρ= -0.26, p= 0.66), though our data suggested a potential negative influence of turbidity on AFDM (ρ= -0.77, p= 0.10). Turbidity was similar across flow regimes (p= 0.40), though *Runoff* streams tended to be more turbid overall and have more variable turbidity (29 ± 22 Nephelometric Turbidity Units (NTU)) than *Groundwater* streams (6 ± 0.9 NTU).

Runoff stream substrate types were primarily bedrock and cobble, while *Groundwater* streams were dominated by pebbles. Mean particle size was 364 ± 88.2 mm^2 in *Runoff* streams and 162 ± 34 mm^2 in *Groundwater* streams, though there was no difference between flow regimes (p= 0.14). Mean particle size was not related to annual GPP (ρ= -0.09, p= 0.92) or annual ER (ρ= -0.26, p= 0.66).

Average annual water temperature was greater in *Runoff* streams (p= 0.02). Water temperature in *Runoff* streams averaged 16.7 ± 0.07 °C over the year, while average annual temperature in *Groundwater* streams was 15.2 + 0.3 °C. There were no significant correlations between annual water temperature and annual GPP (ρ= -0.14, p= 0.80), annual ER (ρ= -0.31, p= 0.56), or annual NEM (ρ= -0.14, p= 0.80). A negative, though not significant, trend with chlorophyll *a* decreasing with greater average water temperature was observed (ρ= -0.77, p= 0.10); this negative relationship was significant when AFDM was compared with average annual water temperature across streams (ρ= -0.88, p= 0.03).

Daily temperature drove daily GPP in all *Runoff* streams, but only one *Groundwater* stream. Conversely, daily water temperature was related to daily ER only in Sylamore Creek (Table 1).

SIMULATIONS

Data simulations to double statistical power revealed greater annual GPP (p= 0.04), ER (p= 0.007) and more heterotrophic NEM (p= 0.01) in *Groundwater* streams. (Figure 9). Average annual PAR (p= 0.3) and canopy cover were similar across flow regimes (p= 0.10). Annual discharge was similar between flow regimes (p= 0.25). Simulated *Runoff* streams experienced more no flow days (p= 0.001), though high flow days were similar in both flow regimes (p=0.07). Similar to my field results, chlorophyll *a* (p<0.0001) and AFDM (p<0.0001) were greater in *Groundwater* streams. Total nitrogen was greater in *Groundwater* streams ($p = 0.0004$), and total phosphorus also tended to be greater in *Groundwater* streams ($p = 0.06$). Simulated *Runoff* streams were more turbid ($p = 0.01$) and warmer ($p < 0.0001$) than *Groundwater* streams. Benthic substrate sizes were larger in *Runoff* streams ($p = 0.0006$).

Simulated annual GPP was not related to annual ER in *Groundwater* streams ($\rho = 0.42$, $p = 0.12$) or *Runoff* streams ($\rho = 0.41$, $p = 0.13$) (Figure 10). ER was the primary driver of net NEP across flow regimes (*Runoff*: $\rho = -0.83$, $p = 0.0002$; *Groundwater*: $\rho = -0.97$, $p < 0.0001$). GPP responded positively to TN in *Runoff* streams ($\rho = 0.63$, $p = 0.01$), while *Groundwater* stream respiration exhibited a negative response to TN ($\rho = 0.68$, $p = 0.007$). Overall, simulated *Groundwater* streams were more autotrophic with increasing nitrogen concentrations ($\rho = 0.66$, $p = 0.009$). GPP responded negatively to canopy cover in both flow classes (*Runoff*: $\rho = -0.53$, $p = 0.04$; *Groundwater*: $\rho = -0.67$, $p = 0.01$). Flow regimes exhibited differing responses of primary production to changes in benthic substrate size. *Runoff* stream GPP rates were greater in the presence of larger substrate ($\rho = 0.63$, $p = 0.01$), while *Groundwater* stream GPP rates were reduced with increasing substrate size ($\rho = -0.54$, $p = 0.04$). *Groundwater* streams exhibited a positive relationship between production rates and AFDM ($\rho = 0.54$, $p = 0.04$) and streams with greater algal biomass were more heterotrophic ($\rho = -0.53$, $p = 0.04$) (Figure 11).

Simulated annual GPP exhibited a negative relationship with *Runoff* streams' number of high-flow days (GPP: $\rho = -0.57$, $p = 0.03$). . The number of no-flow days at *Runoff* sites tended to depress GPP and ER (GPP: $\rho = -0.52$, $p = 0.05$; ER: $\rho = -0.49$, $p = 0.06$). *Runoff* annual GPP also tended to decrease with greater annual discharge (GPP: $\rho = -0.52$, $p = 0.05$). High-flow days tended to depress *Groundwater* stream respiration rates ($\rho = -0.50$, $p = 0.06$), driving streams to be more autotrophic in streams with more high-flow days over the year ($\rho = 0.53$, $p = 0.04$) (Figure 11).

DISCUSSION

METABOLISM

These data provide insight into intra-biome variation in metabolism within a land-use category across two hydrologic regimes. The rates reported for metabolism are well within range for forested stream daily GPP (0.05 to 16.2 g O_2 m^{-2} day^{-1}) (Mulholland et al. 2001, Acuña et al. 2004, Uehlinger 2006, Bernot et al. 2010) reported in studies conducted in single streams as well as in multiple streams across biomes. Headwater streams are notoriously variable in their metabolic regimes, and elucidating regional predictors of drivers continues to be an elusive goal (Bernhardt et al. 2017). Although annual comparisons of GPP and ER did not yield statistically significant differences by flow regime, these results provide a glimpse of potential differences in rates of annual metabolism. Additionally, the simulated dataset results suggest that the lack of statistically significant differences may be an artifact of low sample size. This lack of statistical power in my field data underscores the need for continued work in a greater number of streams across flow regimes. Few published metabolism studies have examined annual trends in more than one stream; the data presented here provide both spatial and temporal replication, but three replicate reaches may not be enough to draw strong conclusions regarding flow regime differences and trends. Greater GPP in *Groundwater* streams is likely a byproduct of the high light and shallow channels coupled with low turbidity; the benthos of a *Runoff* stream is difficult to observe due to the presence of minerals common to the Boston Mountains ecoregion, where *Runoff* streams are the dominant flow type. While *Runoff* streams were warmer than *Groundwater* streams, the amount of light reaching the streambed and greater turbidity may have restricted primary production potential. Lower primary production would, in turn, produce lower rates of respiration. Overall, trends suggesting greater GPP and ER in groundwater-dominated streams may indicate more abundant biological communities. Further work at the community level would allow for a better understanding of the relative contributions of consumers and producers to metabolism in these systems.

Runoff streams exhibited greater variation in annual GPP. This may have resulted from flow regime differences in biological responses to flow metrics. While *Groundwater* streams exhibited greater variability in annual discharge, *Runoff* streams were more variable in the number of no-flow and high-flow days they experienced over the study year. Simulated *Groundwater* stream metabolism was related to only one flow metric, high-flow days. Simulated *Runoff* stream GPP was significantly related to high-flow days and marginally related to discharge and no-flow days. *Runoff* streams exhibited greater variation than *Groundwater* streams in a few other variables, such as turbidity and annual PAR, but no such variables were related to GPP. The tendency of multiple flow metrics to influence *Runoff* stream production suggests that variation in annual flow may be influencing variation in metabolism more in *Runoff* than in *Groundwater* streams.

Flow regimes were similar in what season they exhibited minimum and maximum daily average GPP. Similar to patterns reported in other forested systems and larger rivers, daily *Groundwater* stream GPP was greatest in summer 2016, while summer 2015 had the greatest average GPP for *Runoff* streams (Mulholland et al. 2001, Acuña et al. 2004, Genzoli and Hall 2016). These trends differ from Roberts et al. (2007) in that GPP at Walker Branch was greatest during the spring and comparatively low during two consecutive summers. Uehlinger (2006) also reported GPP to be greatest in May over a 15-year period and lower in summers. The canopies over study streams were not closed (cover averaged 52% in *Runoff* streams and 58% in *Groundwater* streams), allowing light to reach the benthos in summer despite high leaf cover. *Groundwater* streams experienced four storm events in May and June 2016, which may have stimulated production. *Runoff* streams experienced five storm events during this time, but GPP remained significantly lower in *Runoff* than in *Groundwater* streams potentially due to the differential impacts of high flow on each flow regime. Simulated *Runoff* stream GPP responded negatively to high flow, while high flows elicited a negative response from *Groundwater* stream GPP. *Runoff* stream GPP reached its minimum in spring 2015, coinciding with maximum algal biomass that may have reduced production rates. Daily *Groundwater* stream GPP was lowest in autumn 2015, which may have arisen from competition between benthic producers and microbes for resources. Benthic periphyton AFDM was greatest in autumn 2015 across *Groundwater* streams, while chlorophyll *a* peaked in spring and summer, suggesting that reduced production rates were not due to large algal biomass alone. These results differ from other metabolism studies that have shown lower production in other seasons (e.g. winter and summer), though these studies also present no clear trends that might yield predictable patterns (Uehlinger 2006, Roberts et al. 2007). Respiration peaked in spring 2016 in *Runoff* streams and in summer 2015 in *Groundwater* streams; *Groundwater* stream respiration was twice as large in both summers compared to the rest of the year, and respiration rates were fairly consistent from autumn to spring, maintaining rates around 2.6 g O_2 m^{-2} d^{-1} over most of the year. In contrast, *Runoff* streams exhibited consistently low respiration (<2 g O_2 m^{-2} d^{-1}), increasing in average daily rates as well as variation in these rates in spring 2016. These results reflect seasonal trends in ER similar to other small streams (Roberts et al. 2007). Patterns of seasonal metabolism may reflect an interaction between season and flow regime in which flow regimes respond differently to changes in drivers of metabolism such as water temperature, PAR, and nutrient concentrations (Mulholland et al. 2001). This may have impacted my investigation of drivers of metabolism. Given my small sample size and, in turn, the necessity of examining mechanisms across both flow regimes together, I was unable to ascertain potential relationships between metabolism and associated drivers that may exist seasonally, but differ by flow regime.

Variation in daily metabolism tended to differ seasonally and by flow regime. Variation in daily GPP was largest in summer 2015 for all streams, and ER was likewise most variable in *Groundwater* streams during the summer of 2015. Daily variation in ER did not track GPP at *Runoff* sites; rather, ER was more variable over the spring of 2016. Day-to-day variation in GPP was not related to variation in PAR or discharge. Ecosystem respiration responded similarly to PAR and discharge. However, Murray Creek exhibited the greatest variability in respiration across streams even with comparable changes in incident PAR, and removing Murray Creek revealed that variation in respiration was related to variation in light from either weather or changes in canopy cover over the year. This highlights an important point: the seasonality of variation in metabolism may be a function of landscape differences that give rise to differing flow regimes as well as changes in light availability over a year. Additionally, these results support the necessity of continuous metabolism measurements over shorter sampling periods scaled to annual timesteps, particularly because metabolism calculated from field measurements varied most during seasons that accounted for a significant portion of annual GPP and ER.

Respiration drove overall annual rates of stream metabolism, dominating net ecosystem production in all but one stream. Five out of six sites, while forested, had open canopies that allowed light to reach the channel, supporting production and providing conditions for respiration to outpace production (e.g. shallow depths and high light reaching the channel yielded potential for streams to warm). Spring Creek, a *Groundwater* stream, was the only system that was autotrophic over the study period. This was likely due to the stream's closed canopy (90%) and low temperature, as Spring Creek had the lowest annual temperature of any stream in the study.

METABOLISM AND LIGHT

Daily light drove daily GPP in *Runoff* streams and one *Groundwater* stream, but annual PAR did not drive annual GPP, providing further credence to the influence of different factors at hierarchical temporal scales (Roberts et al. 2007). Daily light has been shown as the primary driver of GPP in streams across biomes (Mulholland et al. 2001), and others have inferred light as a driver of GPP by measuring canopy cover as a proxy indicator (Naiman 1983, Webster et al. 1995, Young and Huryn 1999). Study streams exhibited no significant relationship with canopy cover, though the simulated dataset revealed that canopy cover negatively influenced annual GPP across flow regimes. Even with similar amounts of canopy cover, simulated *Runoff* stream production was still significantly lower than *Groundwater* streams. Rather than canopy influence, incident light in *Runoff* streams may have been mitigated by their greater turbidity from a natural precipitate associated with the karst in the Boston Mountains ecoregion where *Runoff* streams were located. The benthos was difficult to see in *Runoff* streams if the water was more than approximately 0.5 meters deep, whereas *Groundwater* streams were impeccably clear. Alternately, the temporal disparity in maximum PAR measurements (e.g. April in *Runoff* streams and July in *Groundwater* streams) may have interacted with seasonal differences in water temperature to increase *Groundwater* GPP compared to *Runoff* GPP, which may have been constrained by cooler temperature at times of high light This disparity further highlights the importance of flow regime as an ultimate control on mechanisms governing metabolism, as landscape geology, vegetation, and dominant water source can interact to influence, for instance, the light regime of an entire group of streams.

Others have reported PAR to be the primary driver of metabolism at low temporal scales (i.e. minutes) (Mulholland et al. 2001) while not finding any relationships between metabolism and flow, though others have shown that light and discharge can both control metabolism (Roberts et al. 2007, Beaulieu et al. 2013). However, this study took place in one biome across streams with similar light regimes, which may explain the lack of relationships between daily light and daily metabolism. I also did not always find clear flow-ecology relationships in all streams, particularly when data were simulated expand statistical capability to explore relationships within each flow type. Others have asserted that metabolism is influenced by various factors at differing temporal (Uehlinger 2006, Roberts et al. 2007) and spatial scales (Bott et al. 1985, Mulholland et al. 2001, Bernot et al 2010). It may be that the identity and strength of these relationships are, at least in part, a function of flow regime, as no previous studies examined the influence of flow classification within a land use type or biome, though flow variables were included in their analyses. Other studies undertaken in a single stream may have been better able to discern potential flow class-specific relationships since there was no grouping of streams. Thus, there could be no potential grouping of disparate flow regimes that could create noise in flow-ecology data, but most studies do not take flow regime into consideration since many areas still do not have mapped flow classifications.

METABOLISM AND FLOW METRICS

Results from the field campaign indicate a negative trend between discharge and NEM in these systems; this negative trend appears to have been driven in part by the interplay between flow and respiration. Greater annual discharge over the year may drive streams toward heterotrophy by directly affecting the algal community composition. Another possibility may be that grazers are mediating this relationship; herbivores may be more active in streams with lower annual discharge, thinning algal mats and driving up P:R ratios by ameliorating competition among periphyton, removing senescent cells, and ingesting microbes in the mat. It is worth noting that the observed relationships between discharge and metabolism appear to be driven more by *Groundwater* than *Runoff* streams Additionally, *Runoff* streams increased in respiration then decreased at greater discharge. This could reflect potential flow type-specific differences in algal community composition, in which *Runoff* stream communities respond more variably to increases in discharge, though data from a greater number of streams would be needed to support this with any certainty.

My simulated dataset revealed that low sample size may have obscured important distinctions in metabolic responses to flow. Specifically, *Groundwater* stream ER and NEM were influenced by high flow while *Runoff* stream GPP was influenced by high flow, drying, and discharge across sites. This has significant management implications if flow regime mediates whether or not stream production rates are responsive to flow. Environmental flow standards for specific metrics may be more critical for some stream communities within one flow regime than they are for others. Another possibility is that *Groundwater* stream metabolism is simply responsive to other flow metrics not addressed in this study. Streams in different flow classifications may merit more individualized management strategies to maximize community health and ecosystem services.

While data analyzed across both flow regimes revealed no significant relationships with high-flow days, simulated streams revealed that large flows resulting from flood events may have differential impacts on metabolism depending on flow class; specifically, GPP was affected moreso in *Runoff* streams while ER and NEM were influenced in *Groundwater* streams. Similar to field data, simulated data also showed a negative response of *Runoff* stream GPP and ER with number of no flow days. Stream drying can depress annual metabolism by increasing competition for diminishing space and resources or, in some cases, completely eliminate production and respiration for days to months of the year in channels that experience total drying. This abrupt shift in C dynamics may impact riparian communities that utilize streams for nursery habitat or that depend upon subsidies of stream insects as a food source. In systems with communities that have adapted to drying, community structure may differ from perennial (e.g. groundwater-dominated) streams. Community-level data in these flow regimes would allow me to evaluate this hypothesis, and I wish to highlight that metabolism data can be well-complemented by community data that may explain differences and trends I am unable to address with the current study.

As stream flow is altered by climate change-induced modification of weather patterns, ecosystem function (including metabolism) and community structure will also change. Greater magnitude and frequency of floods in some areas may negatively impact production and respiration rates in certain systems, but not others. More extreme droughts will make more systems susceptible to drying, also suppressing production and respiration in some streams. For communities not historically adapted to such flow extremes, the consequences for macroinvertebrate and fish communities could be dire. Even if streams do not completely dry, suppression of primary productivity below levels needed to support a diverse consumer community could negatively impact ecosystem function and services. Reference flow-ecology relationships within the framework of flow regime will allow scientists and policymakers to better understand streams as sentinels of climate change (Williamson et al. 2008).

BIOLOGICAL DRIVERS OF METABOLISM

Even though algal biomass and total AFDM were greater in *Groundwater* streams, neither of these were significant drivers of GPP or ER across flow regimes. However, AFDM was positively related to respiration in simulated *Groundwater* streams, indicating that heterotrophic respiration may have been a key factor in *Groundwater* stream metabolism that was not shown in experimental streams due to low sample size. Ecosystem respiration drove trends in net metabolism in all streams, but microbial activity may yield a stronger influence in groundwater-dominated systems. I explored potential environmental and flow variables whose influence AFDM may be mediating and identified no relationships. However, I did not measure variables that may have also influenced heterotrophic activity, such as benthic organic matter standing stocks or decomposition, and further exploration of these metrics would provide a more complete portrait of C dynamics in these systems.

PHYSICAL AND CHEMICAL PARAMETERS

Total N was not related to GPP or ER in experimental streams, though nitrogen was a positive predictor of GPP in simulated *Runoff* streams. Additionally, nitrogen concentrations depressed respiration in simulated *Groundwater* streams. Others have also found N to be a significant predictor of GPP (Bernot et al. 2010). Total nitrogen was low at all sites, and nitrogen is often a limiting nutrient in streams (Elser et al. 2008). Total nitrogen tended to be lower in *Runoff* streams, and this trend was significant within the simulated dataset, indicating that nitrogen may be limiting in *Runoff*, but not *Groundwater* streams. Streams were all nested within heavily forested catchments (>84% forest), and streams with the largest N concentrations were actually situated within the most heavily forested catchments with very little to no agricultural or residential land use immediately upstream of my study reaches. Given that land use was not controlling N levels, as in other studies (Mulholland et al. 2008), I suggest that nitrogen from pastoral land use outside of experimental *Groundwater* stream watersheds percolated through soil and dissolved in groundwater that eventually became part of those streams' base flows.

Similar to Bernot et al. (2010), I identified no relationships between phosphorus and GPP. However, other inter-regional studies have determined P to be a driver of GPP, and P concentrations were similar to those reported by others in forested systems (Lamberti and Steinman 1997, Mulholland et al. 2001). Both experimental and simulated streams exhibited similarly low P concentrations. It should be noted that there was not a large gradient in TP concentrations, as these streams were minimally-impacted reference systems. Phosphorus may not have been a limiting nutrient in these systems, though it is difficult to say, because while TN drove modeled *Runoff* GPP, neither potentially limiting nutrient drove metabolism in experimental or simulated *Groundwater* streams. Additionally, temporal variation in P and N concentrations may have influenced relationships, as I estimated GPP from continuous data, whereas P and N measurements were taken only once per month. These more infrequent measurements of TP and TN, and the measurement of nutrient concentrations rather than uptake rates (and, in turn, the comparison of a state to a production rate) may have failed to provide a sound estimate of nutrient availability to primary producers. Further, streams used in this study were all considered minimally-impacted, so there was not a large gradient of nutrient concentrations that could have impacted metabolism rates.

Physical habitat heterogeneity has been shown to influence ecosystem processes (Cardinale et al. 2002). Experimental streams did not exhibit significant differences in substrate size, but I argue that this was due to one *Runoff* stream, Big Piney Creek, being dominated by bedrock and cobble. Big Piney had fewer boulders than the other two *Runoff* streams, increasing variation in substrate size for that flow class, which also had more variation in GPP than *Runoff* streams. Importantly, all *Runoff* streams consisted of cobble or boulder substrates on top of bedrock while *Groundwater* streams were predominantly gravel and pebble that, beneath the immediate surface, provided a hyporheic corridor. Creating an expanded dataset revealed clear differences in substrate size as well as flow-type impacts of substrate size on GPP. There were contrasting trends in the data; *Groundwater* streams exhibited a negative trend between production and substrate size, while *Runoff* streams showed a positive trend. Other factors likely exert a larger influence on metabolism. For instance, the more stable substrate in *Runoff* streams might be expected to support more primary production, but the greater turbidity and more unstable annual flow of these streams may prevent algae from maximizing potential colonization. Thus, even though the smaller, more easily disturbed substrate in *Groundwater* streams tends to limit production, substrate size is not small enough to reduce primary production to *Runoff* stream levels. Additionally, hyporheic metabolism may be contributing to these trends (Mulholland et al. 1997), and greater attention to the influence of this habitat to instream C production and utilization may explain some of the trends in these streams.

Temperature has been shown to synergistically increase production with light to enhance the photosynthetic capacity of primary producers. Temperature can also be predictive of respiration, as it exerts control on the speed of organismal metabolism (Hill et al. 2000, Mulholland et al. 2001, Acuña et al. 2008, Beaulieu et al. 2013). However, it can be difficult to consistently predict under what conditions temperature is a dominant control. It may be that the low range of annual temperatures in this study compared to other variables may have led to reduced capability to discern temperature-driven trends in both experimental and simulated data.

Daily temperature drove daily GPP in all *Runoff* streams, but only one *Groundwater* stream. The lack of relationship between daily ER and water temperature at most study sites was unexpected; daily water temperature was related to daily ER only in Sylamore Creek. Others have postulated that the effect of temperature on respiration is small, and others who have not shown relationships between ER and water temperature have attributed the lack of relationship to seasonal rather than annual measurements. This lack of relationship in daily data, for which I only used temperature measured in the field, may have stemmed from the fact that estimates of GPP and ER, along with temperature measurements, were taken predominantly in the spring and summer, as probe malfunctions in the autumn and winter reduced the temporal resolution of estimates in some streams during those seasons. For annual temperature estimates, I filled in these gaps in temperature based on established relationships to gauge-measured temperatures. Similar to others, even the significant relationship between respiration and temperature at Sylamore Creek only explained a small percentage (15%) of the variation in metabolism rates (Sinsabaugh 1997, Uehlinger 2006).

I recognize the limitations of this study given that only three streams per flow regime were included; however, the tendency for GPP to vary more in *Runoff* streams, while ER varies more in *Groundwater* streams is worthy of note and merits further attention. Clear differences in the range of metabolism values between flow regimes reveals that identical management schemes may not work well for both flow regimes, and variation in flow metrics needed to sustain biological communities may indicate the need for close monitoring of a greater number of *Groundwater* streams. Though the spatial scope of this work is restricted, the temporal resolution of these data allows for deeper understanding of drivers and trends in metabolism within streams of the same flow classification.

It is important to note that flow regimes are spatially clustered within established ecoregions, and dominant hydrology across northern Arkansas appears to be demarcated by these ecoregion distinctions. *Runoff* streams are dominant in the Boston Mountains ecoregion of Arkansas, while *Groundwater* streams account for the majority of streams in the Ozark Highlands. Ecoregions represent areas of distinct habitat and are themselves nested within biomes (Dasmann 1973, Olson et al. 2001). Given the variation of landscape characteristics across ecoregions, they likely play an important role in shaping the mosaic of natural flow regimes within a biome. These findings are not simply a story of flow influences on stream C dynamics, but of landscape-level variables such as geological and soil characteristics, topography, and local climate that affect both terrestrial as well as aquatic production. A greater focus on ecoregion-level differences may reveal a more detailed portrait of controls and drivers of stream production and respiration.

This comparison of forested systems across flow types provides a foundation for refining comparisons of stream metabolism across systems that may be similar in surrounding land use, but differ in flow regime metrics, such as intermittency, flood regime, and dominant water source. This is crucial for ensuring scientists and managers do not conflate natural variation based on differences in flow versus anthropogenic impacts and hydrologic alteration. Further, others have highlighted differences in metabolism across biomes (Mulholland et al. 2001) and land use categories (Bernot et al. 2010). My efforts reveal that metabolism may exhibit differences in functional variation even within a single biome, land cover classification, and even within a single flow regime. While biome and land use certainly play key roles in determining stream metabolism, I urge others to consider and incorporate flow regime into experimental designs and management decisions whenever possible. If the valley rules the stream (Hynes 1975), landscape variation across a single biome or land use category likely gives rise to various stream characters and flow types, and even variation in hydrology within a flow type, which exert their own unique influence on ecosystem function. These variable controls and flow-metabolism relationships must not be overlooked as freshwater scientists seek to characterize patterns in productivity that have thus far proven elusive (Bernhardt et al. 2017).

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APPENDIX

TABLES

Table 1. Spearman's rank correlations between daily metabolism metrics (GPP= gross primary production, ER= ecosystem respiration) and daily discharge, temperature, and light (PAR). Significant correlations are marked with an asterisk (*). BPC (Big Piney Creek), LPC (Little Piney Creek) and Murray are *Runoff* sites. Roasting Ear, Spring, and Sylamore are *Groundwater* sites.

Site	**Variable 1**	**Variable 2**	**r**	**p-value**
BPC	GPP	Discharge	-0.49	<0.0001*
	ER	Discharge	-0.03	0.64
	GPP	Temperature	0.28	<0.0001*
	ER	Temperature	0.04	0.04
	GPP	PAR	0.33	<0.0001*
LPC	GPP	Discharge	-0.49	<0.0001*
	ER	Discharge	-0.03	0.64
	GPP	Temperature	0.46	<0.0001*
	ER	Temperature	0.01	0.13
	GPP	PAR	0.33	<0.0001*
Murray	GPP	Discharge	-0.10	0.19
	ER	Discharge	-0.24	0.002*
	GPP	Temperature	0.23	<0.0001*
	ER	Temperature	0.05	0.83
	GPP	PAR	0.19	0.01*
Roasting Ear	GPP	Discharge	-0.56	<0.0001*
	ER	Discharge	0.70	<0.0001*
	GPP	Temperature	0.003	0.44
	ER	Temperature	0.001	0.62
	GPP	PAR	-0.01	0.86
Spring	GPP	Discharge	0.01	0.88
	ER	Discharge	0.35	<0.0001*
	GPP	Temperature	0.004	0.44
	ER	Temperature	0.01	0.21
	GPP	PAR	0.04	0.56
Sylamore	GPP	Discharge	0.35	<0.0001*
	ER	Discharge	-0.59	<0.0001*
	GPP	Temperature	0.44	<0.0001*
	ER	Temperature	0.15	<0.0001*
	GPP	PAR	0.47	<0.0001*

Table 2. Site abbreviations and flow metrics measured over the study duration at each site.

Annual Discharge	**Site**	**Abbreviation**	**Mean Annual Discharge (m^3 s^{-1})**	**No Flow Days**	**High Flow Days**	**Floods**	**Annual Rain (cm)**
Runoff	Big Piney	BPC	3.65	33	146	11	153.85
Runoff	Little Piney	LPC	1.49	47	106	11	141.22
Runoff	Murray	MRY	1.16	0	121	11	141.22
Groundwater	Roasting Ear	REC	1.87	9	107	12	151.18
Groundwater	Spring	SPR	1.54	0	137	13	137.64
Groundwater	Sylamore	SYL	5.54	0	107	15	151.18

FIGURES

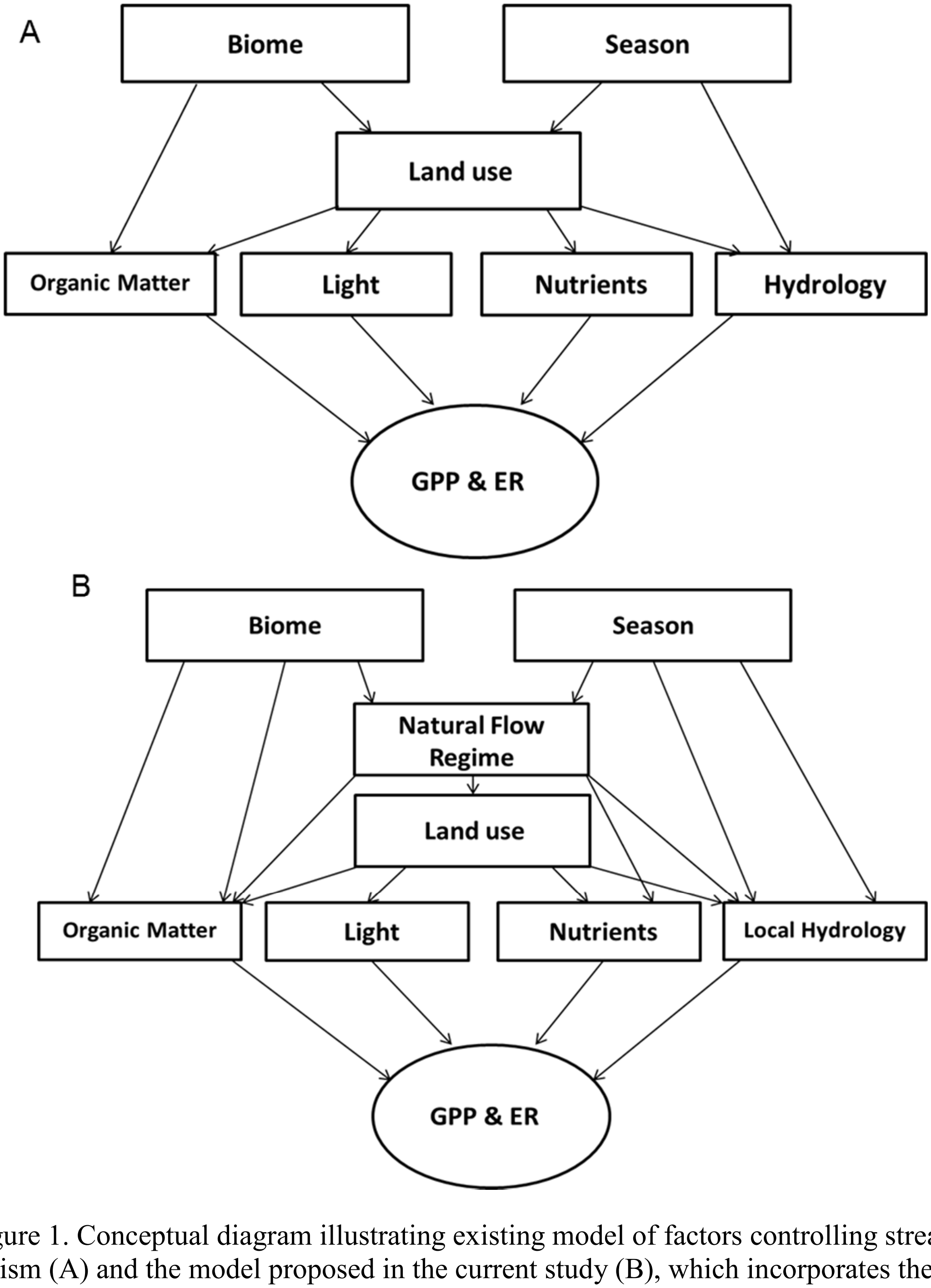

Figure 1. Conceptual diagram illustrating existing model of factors controlling stream metabolism (A) and the model proposed in the current study (B), which incorporates the natural flow regime. GPP and ER denote gross primary production and ecosystem respiration, respectively.

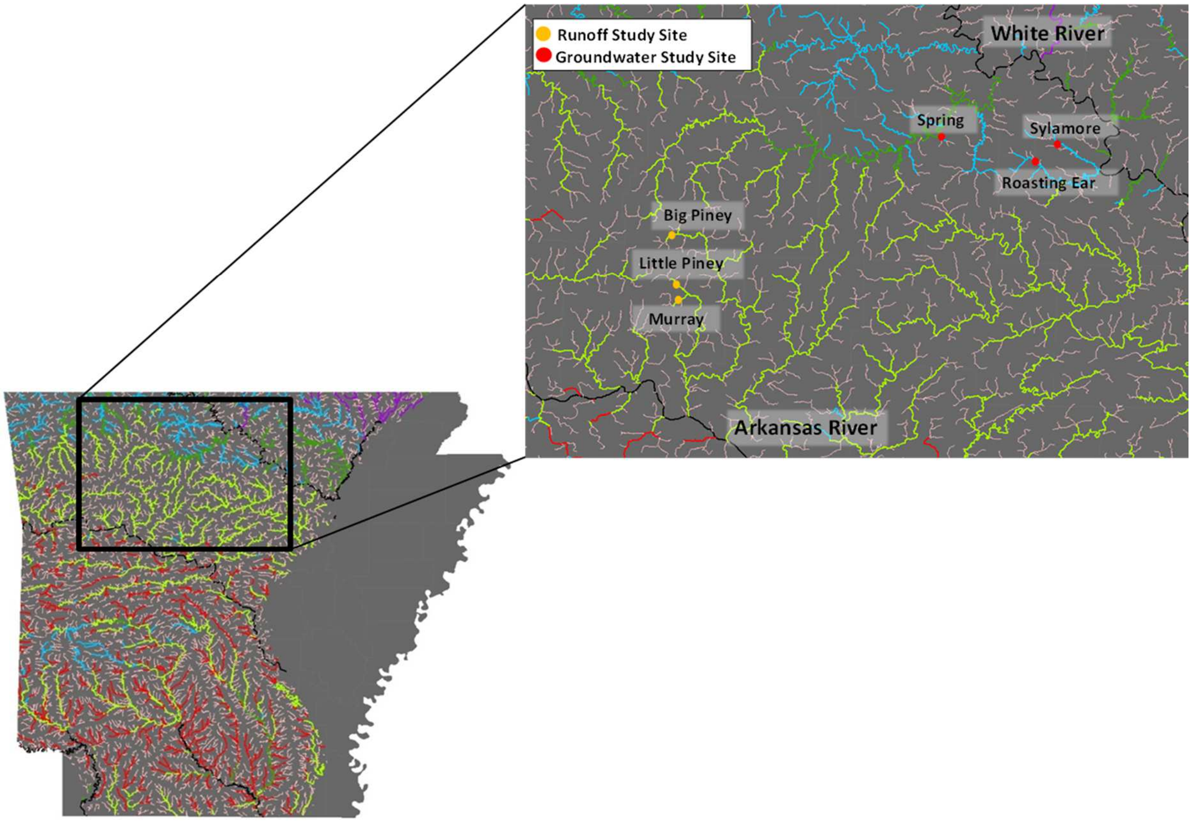

Figure 2. Map of flow regimes in the Ozark and Ouachita Interior Highlands based on Leasure et al. (2016). Highlighted area shows individual study sites sampled from 2015-2016 across northern Arkansas. Teal streams in map represent *Groundwater* streams. Light green streams in map represent *Runoff* streams.

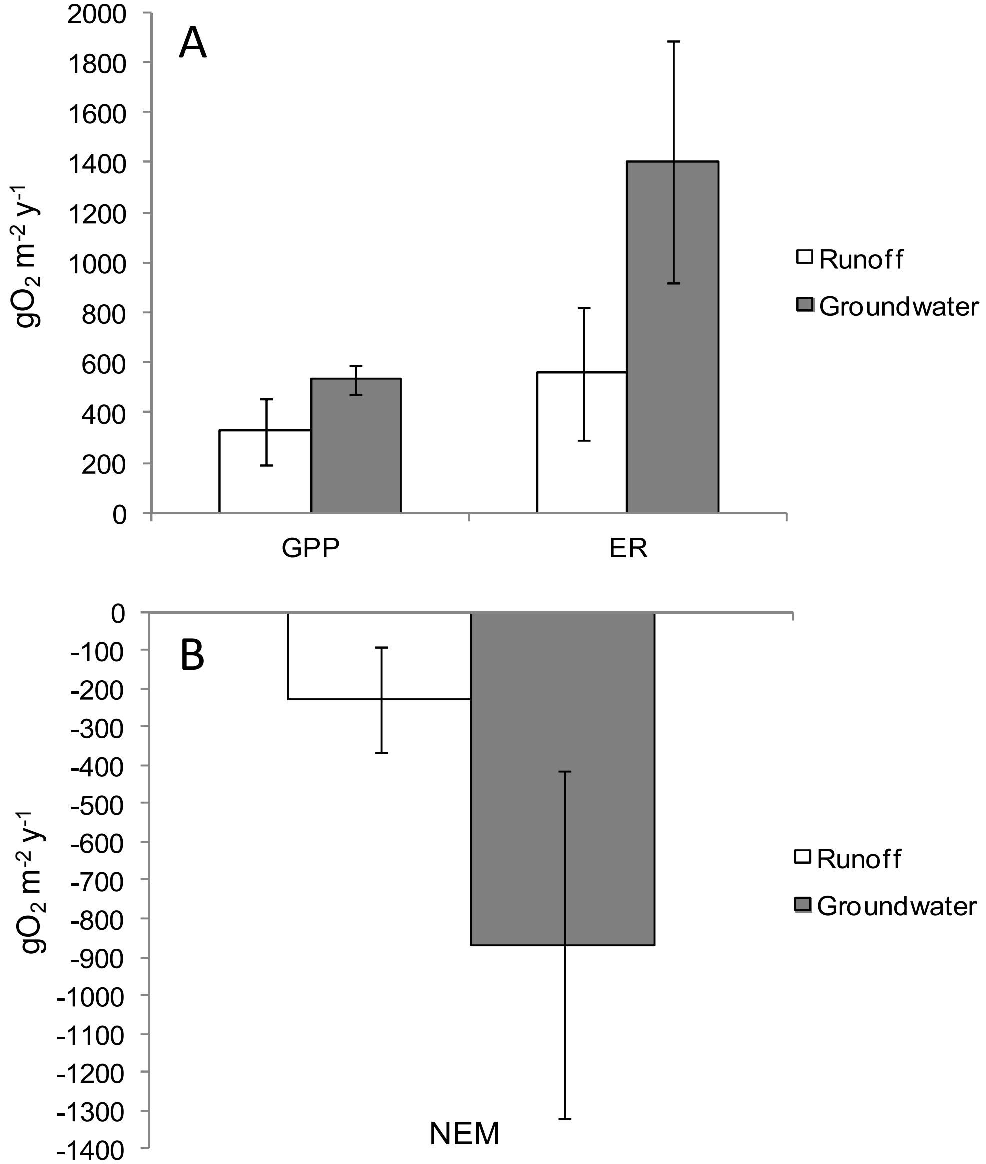

Figure 3. Annual gross primary production (GPP), ecosystem respiration (ER) (A), and net ecosystem metabolism (NEM) (B) in *Runoff* (white) and *Groundwater* (gray) streams. Error bars denote ± 1 standard error. n= 3 per flow regime.

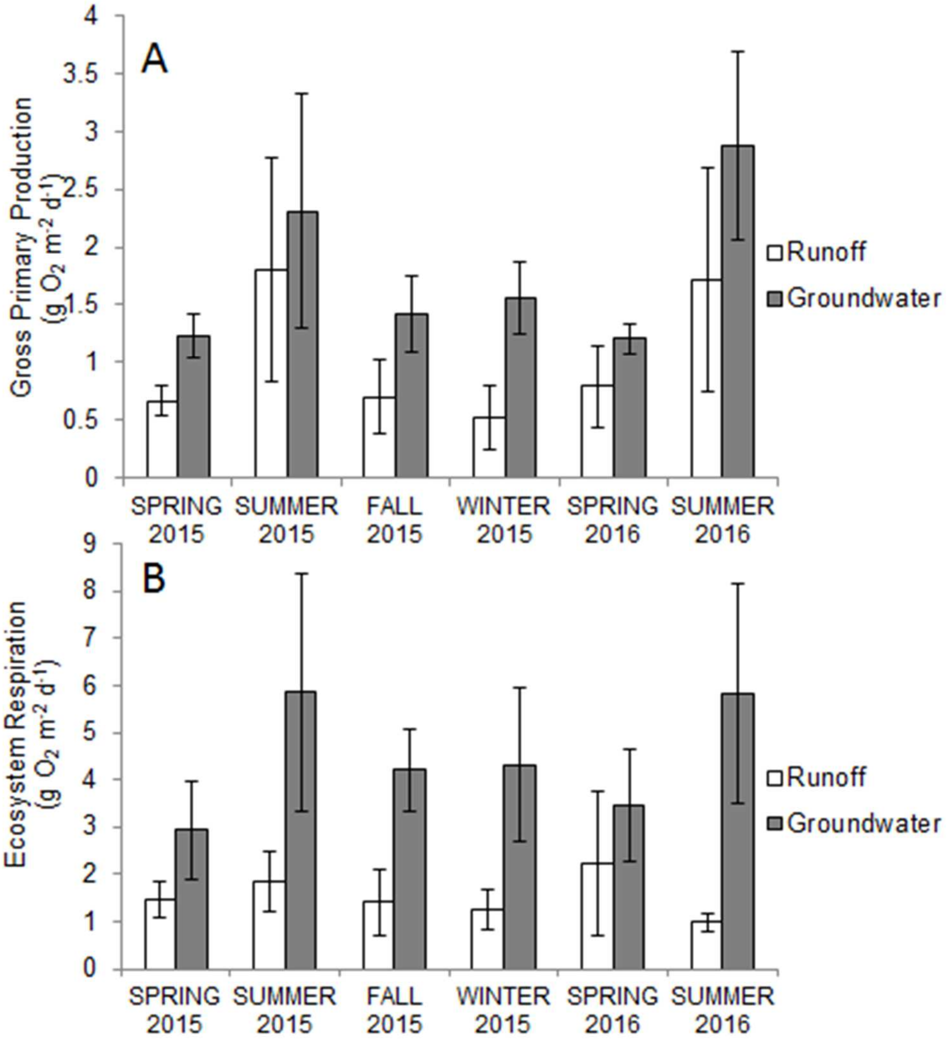

Figure 4. Seasonal average gross primary production (A) and respiration (B) in *Runoff* (white) and *Groundwater* (gray) streams. Error bars denote ± 1 standard error. n= 3 per flow regime.

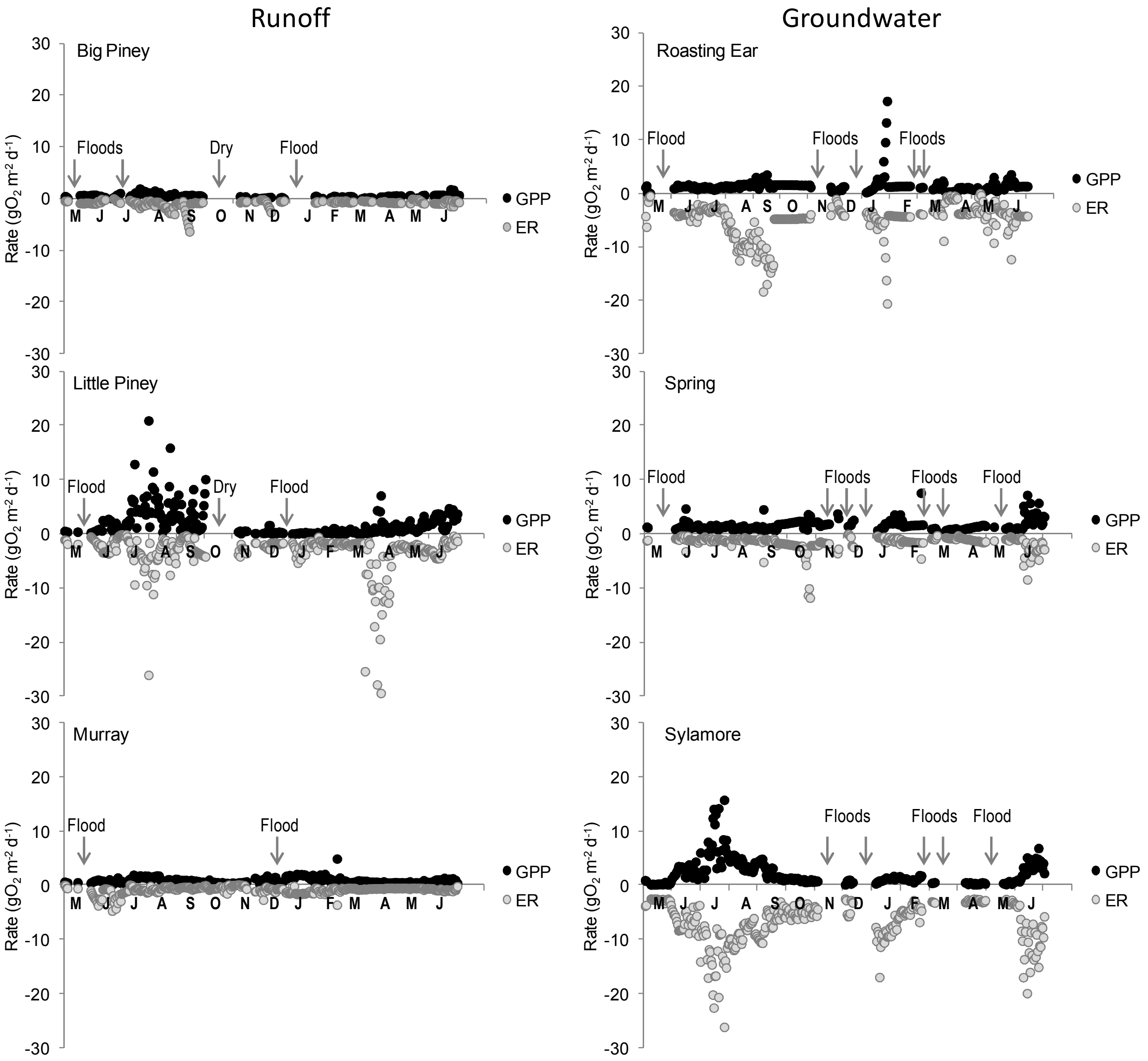

Figure 5. Daily rates of production (black) and respiration (gray) in Big Piney, Little Piney, Murray, Roasting Ear, Spring, and Sylamore from May 2015 to June 2016. *Runoff* streams are shown in panels on the left, *Groundwater* streams are represented in panels on the right. Gaps in data points not marked as “dry” show storm events and seven days of recovery under which GPP and ER were assumed to be close to zero.

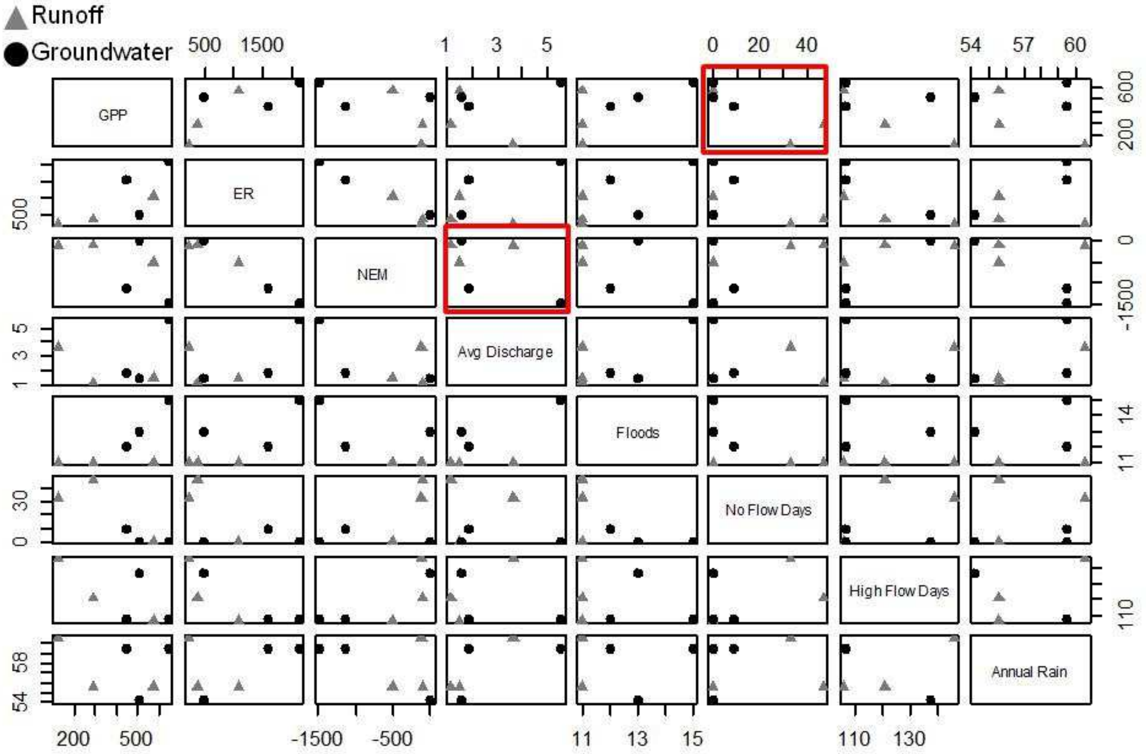

Figure 6. Spearman's rank correlations between annual metabolism metrics and flow variables across *Runoff* and *Groundwater* streams. Significant relationships between metabolism and flow metrics are highlighted with a red rectangle. GPP, ER, and NEM denote gross primary production, ecosystem respiration, and net ecosystem metabolism, respectively. Average discharge was flow averaged over the study year. Floods were defined as discrete hydrograph peaks exceeding the 100th percentile of mean annual flow. High flow days were defined as flows exceeding the 75th percentile of mean annual flow.

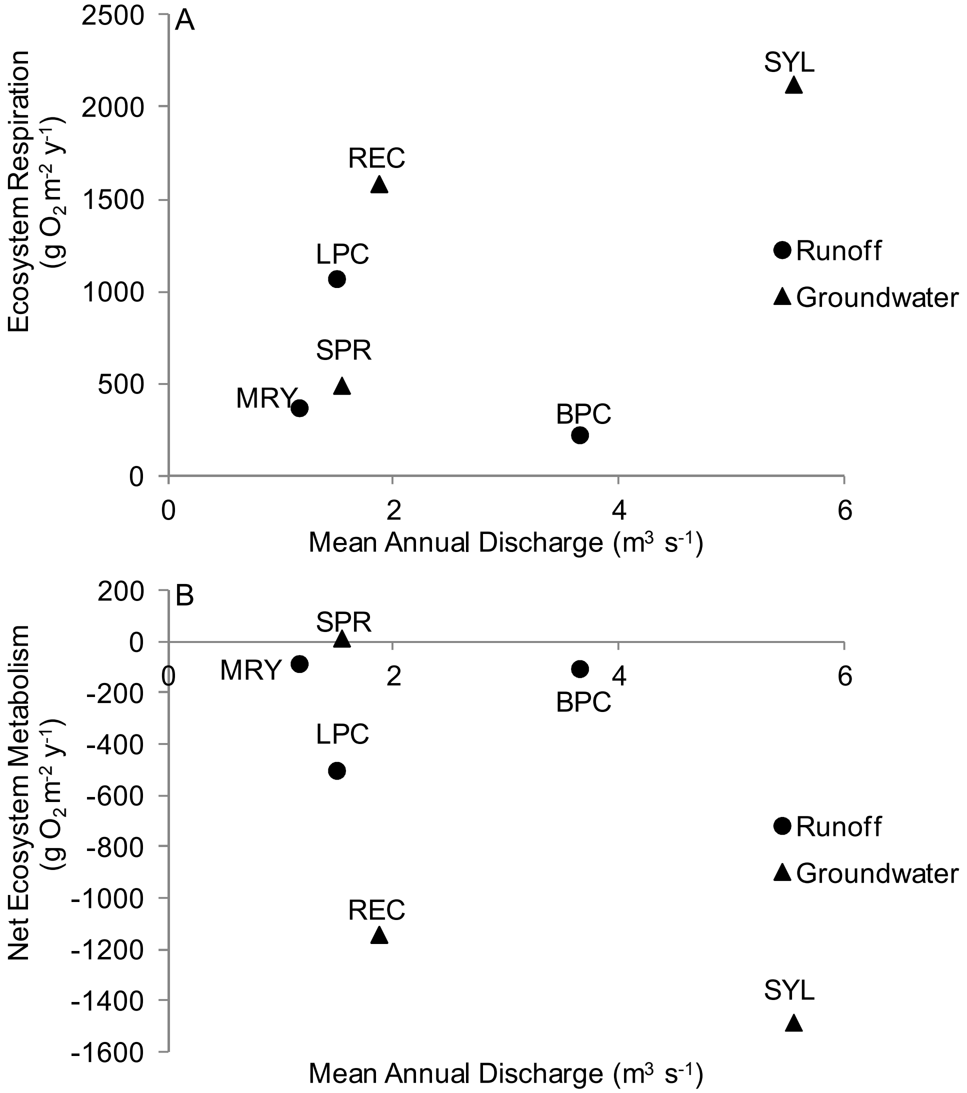

Figure 7. Ecosystem respiration (A) and net ecosystem production (B) compared with discharge across flow regimes.

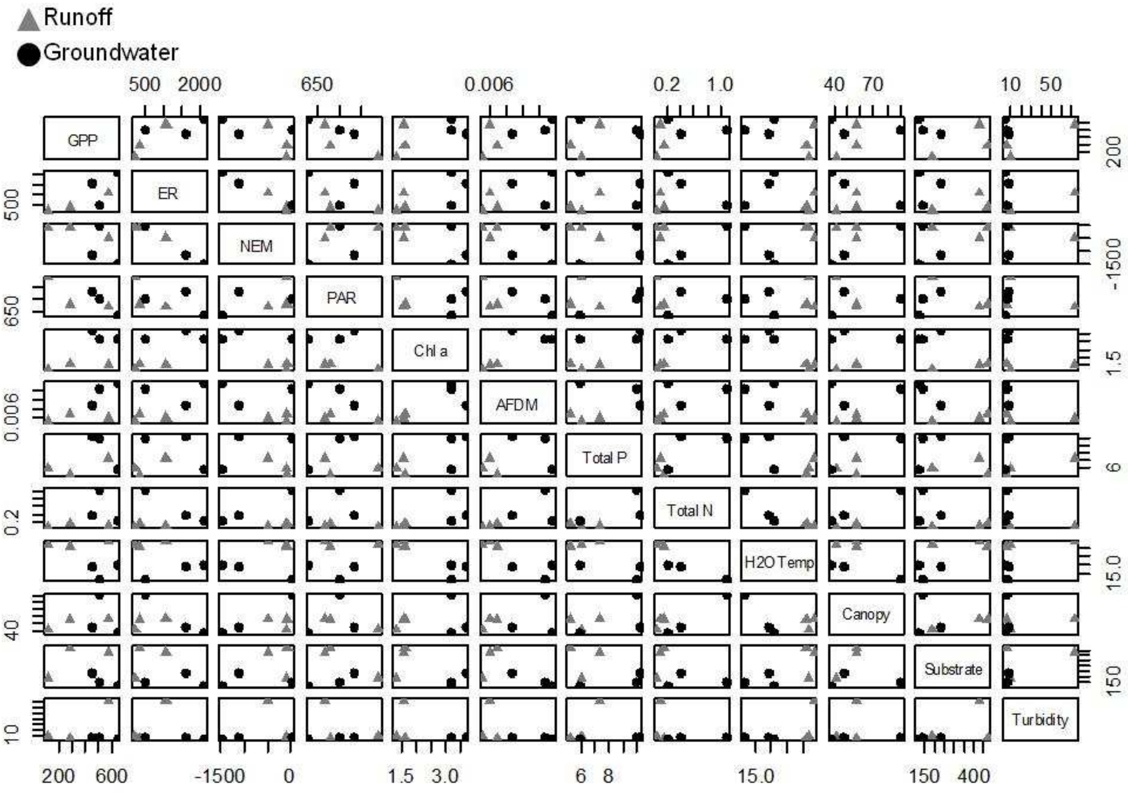

Figure 8. Spearman's rank correlations for biological, physical, and chemical parameters across *Runoff* and *Groundwater* streams. n= 3 per flow regime. Gross primary production, ecosystem respiration, and net ecosystem metabolism are denoted by GPP, ER, and NEM, respectively. Instream variable abbreviations are: Light as photosynthetically active radiation = PAR, algal biomass measured as chlorophyll a= Chl a, periphyton ash-free dry mass= AFDM, total phosphorus= Total P, total nitrogen= Total N, water temperature= H2O Temp, canopy cover= Canopy, substrate size= Substrate.

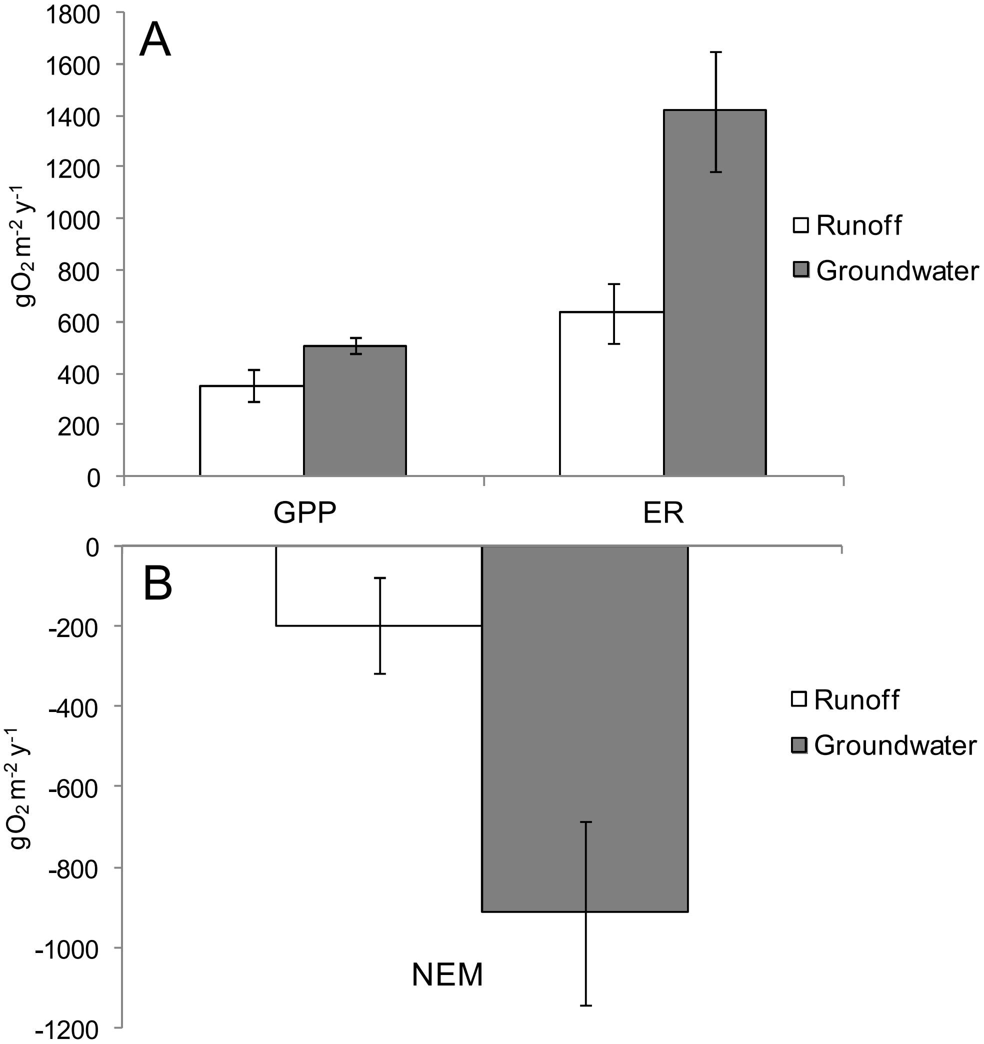

Figure 9. Gross primary production (GPP) and ecosystem respiration (ER) in simulated *Runoff* (white) and *Groundwater* streams (A). Panel (B) shows net ecosystem metabolism (NEM) between modeled flow regimes. Error bars denote ± 1 standard error. n= 15 per flow regime.

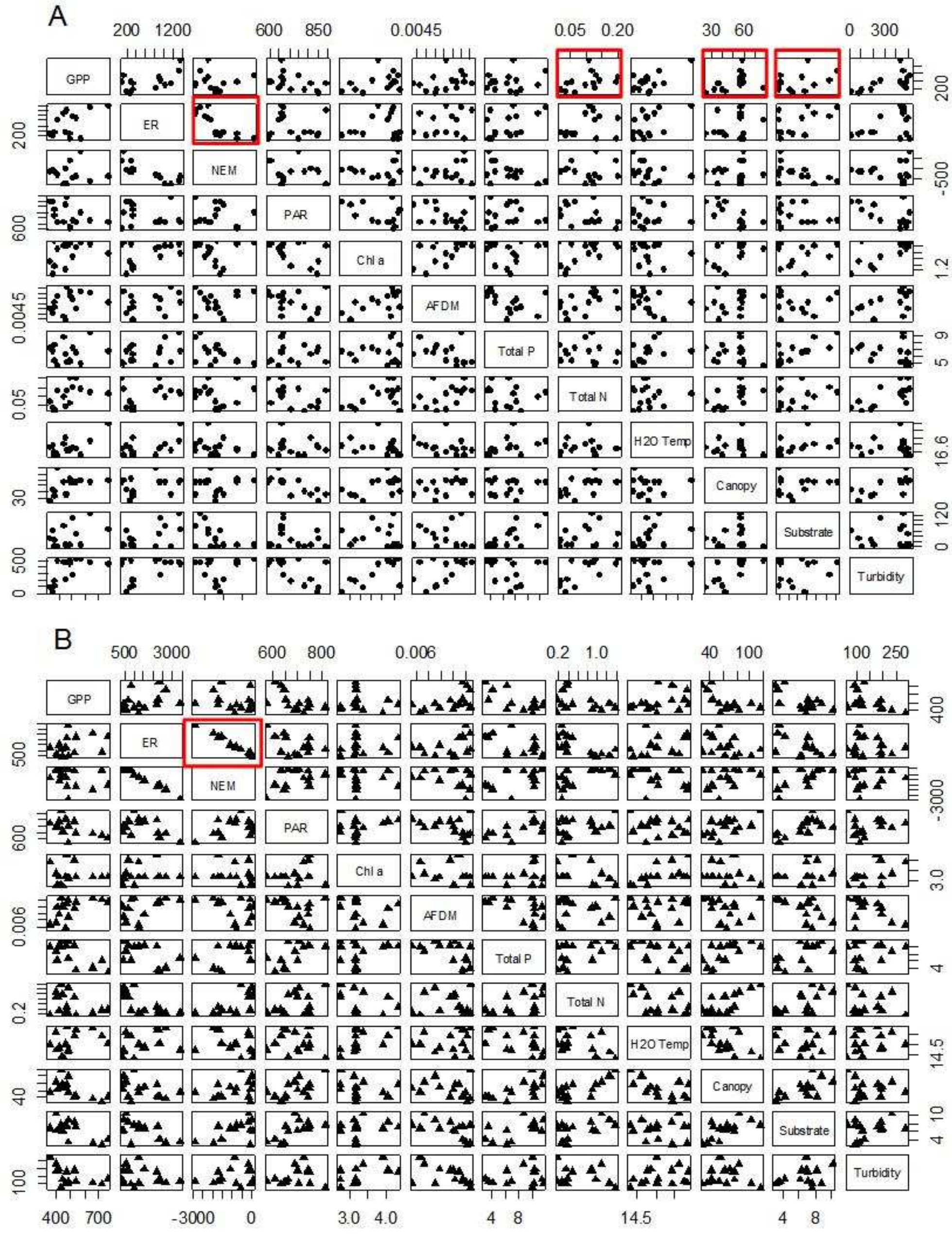

Figure 10. Spearman's rank correlations between modeled metabolism and environmental variables in simulated *Runoff* (A) and *Groundwater* (B) streams. Correlations evaluated relationships within each individual flow regime. Significant correlations are highlighted in red rectangles. n= 15 per flow regime. Gross primary production, ecosystem respiration, and net ecosystem metabolism are denoted by GPP, ER, and NEM, respectively. Instream variable abbreviations are: Light as photosynthetically active radiation = PAR, algal biomass measured as chlorophyll a= Chl a, periphyton ash-free dry mass= AFDM, total phosphorus= Total P, total nitrogen= Total N, water temperature= H2O Temp, canopy cover= Canopy, substrate size= Substrate.

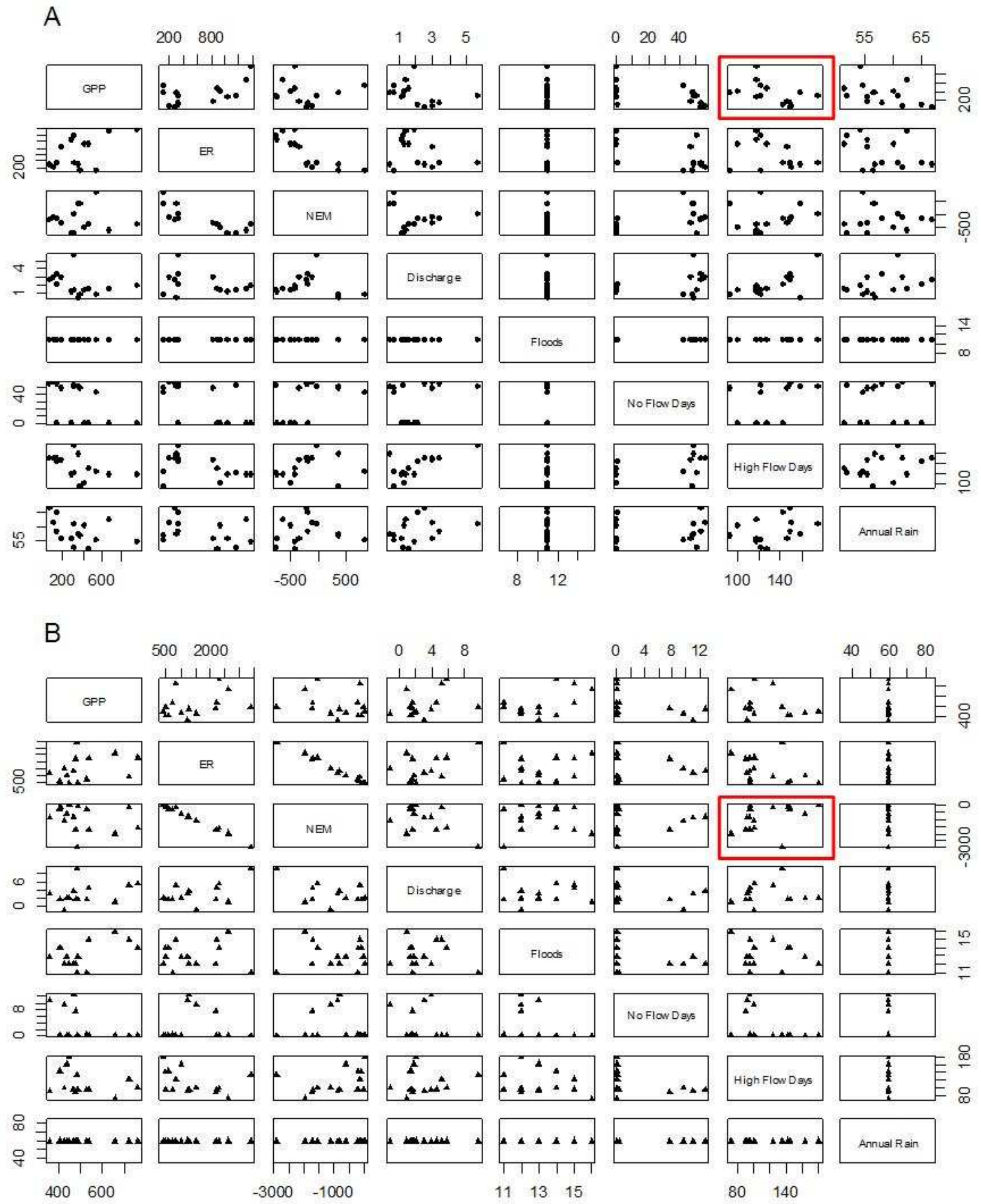

Figure 11. Spearman's rank correlations between modeled metabolism and flow metrics in simulated *Runoff* (A) and *Groundwater* (B) streams. Correlations evaluated relationships within each individual flow regime. Significant correlations between metabolism and flow metrics are highlighted in red rectangles. n= 15 per flow regime. GPP, ER, and NEM denote gross primary production, ecosystem respiration, and net ecosystem metabolism, respectively. Average discharge was flow averaged over the study year. Floods were defined as discrete hydrograph peaks exceeding the 100th percentile of mean annual flow. High flow days were defined as flows exceeding the 75th percentile of mean annual flow.

CHAPTER TWO

The influence of flow regime on sources and factors related to greenhouse gas emissions from Ozark streams

ABSTRACT

Streams are significant contributors of carbon dioxide (CO_2) and methane (CH_4) to the atmospheric carbon (C) sink. The effects of land use and biome on the magnitude of these emissions have been the subject of a small number of studies. However, flow regime may account for a portion of the variation in C gas emissions within a biome or land use category. Flow regime is comprised of the magnitude, timing, duration, predictability, and rate of change of streamflow. No studies to date have examined how flow regime within a biome and land cover category may influence CO_2 and CH_4 emissions, as well as factors related to C emissions. Two dominant flow regimes, Runoff Flashy (*Runoff*) and Groundwater Flashy (Groundwater) systems, exist within northern Arkansas. These flow regimes differ in dominant water source, intermittency, and flood frequency. Carbon dioxide and CH_4 fluxes and $\delta^{13}C$ values were measured to estimate relative source contributions in six temperate, forested-headwater streams on six dates from May 30- September 2, 2017. I also quantified instream biological, physical, and chemical variables that were hypothesized to be related to C gas emissions. Field data were used to produce a dataset of 15 simulated streams per flow regime (N=30 modeled streams) to determine whether increased sample size may affect results and to explore variables that may explain variation in C gas fluxes. *Runoff* stream CO_2 fluxes averaged 0.12 (± 0.02 SE) mol m^{-2} d^{-1} over the study period while *Groundwater* streams emitted 0.18 (± 0.12 SE) mol m^{-2} d^{-1}. *Runoff* stream CH_4 flux rates averaged 0.22 (± 0.10 SE) mmol m^{-2} d^{-1} while *Groundwater* stream CH_4 fluxes averaged 0.46 (± 0.25 SE) mmol m^{-2} d^{-1}. Carbon dioxide and CH_4 fluxes did not differ across flow regimes in the six field sites (CO_2: p= 0.63 CH_4: p= 0.42). Sources of CO_2 across flow regimes based on $\delta^{13}C$ data were the product of mixing between soil-respired and atmospheric CO_2 ($\delta^{13}C$= -17.6 to -22.0 per mil). Thermogenic CH_4 was also found in streams of both flow regimes, but *Groundwater* streams also revealed areas evading biogenic CH_4 (*Runoff* $\delta^{13}C$ = -38.8 to -43.7 per mil, *Groundwater* $\delta^{13}C$ = -32.5- 60.0 per mil). Negative relationships were identified between dissolved organic carbon (DOC) and CO_2 flux during two of the sampling events (July: R^2= 0.70, p= 0.04, mid-August: R^2= 0.85, p= 0.03). On the July date, total organic carbon (TOC) was also negatively related to CO_2 flux (R^2= 0.76, p= 0.02), while on the other sampling date, ER exhibited a negative relationship with CO_2 along with DOC (R^2= 0.92, p= 0.04). Dissolved organic carbon was positively related to CH_4 flux in mid-August (R^2= 0.50, p= 0.03). Modeled streams exhibited no difference between CO_2 or CH_4 fluxes, but multiple linear regressions revealed that global models explained the most variation in CO_2 fluxes (97% in *Runoff* streams, 83% in *Groundwater*s treams), with different variables of greatest significance within each natural flow regime. The global model also explained the 95% of the variation in CH_4 emissions in *Runoff* streams, but no candidate models explained variation in *Groundwater* CH_4. These results provide a case for assessing sources and controls on C gas dynamics within the context of flow regime by revealing different sources and drivers of CH_4 fluxes from stream surfaces.

INTRODUCTION

Inland freshwaters are integral components of the global carbon (C) cycle, as streams and rivers act as conduits through which carbon moves from terrestrial ecosystems to oceans. A growing body of research has revealed that streams are not merely passive pipes transporting C to marine environments, but rather reactors that transform organic carbon and emit excess in the form of CO_2 and CH_4 (Battin et al. 2009, Butman and Raymond 2011) (Figure 1). Terrestrial C budgets have likely grossly overestimated the size of the terrestrial C sink by attributing C that enters the aquatic pool to terrestrial storage or uptake (Cole et al. 2007, Battin et al. 2008).

Carbon is then utilized by aquatic biota to meet energetic demands, transported down the river network to adjacent streams, wetlands, and lakes, and/or evaded to the atmosphere. Carbon dioxide and methane emitted from streams also come from microbial metabolism and biological activity in the stream and surrounding watershed soil. Carbon dioxide is a byproduct of aerobic respiration, while methane originates from anaerobic methanogenesis (Hotchkiss et al. 2015, Stanley et al. 2016). Abiotic processes can also introduce C species into a stream. Carbon dioxide as well as methane may diffuse into the water column (Figure 1, Flux A) or seep through groundwater upwellings in the stream bottom (Figure 1 Flux H); CH_4 also moves from sediments through the water column (Figure 1; Flux I). Although streams make up a small percentage of the landscape area, they are hot spots for CO_2 and CH_4 emissions as byproducts of a number of biotic and abiotic processes, and thus must be included in C budgets to fully characterize landscape C dynamics (Raymond et al. 2013).

Methane was once thought to be negligible in streams due to aerated conditions (Dahm et al. 1991). However, streams are indeed significant sources of this potent greenhouse gas; while CH_4 comprises a small portion of watershed C budgets, streams are significant sources of CH_4 to the atmosphere at the watershed-scale (Stanley et al. 2016). Methane has received less attention than CO_2 with regard to stream C gas footprints, an unfortunate oversight given that CH_4 is nearly four times as potent as CO_2 with respect to atmospheric warming potential (Lashof and Ahuja 1990).

Climate change as a result of anthropogenic greenhouse gas emissions is an urgent problem that requires a detailed understanding of global C sources and sinks to develop mitigation strategies and potential solutions (King 2004, Palmer et al. 2008). Carbon budgets provide an estimate of natural and anthropogenic variation in CO_2 and CH_4 across the landscape, and are of paramount importance in mitigating the effects of climate change by quantifying anthropogenic and natural C sources and sinks. However, few studies have addressed the flux of CO_2 between inland freshwaters and the atmosphere using direct measurements (Cole et al. 2007), which is likely significant enough to affect the global C budget (Butman and Raymond 2011). Further, data characterizing CH_4 emissions are extremely limited in spatial and temporal coverage, underscoring the need for further investigation to reveal potential landscape-level patterns in stream CH_4 loss over time.

No work has quantified CO_2 flux within the context of hydrologic regime, which can exert control over C emissions via differences in water sources (e.g. groundwater versus runoff-dominated systems) as well as geomorphology, intermittency, and frequency of flood events (Striegl and Michmerhuizen 1998) over the seasonal or annual time scales used to categorize natural flow regimes within biomes (Leasure et al. 2016). For example, streams with significant contributions from CO_2-saturated groundwater may emit more greenhouse gases to the atmosphere, while drying should reduce emissions, though dry streambeds should still produce CO_2. Rewetting of the dry channel and flooding should both increase C gas flux rates (Gomez-Gener et al. 2016). Additionally, there is a paucity of data investigating the relationship between instream metabolism and greenhouse gas emissions (Hotchkiss et al. 2015). Further, measuring greenhouse gas fluxes across flow regimes will aid in model parameterization across six ecoregions (e.g. the Ozark Highlands, Boston Mountains, South Central Plains, Arkansas River Valley, Ouachita Mountains, and High Plains) (Woods et al. 2004, Woods et al. 2005), allowing for model development needed to provide regional predictions regarding CO_2 and CH_4 dynamics in streams and changes to watershed C budgets as the effects of climate change become more pronounced.

Recent work has revealed temperate streams to be significant C sources, emitting an estimated 0.5 petagrams of C to the atmosphere each year. However, this prediction was generated with uncertainties regarding three controls on C gas evasion: variation in gas transfer velocities across streams, no information regarding the actual concentration of CO_2 in water, and high resolution with respect to the global surface area of streams and rivers (Butman et al. 2013). While the importance of these systems to atmospheric greenhouse gas concentrations is becoming more apparent, data quantifying variation across systems is scarce (Cole et al. 2007, Battin et al. 2009, Wallin et al. 2012), leading to an inadequate understanding of how gas transfer may vary among headwater systems. Spatial coverage of greenhouse gas evasion is lacking as well, likely resulting in continued underestimation of the role of streams in landscape C cycling. While others have provided estimates of C emissions in the United States, (Butman and Raymond 2011, Raymond et al. 2013, Hotchkiss et al. 2015), the focus of these efforts was on river size. These studies relied on proxy measurements (e.g. alkalinity, temperature, and pH) rather than direct measurements of greenhouse gas evasion, and most direct measurements of C gas emissions are recorded in lakes. Alkalinity, pH, and temperature each exhibit a positive relationship with dissolved CO_2; fluxes are then calculated from dissolved measurements by utilizing the gas exchange coefficient of CO_2 along with the concentration of CO_2 in the atmosphere (Butman and Raymond 2011).

Direct measurements are preferable to proxy measurements because C gas flux may be affected by certain parameters that do not alter physicochemical variables, thereby leading to measurement inaccuracy. For example, CO_2 exchange with the atmosphere and photosynthesis both affect CO_2 emissions from streams, but have no effect on alkalinity, and while calcification (i.e. removal of calcium and bicarbonate from water by organisms to create shells) can affect both CO_2 and alkalinity, it does not affect pH (Libes 2009). Methane has been directly quantified, but efforts have been primarily focused on boreal streams, and more data characterizing CH_4 emissions are needed throughout the United States since organic matter contributions to streams, and therefore CO_2 and CH_4 production, will likely differ at least by biome. Data focused on boreal networks may yield higher estimates for CH_4 emissions given the greater amount of soil organic matter found in northern systems. Thus, anaerobic hotspots of CH_4 production may occur with greater temporal or spatial extent in boreal systems than in temperate streams (Crawford et al. 2013, Crawford et al. 2014). A striking paucity of work has directly measured emissions of CO_2 and CH_4 from temperate streams in particular, and I hypothesize that work focused on C gas emissions has ignored the primary driver of all stream patterns and processes: flow regime.

Greenhouse gas evasion and flow regime

The natural flow regime is characterized by the timing, quantity, and variability of stream flow, governing spatiotemporal variation in water quality and quantity (Poff et al. 1997). Flow within the channel may vary based on the water source (e.g. groundwater versus runoff). Flow regime likely governs stream CO_2 and CH_4 fluxes as well. However, current management and policy makers overlook the primacy of flow regime when making regulatory decisions (Poff et al. 2010). This is problematic given that the flow regime directly influences ecosystem services such as providing adequate amounts of clean water for human consumption, serving as habitat for an abundance of wild and game freshwater species, and providing basal resources for downstream and surrounding terrestrial habitats (Millenium Ecosystem Assessment 2005, Palmer et al. 2014). Additionally, flow alteration from anthropogenic activities negatively impacts stream ecosystems by altering stream temperature and dissolved oxygen (DO) concentrations (Nelson and Palmer 2007), changing or removing habitat, and disrupting natural flow variation that is critical to many aquatic animals' life cycles (Poff et al. 2010, Poff and Zimmerman 2010). These changes are likely associated with changes in C emissions from the stream channel to the atmosphere. It is imperative to ascertain how C storage and flux is influenced by hydrologic regime, as such data would provide a baseline by which to compare streams that have been disturbed by land use and climate change.

Headwater streams comprise the majority of streams within a network and half of all river miles in the United States (Leopold et al. 1964), exerting a profound influence over downstream water quality and quantity as well as carbon cycling throughout watersheds and ecoregions (Alexander et al. 2007). Importantly, these small, high gradient streams emit more CO_2 per meter to the atmosphere than large rivers, and spatial coverage of direct measurements of CO_2 evasion is exceedingly poor. Even less work has focused on CH_4 emissions. Further, forested streams emit more CO_2 than row-crop agricultural systems, underscoring the need to account for specifically forested headwater streams, which account for the largest fraction of stream contributions to regional greenhouse gas budgets per unit area (Butman and Raymond 2011). Others have pointed out that CO_2 emitted from streams could account for up to 10% of all natural CO_2 emissions in Sweden (Butman et al. 2016) emphasizing the importance of streams as integral components of the C balance of whole landscapes, and understanding the role of high gradient, temperate forested headwater systems is especially needed given their high gas transfer velocities and terrestrial C inputs (Cole et al. 2007, Butman and Raymond 2011, Raymond et al. 2012).

Recent efforts in the Ozark and Ouachita Interior Highlands, which encompasses much of Arkansas as well as eastern Oklahoma and southern Missouri, have successfully mapped natural flow regimes based upon flow metrics gleaned from reference gauge data along with landscape-level GIS-based variables (Leasure et al. 2016). These flow regime designations provide ample opportunity for questions regarding how hydrologic regime may influence ecosystem function and community dynamics. While others have begun the important work of exploring flow-ecology relationships within these mapped flow types (Bruckerhoff and Magoulick 2017, Lynch et al. 2018), little to no inquiries into ecosystem-level metrics have been undertaken based on these designations, and addressing greenhouse gas emissions represents a further step to explore possible ecohydrological distinctions that may influence C biogeochemistry and thus link hydrology-biology relationships with CO_2 and CH_4 emissions. I sought to determine potential differences and relationships between and across the two dominant flow regimes in northern Arkansas: Groundwater Flashy streams and Runoff Flashy streams (hereafter *Groundwater* and *Runoff*).

The first objective of this study was to determine whether differences existed in CO_2 and CH_4 fluxes as well as C gas sources between *Groundwater* and *Runoff* streams. I expected CO_2 and CH_4 fluxes to be greater in groundwater-dominated streams due to the influx of CO_2-rich groundwater, greater community respiration (Chapter 1), and gravel-dominated substrate (as opposed to bedrock in *Runoff* streams) that may support small anaerobic pockets within the hyporheic zone (Figure 2). The second objective was to determine whether relationships existed between C gas fluxes and selected biological, physical, and chemical parameters across both flow regimes (Table 1). I expected CO_2 flux to be related to daily gross primary production and respiration, pH, and alkalinity given that the latter two metrics can be used to model CO_2 dynamics. The third objective was to use the data from six streams to simulate a larger number of streams to determine potential relationships between factors and gas fluxes within each flow regime that may have been indiscernible with a sample size of three streams per flow regime. Small sample sizes are typical of ecosystem-scale studies (e.g. Bormann et al. 1974, Schindler 1977), but do not have the statistical power of smaller-scale manipulations with greater replication (Quinn and Keough 2002).

METHODS

Study Sites

This study was conducted in six minimally impacted headwater streams with deciduous-forested land cover ranging from 84.8 to 97% of total watershed area (CAST 2007) (Figure 3). I chose three *Groundwater* streams and three *Runoff* streams of similar size and discharge. These two natural flow regimes are spatially clustered within the Ozark Highlands and Boston Mountains ecoregions, respectively, and comprise the two dominant flow regimes in northern Arkansas, southern Missouri, and eastern Oklahoma (Leasure et al. 2016).

Four streams were located upstream of United State Geological Survey gauging stations. Two *Groundwater* streams (Roasting Ear and Spring) were not located directly upstream from gauges; however, these streams were located near streams (within the same watershed) of the same order and similar size with gauges. There were significant relationships between measurements made in those two stream reaches and two “proxy gauges” within the same watershed (one of which was Sylamore Creek, a *Groundwater* stream in this study), allowing for quantification of flow metrics for all six streams over the year. Watershed areas ranged from 20.99 to 38.77 km^2 (Table 1). Both flow types exhibit flashy hydrology marked by floods of large discharge in short duration. The majority of flow in *Runoff* streams originates from overland runoff and precipitation, and *Runoff* streams typically dry for several days to weeks each year. *Groundwater* streams are heavily influenced by spring water inputs and tend to flow year-round.

Experimental Design

I sampled each stream six times over a range of discharges (0.70-7.92 m^3/s) from May 30th, 2017 to September 2nd, 2017. I did not sample Murray Creek, a *Runoff* stream, during the mid-August sampling event due to a large flood that prevented access to the site. Measurements of CO_2 and CH_4 exchange with the atmosphere were collected using a floating chamber constructed from an inverted bucket attached to a sheet of foam approximately 4 centimeters from the edge of the bucket so as to ensure the chamber was completely sealed to the stream surface. I inserted tubing into the top of the chamber that could be clamped shut between samples. The chamber was attached to small weights to anchor it in place in the stream channel. I collected discrete 150 mL gas samples with a 50 mL syringe from the sealed chamber every 10 minutes from 0 to 30 minutes (i.e. four samples in each location) at the top and bottom of each 200-meter stream reach. Air samples were injected into pre-evacuated gas bags. I determined C gas concentrations and C isotopic signatures in each sample by running gas samples into a Picarro G2201-i carbon isotope analyzer (Picarro Inc., Santa Clara, CA). I calculated CO_2 and CH_4 fluxes based on the gas concentration change within the sealed chamber over time (i.e. over 10 minutes) according to the equation

$$J_{CO2} = dc/dt * h \qquad [1]$$

where dc/dt is the change in gas concentration in the chamber air in parts per million (ppm) over time in minutes multiplied by the height (h) of the chamber in meters minus the submerged portion (Crawford et al. 2013). I converted fluxes using the ideal gas law and reported measurements in moles $meter^{-2}$ day^{-1} for CO_2 and millimoles $meter^{-2}$ day^{-1} for CH_4.

Continuous DO and temperature were recorded using a MiniDOT logger at the bottom of four of the six reaches per sampling event. Each probe was set to log every 15 minutes for 24 hours, from the evening on the day of chamber deployment until midnight of the following day. Photosynethetically-active radiation (PAR) measurements were logged concurrently with metabolism parameters using an Odyssey PAR light meter (Dataflow Systems Ltd., Christchurch, NZ) positioned in an area near the stream with open canopy. Reaeration coefficients as estimates of air-water gas exchange were determined via a propane and salt release at each stream. Corrections for groundwater contributions to reaches receiving appreciable inputs were made according to Hall and Tank (2005) by measuring DO in water at upwellings as well as discharge down the reach to determine springwater gains and losses from springs to the sonde within the 200-meter study reach. I calculated reach-scale metabolism based on changes in DO, temperature, depth, and light measured over the 24-hour period using the StreamMetabolizer package in R (version 3.4.3) to determine gross primary production and respiration according to a single-station, open-channel metabolism model within a maximum likelihood framework:

$$O_{2(t)} = O_{2(t-\Delta t)} + \left(\frac{GPP_{Total}}{\underline{z}} \times \frac{PAR_{(t)}}{\sum PAR_{24}}\right) + \left(\frac{ER_{Total}}{\underline{z}} \times \Delta t\right) + K_{(t)}\left(O_{2sat(t)} - O_{2(t)}\right)\Delta t \quad [2]$$

where t is time and Δt is the time step between measurements (15 minutes), $\underline{z}$ is mean reach depth, $\sum PAR24$ is daily photosynthetically active radiation, and K(t) is air-water gas exchange corrected for temperature. Solving this equation for GPP_{Total} and ER_{Total} yielded daily rates of production and respiration for each sampling date at each stream that were regressed with CO_2 and CH_4 gas flux measurements across streams on each sampling date to determine whether metabolic parameters were driving C gas evasion on those dates.

I collected triplicate water samples in each reach to determine concentrations of total organic carbon (TOC), dissolved organic carbon (DOC), and dissolved inorganic carbon (DIC). Samples were analyzed on a Shimadzu TOC-V_{csh} analyzer (Shimadzu Corporation, Kyoto, Japan). I also retrieved triplicate samples to determine pH with a Thermo Scientific handheld meter and total alkalinity via acid titration in the laboratory. I collected six cobbles per stream per sampling event to estimate algal biomass as chlorophyll a by ethanol extraction (Sartory and Grobbelaar 1984). I measured discharge using the mid-section method (Gore 2006).

Factors related to CO_2 and CH_4 fluxes across streams were determined by analyzing potential relationships averaging factors across sampling dates as well as on each sampling date. To do this, data were first visually inspected to explore whether potential linear or non-linear relationships existed between fluxes and instream variables (e.g. water chemistry, discharge, and algal biomass). Subsequently, linear regressions were employed to test for significant relationships between C gas fluxes and instream variables. T-tests were used to determine whether differences exist in C gas fluxes and stream variables between *Groundwater* and *Runoff* streams. Variation in data is reported as $\pm$ 1 standard error.

To increase statistical power and explore potential relationships between C gas flux and stream variables within each flow class, I simulated a dataset with 15 streams from each flow regime (N=30). Each set of five simulated streams within a flow class was sampled from a normal distribution using each variable in the field data set from each stream to set minimum, maximum, mean, and standard deviation parameters within each flow class to conserve possible relationships across variables. I again ran t-tests to determine whether differences exist between *Groundwater* and *Runoff* stream gas fluxes and stream variables. I then separated the dataset by flow regime and utilized an information theoretic approach to determine what set of variables best explained variation in CO_2 and CH_4 fluxes, respectively, in *Runoff* and *Groundwater* streams.

RESULTS

All streams in this study were net sources of CO_2 and CH_4 to the atmosphere (Figure 4). Methane fluxes in individual *Runoff* streams ranged from 0.03 to 1.45 ($\pm$ 0.08) mmol m^{-2} d^{-1} and from 0.05 to 2.04 ($\pm$ 0.14) mmol m^{-2} d^{-1} in individual *Groundwater* streams over the study duration (Figure 5). *Runoff* stream CO_2 fluxes ranged from 0.03 to 0.23 ($\pm$ 0.015) mol m^{-2} d^{-1} over the study period while *Groundwater* streams emitted 0.02 to 0.58 ($\pm$ 0.034) mol m^{-2} d^{-1}. There was no significant difference between CO_2 fluxes measured across sites during storms versus during base flow ($p= 0.14$). Methane fluxes were also similar regardless of whether measurements were made during storms or not ($p= 0.75$). Separating sites by flow regime also made no difference; CO_2 fluxes at base flow ($p= 0.57$) and storm flow ($p= 0.24$) did not differ between flow regimes. Methane fluxes at base flow ($p= 0.66$) and storm flow ($p= 0.39$) also did not differ between flow regimes.

Carbon dioxide fluxes did not differ over the study period between flow regimes ($p= 0.63$), though *Groundwater* streams tended to emit more CO_2 and were more variable in CO_2 fluxes than *Runoff* streams (Figure 5). Methane fluxes also did not differ between flow regimes ($p= 0.42$); however, *Groundwater* streams tended to emit more CH_4 and were more variable in the amount of CH_4 produced than *Runoff* streams.

Carbon dioxide $\delta^{13}C$ isotope values ranged from -25.16 to -13.10 per mil in *Runoff* streams and from -23.22 to -14.6 per mil in *Groundwater* streams from May through September (Figure 6). Sources of CO_2 across streams were a mixture of riparian soil respiration and atmospheric CO_2. Methane in *Runoff* streams originated from natural gas ($\delta^{13}CH_4$= -34.29 to -45.42 per mil), while both thermogenic and biogenic CH_4 were measured in *Groundwater* streams ($\delta^{13}CH_4$= -26.35 to -59.8 per mil) (Figure 6).

Groundwater streams tended to yield numerically greater rates of gross primary production (p= 0.09) and respiration (p=0.36), though differences were not significant. Gross primary production ranged from 1.76 to 4.31 g O_2 m^2 day^{-1} over the summer in *Groundwater* streams and from 0.28 to 0.78 g O_2 m^2 day^{-1} in *Runoff* streams. Gross primary production averaged 2.83 (± 0.76) g O_2 m^2 day^{-1} in *Groundwater* streams and 0.57 (± 0.15) g O_2 m^2 day^{-1} in *Runoff* streams. Ecosystem respiration varied from -1.95 to -7.35 in *Groundwater* streams and from -1.65 to -4.16 in *Runoff* streams. *Groundwater* stream mean ER over the summer was -4.75 (± 1.56) g O_2 m^2 day^{-1} while *Runoff* stream ER averaged -2.89 (± 0.73) g O_2 m^2 day^{-1}.

Algal biomass was greater and more variable at *Groundwater* sites over the summer (p= 0.02) (Figure 7a). *Runoff* chlorophyll a concentrations at each site over the study varied from 13.8 to 27.7 mg cm^{-2}. *Groundwater* site algal biomass varied from 144.5 to 210.6 mg cm^{-2}. *Runoff* sites averaged 22.36 (±4.3) mg chlorophyll a cm^{-2}, while chlorophyll a at *Groundwater* sites averaged 199.1 (± 28.79) mg cm^{-2}. Algal biomass consistently tended to be greater in *Groundwater* streams on individual sampling dates. All streams experienced reduced algal biomass following a large flood event on August 15th. Two *Runoff* streams exhibited increasing biomass until the flood, while the third *Runoff* stream, Little Piney Creek, exhibited a reduction in biomass before the storm. *Runoff* stream responses in the flood recovery period were variable; one stream yielded consistent chlorophyll a levels, while algal biomass increased at Little Piney Creek and decreased at Murray Creek. *Groundwater* streams each differed in algal biomass over the summer; Spring Creek was consistent until the large flood, while chlorophyll a in Sylamore and Roasting Ear Creeks increased prior to the flood. Afterward, the Roasting Ear algal community appeared to consistently recover, but the communities at Spring and Sylamore continued to decline through the last sampling date.

Groundwater streams held numerically greater and more variable concentrations of TOC (p= 0.097) and DOC (p= 0.22), averaging 31.3 (± 9.2) mg L^{-1} TOC and 23.6 (± 12.1) mg L^{-1} DOC over the study period (Figure 7b and 7c). *Groundwater* stream TOC at each site ranged from 20.3 to 49.5 mg L^{-1} while DOC ranged from 4.1 to 47.0 mg L^{-1}. *Runoff* stream TOC concentrations were 4.0 (± 0.5) mg L^{-1} while DOC averaged 1.9 (± 0.7) mg L^{-1}. Total organic C concentrations at *Runoff* sites varied from 3.0 to 4.6 mg L^{-1}; DOC varied from 0.9 to 3.1 mg L^{-1}. Total organic C concentrations increased two-to-tenfold across streams during and following the August 15th flood. However, three streams, Murray (*Runoff*), Spring (*Groundwater*), and Roasting Ear (*Groundwater*), dropped to below-flood concentrations in the following weeks as discharge returned to base flow.

Groundwater streams had larger DIC concentrations (p= 0.004) and alkalinity (p= 0.003) (Figure 7d). Dissolved inorganic C values ranged from 31.3 to 36.8 mg L^{-1} in *Groundwater* streams and from 4.9 to 5.0 mg L^{-1} in Runoff streams. Alkalinity varied from 153.7 to 182.5 mg $CaCO_3$ L^{-1} at *Groundwater* sites and from 10.7 to 15.4 $CaCO_3$ L^{-1} at *Runoff* sites. Dissolved inorganic C concentrations averaged 33.3 mg L^{-1} compared to 4.9 mg L^{-1} in *Runoff* streams; mean alkalinity in *Groundwater* streams over the summer was 165.5 mg $CaCO_3$ L^{-1} compared to *Runoff* streams' 12.8 mg $CaCO_3$ L^{-1} (Figure 7e). The pH across flow regimes was similar (p= 0.60), ranging from 7.09 to 7.24 in *Runoff* streams and 7.13 to 7.32 at *Groundwater* sites (Figure 7f). DIC concentrations at *Runoff* sites varied between 6-8 mg L^{-1} across sites during the early portion of the summer, then dropped to 2-5 mg L^{-1} after the August 15th flood event at each site. *Groundwater* streams exhibited an opposite pattern, with DIC concentrations increasing consistently over the summer; values ranged from 28.8 to 34.5 earlier in the summer, then jumped to 32.5 to 38.5 following the flood and continued to steadily increase at all sites but

Spring Creek, which experienced a drop to pre-flood DIC levels by September 6th. Alkalinity in *Runoff* streams was consistent over the study period, albeit more variable following the August 15th flood, while *Groundwater* streams exhibited consistent alkalinity over the entire study. All sites experienced a slight (<0.5) drop in pH following the flood, though Little Piney Creek recovered by the following week, while other sites were consistently lower than pre-flood levels for the remaining two weeks of the study.

Average discharge tended to be greater at *Runoff* streams over the study period ($p=0.10$); average *Runoff* stream discharge was nearly two times greater than *Groundwater* discharge even after removing the August 15th flood event (0.97 vs. 0.49 m^3s^{-1}). Average summer discharge measured during the six sampling events varied from 2.50 to 7.92 m^3s^{-1} at *Runoff* sites; removing the August 15th flood reduces these values from 0.70 m^3s^{-1} to 1.28 m^3s^{-1}. *Groundwater* site discharge varied from 0.30 to 0.90 m^3s^{-1} for the summer.*Runoff* discharge varied over the summer with incident rainfall while *Groundwater* streams exhibited consistently reduced discharge at all sampling events over the summer. Average summer discharge measured during the six sampling events varied from 2.50 to 7.92 m^3s^{-1} at *Runoff* sites; removing the August 15th flood reduces these values from 0.70 m^3s^{-1} to 1.28 m^3s^{-1}. *Groundwater* site discharge varied from 0.30 to 0.90 m^3s^{-1} for the summer.

Linear regressions were used to evaluate relationships since visual inspection of data suggested this approach. Additionally, Pearson's correlation coefficients were greater than Spearman's rank coefficients, suggesting more linear relationships. No significant relationships were identified between CO_2 or CH_4 fluxes and any stream variable averaged over the summer (Table 2). Further, no significant relationships were identified between CO_2 or CH_4 fluxes and discharge on each sampling date at the individual site level. I also explored potential relationships between C gas fluxes and stream metrics on each sampling date, as changes in discharge over the sampling period as well as storm events may have influenced results (Figure 8).

Carbon dioxide flux was negatively related to TOC (R^2= 0.70 p= 0.02) and DOC (R^2= 0.62 p= 0.04) on the July 1st sampling event. Dissolved organic C was also negatively related to CO_2 flux measured in mid-August (R^2= 0.80 p= 0.03). Ecosystem respiration measured during the mid-August sampling event was also negatively related to CO_2 flux (R^2= 0.88 p= 0.04). Dissolved organic C was positively related to CH_4 flux measured during mid-August sampling (R^2= 0.80 p= 0.03) (Figure 9).

SIMULATIONS

The simulated dataset yielded no significant differences between CO_2 flux (p=0.19) or CH_4 flux (p= 0.15) by flow regime (Figure 10). Similar to my field sites, modeled *Groundwater* sites exhibited greater TOC (p= 0.0001), DIC (p< 0.0001), alkalinity (p< 0.0001) and GPP (p< 0.0001). The expanded dataset also revealed greater algal biomass (p< 0.0001) and DOC (p= 0.009) in modeled *Groundwater* streams. Discharge was greater in modeled *Runoff* streams (p< 0.0001).

Variation in simulated *Runoff* stream CO_2 flux was best explained by a global model modified to exclude one outlier in the model, as well as a second data point to improve homoscedasticity (R^2=0.97, p= 0.04) (Table 3). Within the global model, TOC (p= 0.02) and GPP (p= 0.02) were significant variables. A global model also explained the most variation in modeled *Runoff* stream CH_4 flux (R^2=0.95, p= 0.002) (Table 4); algal biomass was the sole significant variable in the global model (p= 0.0009). Simulated *Groundwater* stream CO_2 flux variation was best explained by a global model (R^2=0.83, p= 0.009, Table 5) in which discharge was significant ($p = 0.009$). None of the candidate models explained a significant amount of variation in CH_4 flux (Table 6).

DISCUSSION

All streams in this study were consistent C gas sources to the atmosphere, as others have reported (Butman and Raymond 2011, Crawford et al. 2013, Crawford et al. 2014, Stanley et al. 2016). Carbon dioxide fluxes measured in these streams across flow regimes were within range of those reported in the conterminous United States as well as Alaskan streams (Butman and Raymond 2011, Crawford et al. 2014) (Table 7). However, streams in the present study evaded nearly twice as much CO_2 as boreal systems outside the United States (Jonnson et al. 2007, Teodoru et al. 2009, Kaprivnjak et al. 2010), suggesting that temperate streams are likely significantly greater sources of C to the atmosphere than boreal streams. I compared my CH_4 flux rates to those reported as total fluxes since I did not separate diffusive and ebullitive contributions in these systems. Methane emissions across streams tended to be lower than the mean of published total CH_4 fluxes, though all sites were well within range of published values (Crawford et al. 2014, Stanley et al. 2016).

Given that groundwater is typically supersaturated in CO_2, I initially hypothesized that greater flux rates due to the attribution of carbonate weathering in addition to biological processes. However, even though *Groundwater* streams hold more dissolved organic and inorganic C, they do not emit significantly more CO_2 than *Runoff* streams. Further, while *Groundwater* streams respire more on average than *Runoff* streams, respiration also does not appear to drive up flux rates. In fact, the greatest CO_2 flux rates from this study came from Roasting Ear Creek, a *Groundwater* stream, but CO_2 flux rates at other *Groundwater* sites on most of my sampling dates were similar to *Runoff* stream fluxes. It may be that *Groundwater* streams' greater primary production rates and algal biomass may be responsible for similar flux rates, as algae may be incorporating a significant fraction of DIC into biomass, serving as an instream biogenic C sink during the growing season.

These findings support the assertion that flow regime influences CH_4 sources and dynamics. *Runoff* streams exhibited markedly depressed CH_4 emissions following the mid-August flood that remained low through the final two sampling events; this same pattern was not observed in CO_2 flux. This contrasts with other studies that have found CH_4 emissions to spike during floods. The primary source of CH_4 in *Runoff* streams is from subsurface shale gas, and high flows may have exported dissolved CH_4 that built up during the declining flows of the hot summer out of the reach. Conversely, *Groundwater* CH_4 fluxes were highest on the sampling date immediately following the flood and declined thereafter, having been increasing since the June sampling date. Methane in *Groundwater* streams originated from biogenic as well as thermogenic sources, leaving me to speculate that perhaps changes in water temperature over the summer (e.g. heating then cooling toward the end of the study) may have influenced methanogenic organisms. However, I found no such association; CH_4 emissions actually decreased from late May to mid-June. It is possible that temperature played a role, but other factors unaccounted for in this study led to the decrease in CH_4 flux between those first two sampling events. Nonetheless, my data reveal that unlike boreal systems (Crawford et al. 2013) temperate streams are likely consistent CH_4 sources across flow regimes. Differing sources of CH_4 in this study reveal that flow regime is an important determinant of instream anaerobic processes, and more research is needed to characterize physical, chemical, and biological controls on CH_4 production and emissions in streams and rivers.

I expected to find relationships between C gas fluxes and instream biological and chemical metrics. Specifically, I predicted that CO_2 flux would be related to GPP, ER, pH and alkalinity given that GPP and ER exert significant influence over instream C dynamics while pH and alkalinity can be used to calculate modeled CO_2 fluxes. No relationships were found when variables were averaged over the study duration, likely due to low sample size and the dynamic nature of instream processes, particularly during the warmer growing season. Temporal variation in processes influencing C dynamics led to inconsistent trends in data averaged over the summer.

Evaluating relationships for each discrete sampling event revealed DOC to be a significant predictor of CO_2 flux on two occasions, albeit inconsistently. The direction of the relationship between DOC and CO_2 flux differed over the summer as well- DOC may have differential impacts on C uptake and release depending on instream conditions at the time of sampling. DOC concentrations were negatively related to CO_2 emissions for most of the summer, while the opposite was true in June, when a positive relationship between DOC and CO_2 flux was marginally significant. The difference in the direction of the relationships may reveal how the relative influence of ecosystem processes such as decomposition and respiration (i.e. C release) and primary production (i.e. C uptake) changes temporally. While neither GPP or ER were correlated with DOC on most days in this study, decreasing CO_2 flux in the presence of greater DOC concentrations may indicate that more C was being incorporated into biomass, while greater DOC concentrations with greater CO_2 fluxes may result from oxidative processes such as decomposition and respiration dominating in the channel. Further, ER was also negatively related to CO_2 flux on one of the dates that there was an observed a negative relationship with DOC, supporting the assertion that biological C uptake drove down flux rates.

Interestingly, there was a positive relationship between DOC and CH_4 emissions on the same day that DOC was shown to be negatively related to CO_2. A greater amount of DOC enhances microbial respiration which, in turn, can lead to the development of transient anaerobic pockets supporting CH_4 production, though more data to support this hypothesis are needed. This would only be applicable to *Groundwater* streams, however, as *Runoff* streams only produced CH_4 from natural gas sources- the nature of the relationship between DOC and thermogenic CH_4 ebullition is unclear.

Simulating additional *Runoff* and *Groundwater* streams to improve statistical power allowed me to determine whether potential relationships may exist that may have been difficult to visualize due to low sample size. When divided by flow regime, multivariate models revealed differing controls on stream CO_2 emissions, even while models containing the greatest number of variables for each analysis explained the greatest variation. This is unsurprising given that instream characteristics interact to give rise to patterns and variation in ecosystem function. Simulated *Groundwater* stream discharge was the most important variable for predicting CO_2 emissions. Similarly, other studies have shown discharge to control CO_2 dynamics due to changes in gas transfer velocities and CO_2 partial pressures (Jones and Mulholland 1998, Dinsmore et al. 2013). However, biological rather than physical parameters were most important for explaining variation in simulated *Runoff* streams, as TOC and GPP were the most significant variables in the global CO_2 flux model. Gross primary production in *Runoff* streams is constrained by lower light reaching the benthos due to a natural precipitate (sandstone), and this lower GPP yields less dissolved CO_2 from respiration than what was observed in *Groundwater* streams. Greater TOC may have interacted with production to yield even lower rates of CO_2 emissions, as TOC was taken up by autotrophs and heterotrophs.

Flow regimes differed in controls on CH_4 evasion. The global model best explained CH_4 emissions in *Runoff* streams, with algal biomass being the most significant variable in the model. This was unexpected given that *Runoff* stream CH_4 likely originated from thermogenic processes in the shale rock below the channels' bedrock bottoms. The second best explanatory model consisted of biological metrics- algal biomass, GPP, and ER. Gross primary production has been shown to positively effect methanogenesis rates (West et al. 2015); however, West et al. (2015) took place in lake systems, which are more susceptible to anaerobic conditions in benthic sediments. Further, CH_4 from *Runoff* streams was not biogenically produced.

Conversely, no candidate model explained variation in simulated *Groundwater* stream CH_4 evasion, even though methanogenic organisms were responsible for some of the CH_4 evading from the water's surface. Methanogenesis has been linked to productivity in lentic systems (West et al. 2015), and even though productivity was greater in *Groundwater* streams, I found no such association. Biogenic sources comprised a minority of gas samples, and I averaged measured flux rates together regardless of source to address my study objectives. The dual sources of CH_4 in the field may each be related to different factors, and thus more work could be done to parse out sources and quantify potential mechanisms near biogenic hotspots. Another possibility is that instream processes are not as important as soil and groundwater conditions for controlling CH_4 emissions in groundwater-dominated systems.

While I did not find differences in flux rates, it is clear that more research is needed in order to understand the instream processes giving rise to patterns in CO_2 and CH_4 fluxes and how these may differ based on flow characteristics. To date, few studies have addressed how flow source and dynamics influence greenhouse gas evasion rates. Some studies have shown differences in CO_2 and CH_4 fluxes across streams to be directly related to variation in groundwater inputs (Jones and Mulholland 1998). Carbon dioxide flux rates from the forested headwater streams in this study across flow regimes were greater than reports for forested and agricultural soils as well as large rivers (Raich et al. 1995, Butman and Raymond 2011), illustrating the importance of low-order systems to C transport from the terrestrial sink to the atmosphere at broad spatial scales (Kling et al. 1991, Billett et al. 2004, Cole et al. 2007). Stream gas emissions, even from small headwater systems, are large enough to affect regional C balances (Crawford et al. 2013), and it is imperative that CO_2 originating from terrestrial respiration that may reach stream channels through groundwater or soil water be accounted for to ensure accurate C budgets in the face of a changing climate (Richey et al. 2002, Johnson et al. 2008, Wallin et al. 2013).

Given the influences of CO_2 and CH_4 on atmospheric warming, more work is needed to clearly define factors influencing emissions from headwater streams, as these data show that differing flow classifications support conditions that lead to variability in controls on CH_4 fluxes within a biome and land use category. This finer scale of resolution- flow regime- appears promising for headwater streams, which tend to be more variable with respect to C dynamics than larger systems (Jones and Mulholland 1998, Teodoru et al. 2009, Butman and Raymond 2011). This high variability and potential for greater explanatory power by classifying streams by flow regime or, at the very least, flow source, underscores the need for spatially as well as temporally comprehensive datasets. Other studies have addressed the effects of land use (Beaulieu et al. 2013) and biome (Raymond et al. 2013). Future work exploring the relative influences of biome, land use, and flow regime, as well as how these three interact, may be especially helpful to our understanding of sources and processes controlling emissions, especially given headwater streams' close connectivity to the surrounding landscape.

Recent efforts have yielded useful frameworks of CO_2 and CH_4 emissions from streams and rivers (Hotchkiss et al. 2015, Stanley et al. 2016). The majority of my data falls within and supports these conceptual models. However, some of my results appear to contradict the predicted influence of DOC on CO_2 emissions. Dissolved organic carbon is predicted to be a substrate for ER, increasing CO_2 loss to the atmosphere. The opposite was true in these streams, with lower CO_2 evasion measured with increasing DOC. My finding that ER was negatively related to CO_2 flux during mid-August also contrasts with conceptual model predictions that ER directly contributes, and thus directly increases, CO_2 flux rates. This underscores the high variability in headwater streams, and the need for more direct measurements of CO_2 evasion in small systems is needed to determine why some systems emit more or less CO_2 in the presence of more DOC and greater rates of respiration.

Work by Stanley et al. (2016) set forth a framework for CH_4 dynamics within streams for which this work provides further support. I identified a positive relationship between organic C and CH_4 flux in the field across flow regimes on one sampling date, and a near-significant relationship on a second date, even though DOC did not explain a significant amount of variation in simulated stream candidate models. However, this does not mean that DOC is not influencing CH_4 dynamics, but rather that there is temporal variation in this relationship. Additionally, other instream processes may be mediating the effect of DOC concentrations on CH_4 fluxes through variation in discharge, biological uptake and release, or a combination of the two.

Stanley et al. (2016) also point out that hydrology has a large impact on instream CH_4, while also influencing nutrient concentrations, which may further affect CH_4 dynamics. The present study was conducted in forested headwater streams in an attempt to control for differences in nutrient concentrations that may arise from anthropogenic land use, and thus did not address that in this work. Additional work to discern the influence of changing nutrient concentrations on greenhouse gas fluxes would be useful. I also did not address other terminal electron acceptors or delve into how differences in specific aspects of geomorphology play a role, though I assert that this was addressed in a broad sense with the flow regime approach. Stream flow classifications include differences in geomorphological variables, such as substrate size and geological parent materials that can influence instream C uptake, release, and processing.

The relationship between stream metabolism and greenhouse gas fluxes from streams cannot be overlooked. This work shows that metabolic parameters do indeed explain CO_2 fluxes in one flow regime, and instream metabolism has been shown to contribute substantially to CO_2 off-gassing, accounting for greater portions of emitted CO_2 as streams move from mouth to headwaters. Most studies into lotic-atmospheric CO_2 dynamics address the influence of aerobic metabolism (Cole et al. 2001, Crawford et al. 2014, Hotchkiss et al. 2015). However, the same attention has not been given to CH_4. Given that streams are consistent CH_4 sources, adding defined and quantified anaerobic processes such as methanogenesis and methanotrophy to our understanding of whole-stream metabolism is clearly necessary. To date, a stark paucity of research has sought to quantify these processes- perhaps this area signifies another frontier in our quest to fully elucidate stream C budgets.

These results reveal that flow regime and one of the factors responsible for distinct flow characteristics, water source, influence not only where a sizeable portion of greenhouse gases originate (i.e. groundwater, soil water, precipitation), but also what instream variables control rates of gas evasion. It is imperative to account for reach and landscape-level controls of greenhouse gas fluxes as I have done here to construct C budgets that integrate fluxes from the terrestrial sink to streams and rivers and to gain a more detailed picture of sources controlling C movement in headwater streams, which are notoriously both spatially and temporally variable with respect to ecosystem processes and functions (Gomi et al. 2002). Increased warming and changes in storm magnitude and frequency are already shifting hydrologic regimes (Palmer et al. 2008) by increasing droughts and enhancing the magnitude and frequency of large flood events, in turn altering rates of C production, processing, and emissions. Understanding the interplay between rivers and their catchments will increase our predictive power as ecosystems continue to be altered at a rapid pace from land use and climate change (Allan et al. 2004, Palmer et al. 2008, Hotchkiss et al. 2015). Additionally, environmental flow management to meet biological and human needs in an age of increased climate volatility requires a detailed understanding of how flow regime as well as changes in flow (e.g. from perennial to intermittent flow, or fewer/more major floods) may influence instream C dynamics and, in turn, regional C balances.

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APPENDIX

TABLES

Table 1. Flow classifications, watershed areas and percent forested land cover for study sites.

Flow Regime	Site	Watershed Area (km^2)	Forested Land Cover (%)
Runoff Flashy	Big Piney	20.99	97.0
Runoff Flashy	Little Piney	25.35	95.4
Runoff Flashy	Murray	25.35	95.4
Groundwater Flashy	Roasting Ear	34.87	86.1
Groundwater Flashy	Spring	38.77	84.8
Groundwater Flashy	Sylamore	30.15	95.5

Table 2. Results of linear regressions between overall means in C gas fluxes and instream variables in the six study streams. GPP and ER denote gross primary production and ecosystem respiration, respectively. TOC and DOC are total organic carbon and dissolved organic carbon, respectively. DIC is dissolved inorganic carbon.

Predictor	Response	R^2	p-value
logGPP	logCO_2 flux	0.008	0.86
logGPP	logCH_4 flux	0.25	0.31
logER	logCO_2 flux	0.46	0.14
logER	logCH_4 flux	0.05	0.68
logChlorophyll a	logCO_2 flux	0.018	0.78
logChlorophyll a	logCH_4 flux	0.13	0.48
logTOC	logCO_2 flux	0.002	0.93
logTOC	logCH_4 flux	0.28	0.28
logDOC	logCO_2 flux	0.23	0.33
logDOC	logCH_4 flux	0.48	0.13
logDIC	logCO_2 flux	0.01	0.85
logDIC	logCH_4 flux	0.13	0.49
logpH	logCO_2 flux	0.19	0.38
logpH	logCH_4 flux	0.28	0.28
logAlkalinity	logCO_2 flux	0.005	0.89
logAlkalinity	logCH_4 flux	0.13	0.49
logDischarge	logCO_2 flux	0.07	0.61
logDischarge	logCH_4 flux	0.03	0.74

Table 3. Results of multiple linear regression models in simulated *Runoff* streams with CO_2 flux as the response variable. GPP and ER denote gross primary production and ecosystem respiration, respectively. TOC and DOC are total organic carbon and dissolved organic carbon, respectively. DIC is dissolved inorganic carbon. $\delta^{13}C$ represents CO_2-carbon's isotopic signature.

Response	Predictors	Multiple R^2	p-value	AIC	Model Notes
logCO_2 flux	logTOC + logDOC + logDIC + logChlorophyll a + logDischarge + logGPP + logER + $\delta^{13}C$ + logpH	0.97	0.04	-36.25	Global model: Removed one outlier, removed second data point to improve homoscedasticity
logCO_2 flux	logTOC + logDOC + logDIC + logChlorophyll a + logDischarge + logGPP + logER + $\delta^{13}C$ + logpH	0.87	0.16	-22.32	Global model: Removed one outlier
logCO_2 flux	logDischarge +$\delta^{13}C$	0.47	0.02	-19.34	NA
logCO_2 flux	logDOC + logTOC	0.32	0.1	-15.42	NA
logCO_2 flux	logChlorophyll a + logGPP + logER	0.34	0.19	-13.95	NA
logCO_2 flux	logTOC + logDOC + logDIC + logChlorophyll a + logDischarge + logGPP + logER + $\delta^{13}C$ + logpH	0.67	0.47	-12.41	Global model with all data
logCO_2 flux	logDIC + logpH	0.01	0.94	-9.84	NA

Table 4. Results of multiple linear regression models in simulated *Groundwater* streams with CO_2 flux as the response variable. GPP and ER denote gross primary production and ecosystem respiration, respectively. TOC and DOC are total organic carbon and dissolved organic carbon, respectively. DIC is dissolved inorganic carbon. $\delta^{13}C$ represents CO_2-carbon's isotopic signature.

Response	Predictors	Multiple R^2	p-value	AIC
logCO_2 flux	logDOC + logDIC + logDischarge + logGPP + logER + logAlkalinity	0.83	0.009	6.08
logCO_2 flux	logDOC	0.35	0.02	16.04
logCO_2 flux	logDIC + logAlkalinity	0.01	0.94	16.14
logCO_2 flux	logGPP + logER	0.27	0.15	19.71
logCO_2 flux	logDischarge	0.43	0.31	21.22

Table 5. Results of multiple linear regression models in simulated *Runoff* streams with CH_4 flux as the response variable. GPP and ER denote gross primary production and ecosystem respiration, respectively. TOC and DOC are total organic carbon and dissolved organic carbon, respectively. DIC is dissolved inorganic carbon. $\delta^{13}C$ represents CH_4-carbon's isotopic signature.

Response	Predictors	Multiple R^2	p-value	AIC
logCH_4 flux	logTOC + DOC + logDIC + logChlorophyll a + logDischarge + logGPP + ER + logpH	0.95	0.002	-7.00
logCH_4 flux	logChlorophyll a + logGPP + ER	0.88	0.00002	-2.21
logCH_4 flux	logDischarge	0.48	0.004	15.51
logCH_4 flux	DOC + log TOC	0.3	0.12	22.11
logCH_4 flux	logDIC + logpH	0.14	0.42	25.22

Table 6. Results of multiple linear regression models in simulated *Groundwater* streams with CH_4 flux as the response variable. GPP and ER denote gross primary production and ecosystem respiration, respectively. TOC and DOC are total organic carbon and dissolved organic carbon, respectively. DIC is dissolved inorganic carbon. $\delta^{13}C$ represents CH_4-carbon's isotopic signature.

Response	Predictors	Multiple R^2	p-value	AIC
logCH_4 flux	logDOC	0.007	0.77	42.41
logCH_4 flux	logDischarge	0.0007	0.93	42.50
logCH_4 flux	logDIC + logAlkalinity	0.05	0.73	43.73
logCH_4 flux	logGPP + logER	0.04	0.80	43.94
logCH_4 flux	logDOC + logDIC + logDischarge + logGPP + logER + logAlkalinity	0.11	0.99	52.75

Table 7. Summary of means and ranges from this study and other published values of CO_2 and CH_4 fluxes.

Variable	Study	Sample Size	Mean (mol m^{-2} d^{-1})	Range (mol m^{-2} d^{-1})
CO_2 Flux	This study	6	0.15	0.02-0.58
CO_2 Flux	Jonsson et al. (2007)	7	0.03	0.01-0.05
CO_2 Flux	Teodoru et al. (2009)	790	0.07	0.02-0.07
CO_2 Flux	Kaprivnjak et al. (2010)	18	0.04	0.02-0.09*
CO_2 Flux	Butman & Raymond (2011)	**	0.15	-
CO_2 Flux	Crawford et al. (2014)	93	0.51	0-2.03
			(mmol m^{-2} d^{-1})	(mmol m^{-2} d^{-1})
CH_4 Flux	This study	6	0.35	0.03-2.04
CH_4 Flux	Crawford et al. (2014)	42	8.46	0-60.1
CH_4 Flux	Stanley et al. (2016)	26	4.23	<0.0001-40.49

*Listed mean and range represent summer values from Kaprivnjak et al. (2010).

**Butman & Raymond calculated fluxes based on U.S. stream surface area of 230,000 km^2. Listed mean represents average over all stream miles.

FIGURES

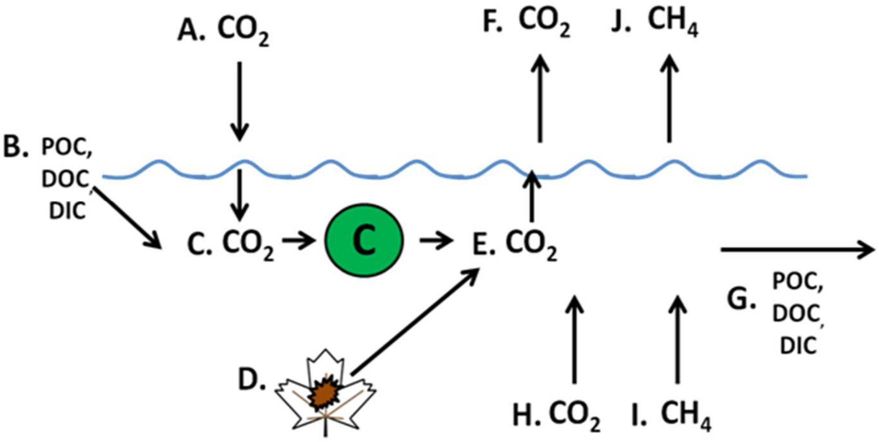


Figure 1. Sources and fates of carbon in streams. Carbon enters streams through (A) diffusion of atmospheric CO_2 directly into the stream or via rainwater and (B) as particulate or dissolved organic matter from terrestrial organic matter as well as dissolved inorganic carbon in soil water. CO_2 in stream water is then used to support instream carbon production (C) in the form of primary production. These primary producers along with microbial heterotrophs utilizing organic substrates (D) and other biota respire CO_2 (E), which is then either transported downstream along with particulate and dissolved organic carbon (G) or evaded to the atmosphere (F). CO_2 may also enter from groundwater seepage (H). CH_4 enters the stream from anoxic pockets in sediments on the channel bottom from biogenic and/or thermogenic sources (I), which is then evaded to the atmosphere (J).

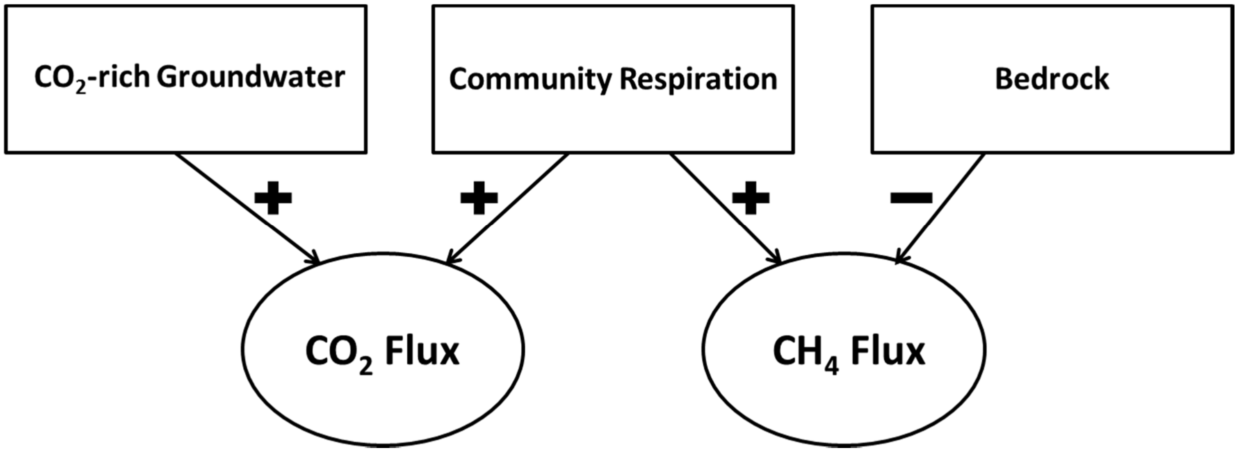


Figure 2. Conceptual model of hypothesized primary influences driving predicted differences in carbon gas fluxes in *Runoff* and *Groundwater* streams. *Groundwater* streams have greater groundwater inputs and community respiration and less bedrock than *Runoff* streams, which should result in greater CO_2 and CH_4 emissions from *Groundwater* streams.

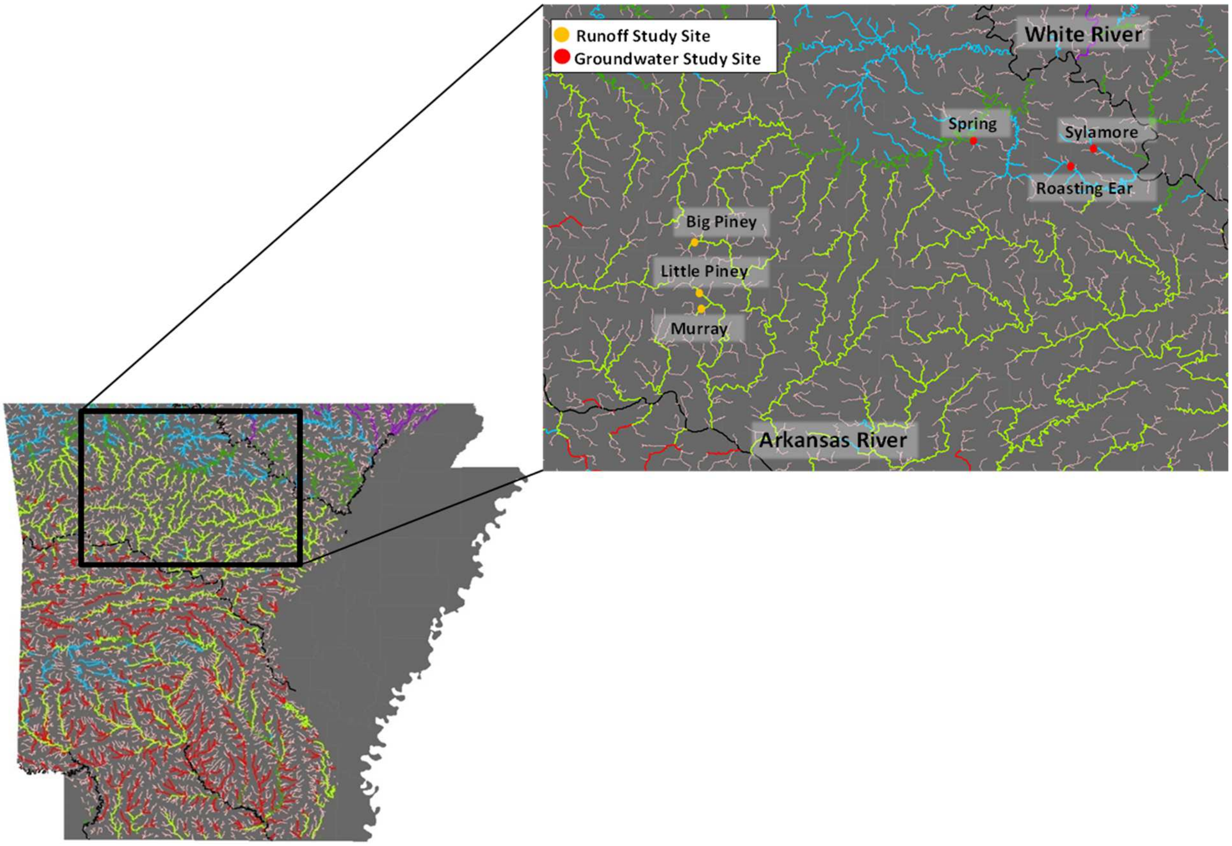


Figure 3. Map of flow regimes in the Ozark and Ouachita Interior Highlands based on Leasure et al. (2016). Highlighted area shows individual study sites sampled in summer 2017 across northern Arkansas.

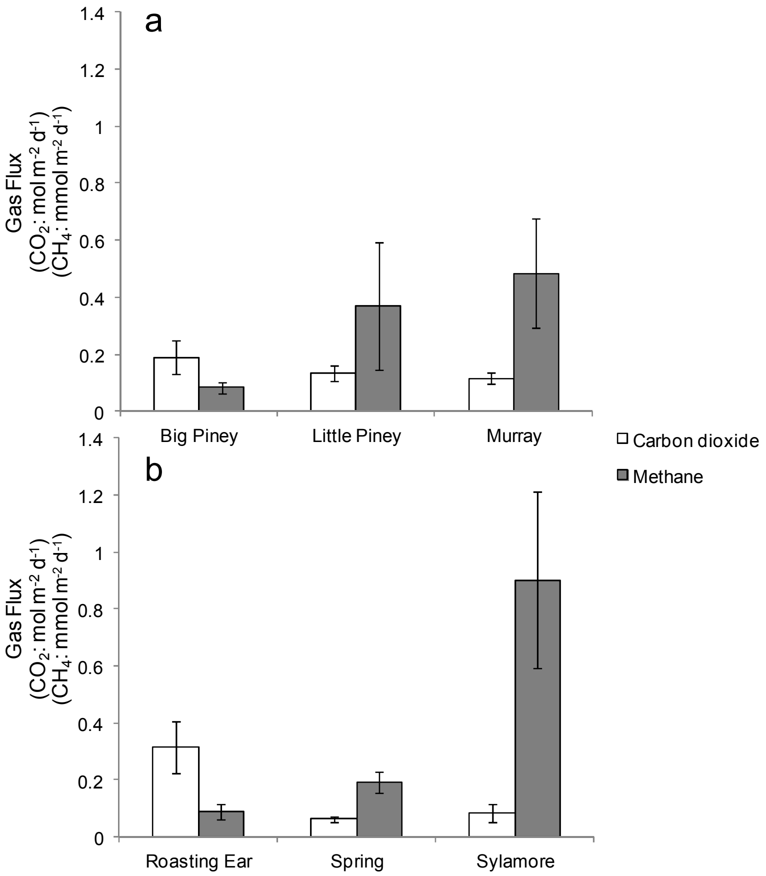


Figure 4. Carbon dioxide and methane fluxes in each study stream over the study duration in (a) *Runoff* and (b) *Groundwater* streams. Error bars denote ± 1 standard error. N=6 dates per stream.

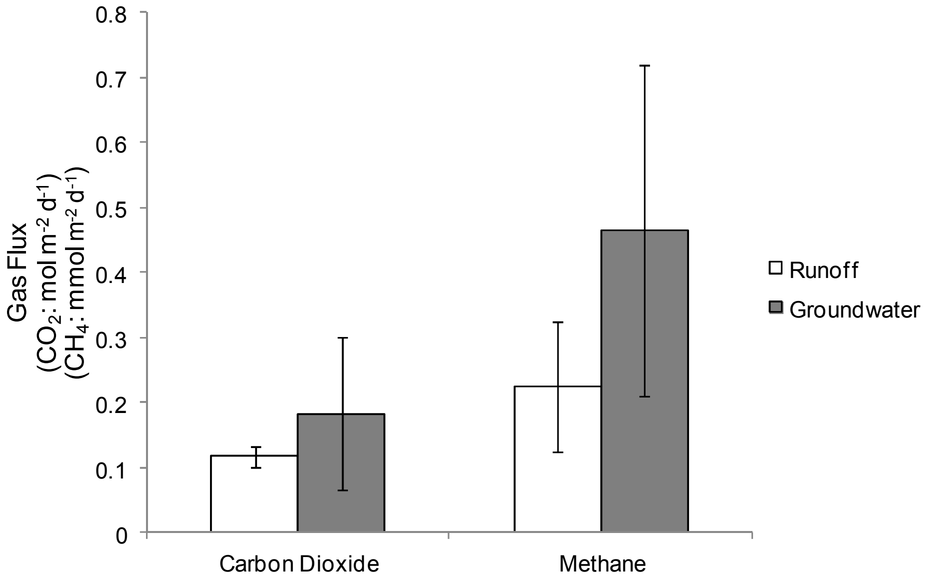


Figure 5. CO_2 and CH_4 fluxes from field sites averaged over summer 2017. Error bars denote ± 1 standard error. n=3 per flow regime.

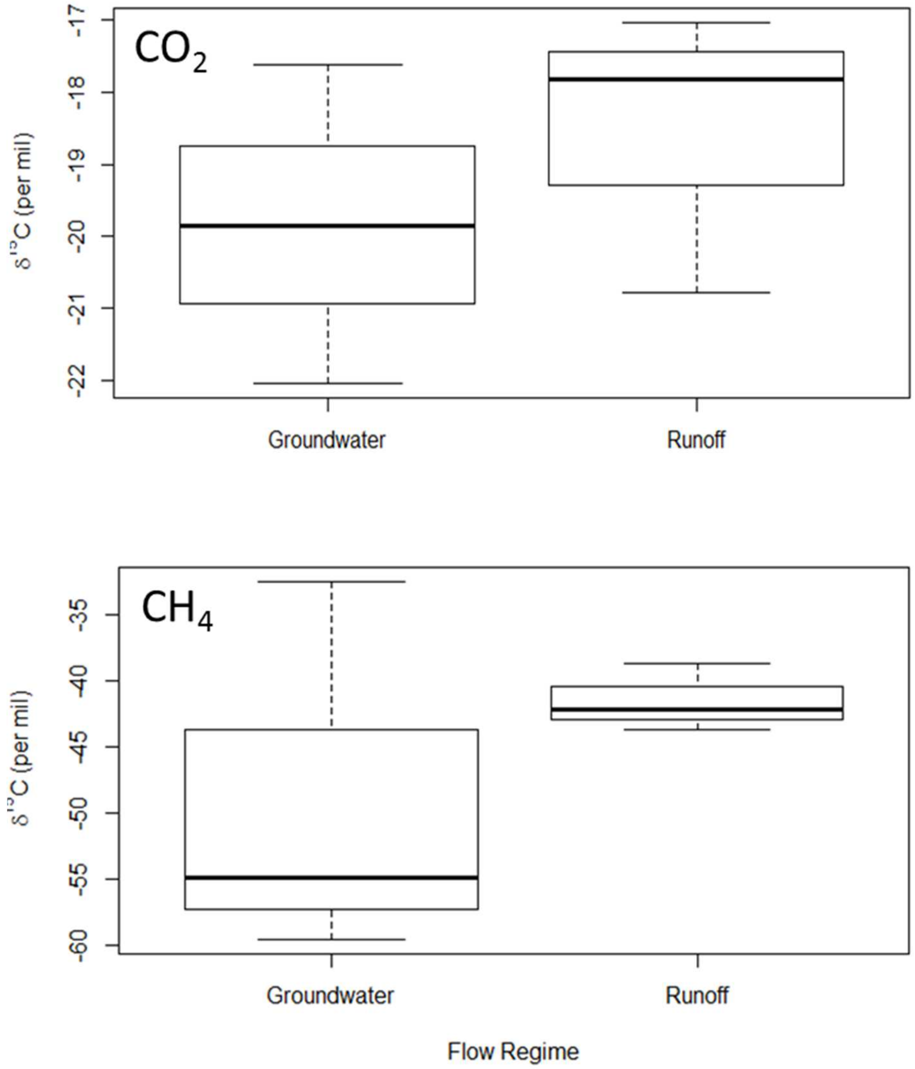


Figure 6. $\delta^{13}C$ signatures for C found in CO_2 and CH_4 gas samples across *Groundwater* and *Runoff* streams. Error bars denote full range of isotope values. n=3 per flow regime.

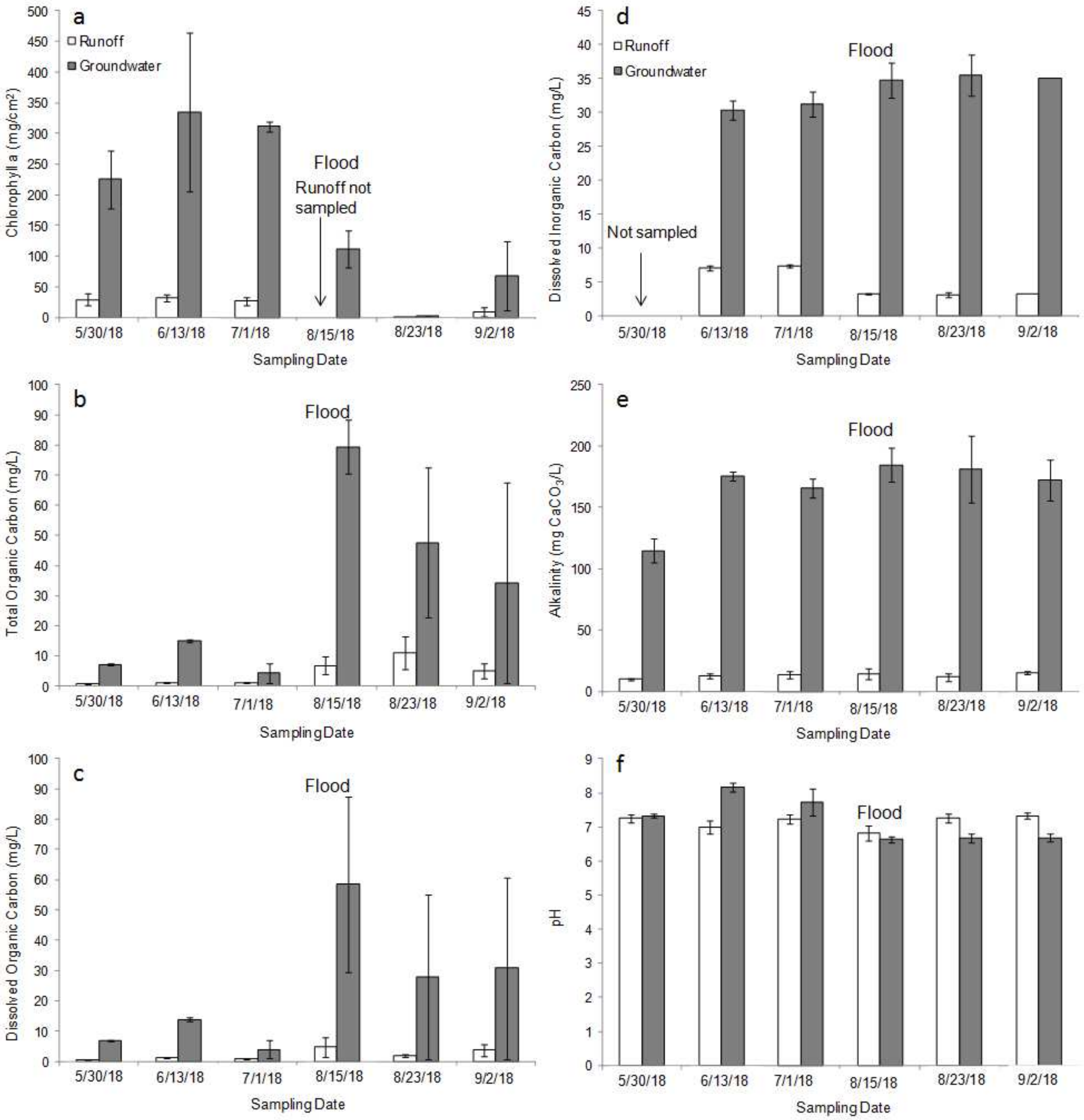
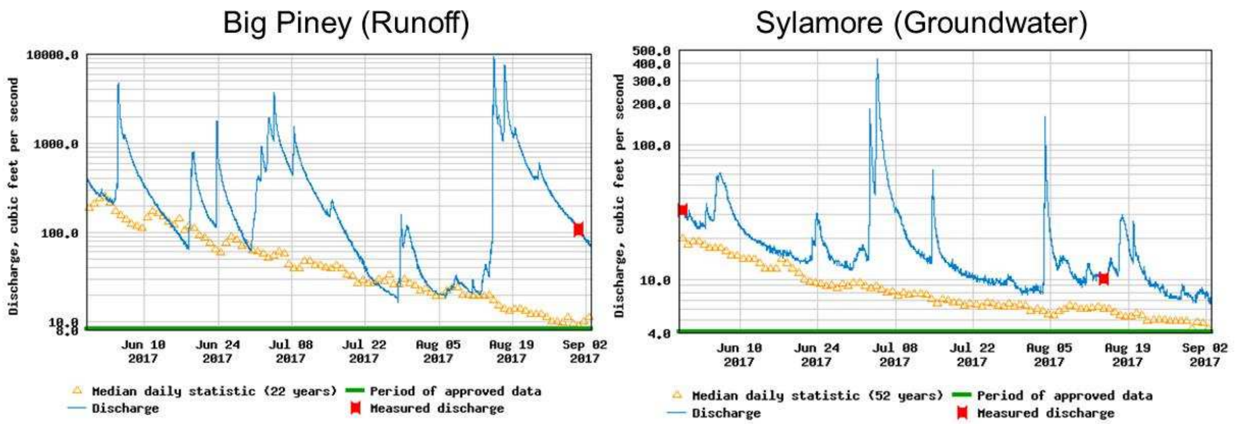


Figure 7. Average chlorophyll a (a), TOC (b), DOC (c), DIC (d), alkalinity (e), and pH (f) values on each sampling date over the study period. Dates denote first day of sampling event. Error bars denote ± 1 standard error. n=3 per flow regime.


Big Piney (Runoff)

Sylamore (Groundwater)

Figure 8. USGS gauge discharge data for Big Piney and Sylamore creeks over the study period. Note that hydrograph data are for downstream gauges with established relationships to upstream study reaches. Hydrographs are representative of flow over the summer within each flow class.

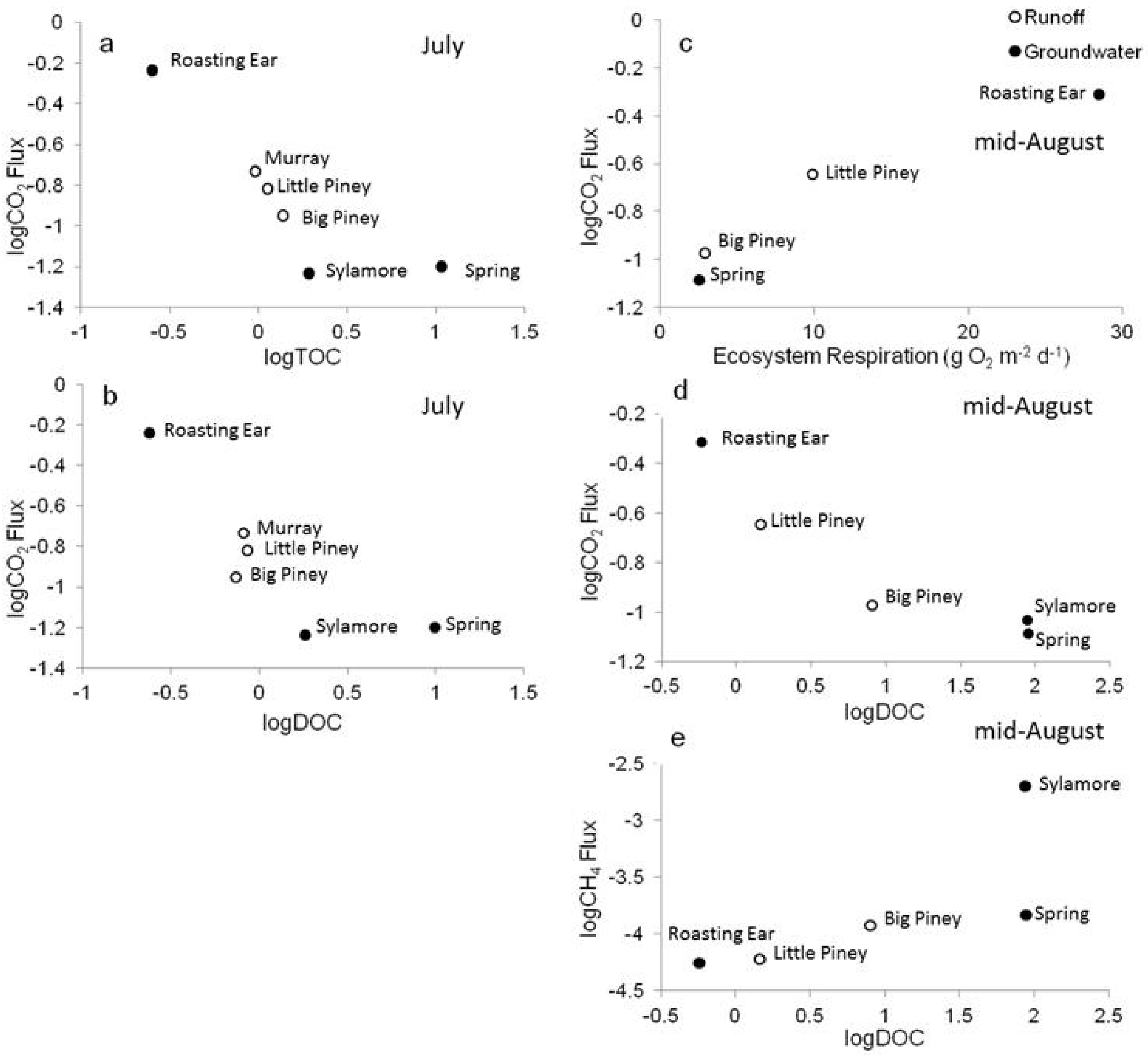


Figure 9. Results of significant linear regression models comparing C gas fluxes and instream variables across flow regimes on individual sampling dates. Panels (a) and (b) show significant relationships found during July sampling event. Panels (c), (d), and (e) represent significant relationships from mid-August sampling. TOC= total organic carbon, DOC= dissolved organic carbon.

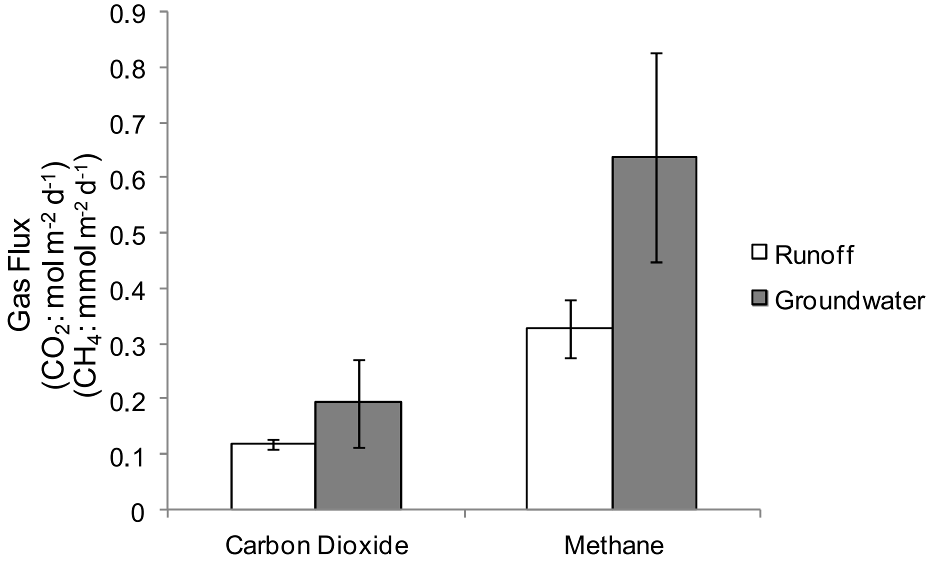


Figure 10. Average CO_2 and CH_4 fluxes from simulated streams. Error bars denote ± 1 standard error. n=15 per flow regime.

CHAPTER THREE

Soil carbon dioxide fluxes from riparian areas in two distinct hydrogeomorphic settings

ABSTRACT

Riparian buffers serve as a conduit through which carbon (C) can move from the terrestrial landscape to aquatic systems and, ultimately, to oceanic or atmospheric sinks. Riparian areas have also been identified as zones of concentrated biogeochemical activity in the terrestrial landscape that often produce greater amounts of greenhouse gases than neighboring more upland terrestrial and adjacent aquatic systems. Little work has explored how ecoregion and hydrologic classification of a stream may influence the magnitude of riparian soil processes. A three-way factorial field study examined soil carbon dioxide (CO_2) fluxes across flow regime (*Groundwater* and *Runoff* streams), season (autumn, winter, spring, and summer), and perpendicular distance from stream edge (0, 10, and 20 m). Stream-surface CO_2 fluxes were also measured and compared to soil fluxes in June. Relationships between CO_2 flux and soil temperature and moisture at seasonal temporal scales at the site level were examined. Soil CO_2 flux differed between flow regimes among seasons ($F_{(3,40)}$= 3.95, p= 0.01). *Post-hoc* tests revealed that *Runoff* sites had greater spring soil surface CO_2 flux (i.e., soil respiration mean $\underline{+}$1 SE= 2.98 $\underline{+}$ 0.29 μmol CO_2 m^{-2} s^{-1}) than did *Groundwater* sites (1.40 $\underline{+}$ 0.30 μmol CO_2 m^{-2} s^{-1}) (p= 0.01). Soil moisture was positively related to autumn soil CO_2 flux across all sites (ρ= 0.94, p= 0.005). Soil CO_2 fluxes at each *Runoff* site were related to soil temperature and moisture, but fluxes were only related to soil temperature at each *Groundwater* site. Summer soil respiration rates were greater (mean $\underline{+}$1SE = 7.82 $\underline{+}$ 1.42 μmol CO_2 m^{-2} s^{-1}) than summer stream CO_2 fluxes across all sites (1.74 $\underline{+}$ 0.64 μmol CO_2 m^{-2} s^{-}1) (p= 0.005). Results suggest the addition of the riparian zone and demonstrated differences in flow regime are needed to understand stream C budgets and to evaluate flow-ecology relationships. Anthropogenic landscape alteration and

climate change can alter the natural flow regime which, in turn, may have consequences for aquatic C dynamics in streams draining altered riparian zones.

INTRODUCTION

Soil surface carbon dioxide (CO_2) flux from biological respiration forms an important component of the C cycle in terrestrial as well as adjacent aquatic systems. A significant portion of the CO_2 that is emitted from streams is terrestrially-derived, as terrestrial organic matter is broken down by respiring soil microbes. Characterizing patterns and drivers of riparian soil C flux will enhance the capacity to predict greenhouse gas emission rates in adjacent and downstream systems, and comparing soil and stream C gas dynamics can provide a more holistic view of local biogeochemical processes at the aquatic-terrestrial interface. Though often overlooked, it is critical to integrate measures of aquatic and terrestrial C cycling to construct accurate and mechanistically-based C budgets (Cole et al. 2007, Buffam et al. 2011).

Biological activity, specifically plant root respiration and microbial decomposition of organic matter, are the primary drivers of soil CO_2 flux (Hogberg et al. 2001, Risk et al. 2002). Soil temperature is considered the primary control on fluxes, while precipitation and, in turn, soil water content are secondary influences (Rustad et al. 2000, Davidson et al. 2002). Temperature and water content influence soil C fluxes by increasing microbial activity in warm, moist soil conditions, but depresses microbial activity in cold, dry or extremely wet soil conditions, resulting in seasonal patterns of soil respiration (Raich and Potter 1995, Davidson et al. 1998, Chen et al. 2002, Xu and Qi 2008). The soil temperature, moisture, soil CO_2 flux interactions are typically described by exponential equations between soil temperature and soil CO_2 flux, but soil moisture and soil CO_2 flux have often exhibited linear, parabolic, quadratic, and logarithmic relationships depending on the specific soil water content variable measured (e.g., matric potential, water-holding capacity, and gravimetric or volumetric water content) (Davidson et al.

2000). Soil conditions are known to be heterogeneous (Raich and Schlesinger 1992, Raich and Potter 1995, Fang et al. 1998, Rayment and Jarvis 2000, Epron et al. 2004). Proximity to a wetted channel and differences in surrounding topography, such as in the case of differing ecoregions, may result in distinct temporal (i.e., seasonal and/or annual) and spatial patterns in soil conditions within a riparian corridor. However, spatial heterogeneity of plant root and soil microbial activity, along with the tendency for factors such as soil moisture and temperature to covary, makes it difficult to construct models that accurately capture the extent of spatial and temporal variation in soil respiration (Davidson et al. 2002). Additionally, areas where aquatic and terrestrial systems adjoin have been shown to produce concentrated biogeochemical activity in time and space [i.e. hot moments and hot spots, respectively (McClain et al. 2002, Scott et al. 2004)], though the specific mechanisms responsible for up-regulation of aerobic respiration in these areas still requires further study.

Riparian areas are distinct from upland soils with respect to hydrologic characteristics, and riparian areas adjacent to streams with differing flow characteristics and geomorphology may have even further unique soil characteristics, microbial communities, and, in turn, soil respiration. Little work has been conducted specifically within the riparian areas of minimally-disturbed systems (Griffiths et al. 1997, Tufekcioglu et al. 1998), and, to my knowledge, no studies have sought to ascertain natural variation in biological activity that may exist within a biome as a result of a neighboring stream with a distinct flow regime. Additionally, few studies have evaluated soil respiration within clearly-defined, longitudinal transects at various distances from the stream, even though soil conditions likely change from the immediate stream bank back into the zone of riparian soil and vegetation. Previous studies have sought to predict in-stream organic C fluxes using soil metrics (Aitkenhead and McDowell 2000), but there is a clear lack of data revealing patterns in riparian soil respiration, as well as linking respiration to stream CO_2 off-gassing.

Climate change has already begun to alter air temperature and precipitation patterns, with consequences to soil conditions and, in turn, terrestrial C storage and loss (Heimann and Reichstein 2008). Field measurements of soil respiration, soil temperature, and soil water content thus far have revealed few broad-scale patterns, as most relationships are site-specific (Davidson et al. 2005). However, riparian soils have been shown to produce greater respiration rates than adjacent upland hillslope positions due to greater soil moisture in the riparian, signaling the importance of these areas to watershed and regional C balances (Pacific et al. 2008, 2010). Furthermore, riparian areas affected by flooding have been shown to be even more spatially variable in C efflux rates, implying that differences in flood regime can affect local atmospheric and terrestrial C dynamics (Samaritani et al. 2011). However, these trends may be altered, as some areas experience greater air temperatures and increasing flood and drought intensities (Rosenzweig et al. 2001, Barnett et al. 2004), but these changes may be overlooked in the absence of adequate data to provide a foundation for assessing climate change impacts on soil C fluxes. Further, characterizing riparian soil respiration and potential correlative soil characteristics, such as soil temperature and moisture, can reveal areas that may be better- or less-suited as sinks for potential stream pollutants, such as nutrients, sediments, and pesticides, while also providing an index of stream susceptibility to pollutant enrichment (Jacinthe et al. 2003, Vidon and Hill 2007).

The objective of this study was to evaluate riparian soil CO_2 fluxes between two dominant flow regimes in northern Arkansas. The two flow regimes, Runoff Flashy and Groundwater Flashy (hereafter *Runoff* and *Groundwater*), form distinct hydroecological areas that are demarcated by ecoregion. Flow regime is comprised of the timing, duration, magnitude, frequency, and rate of change of flow in a system, and is influenced by factors that also influence soil microbial activity, such as climate and geology (Poff et al. 1997, Allan and Castillo 2007). It may be that differences in soil C dynamics are influencing C uptake and transport within these systems. It was hypothesized that the alluvial soils adjacent to *Groundwater* streams will have greater soil respiration based on previous work that revealed the tendency for greater instream respiration in *Groundwater* compared to *Runoff* streams (Chapter 1). Further, *Groundwater* streams have also been found to off-gas more CO_2 to the atmosphere than *Runoff* sites. $\delta^{13}C$ data revealed that CO_2 coming from *Groundwater* streams contained more C from soil respiration than *Runoff* sites (Chapter 2). In sum, these trends provide a glimpse of biological activity at *Groundwater* and *Runoff* sites, and the greater amounts of CO_2 coming from *Groundwater* sites may be partially driven by a more active soil microbial community. Therefore, greater soil respiration was expected in *Groundwater* versus *Runoff* stream riparian zones. Additionally, *Groundwater* sites in the region tend to be flanked by open-canopy, alluvial gravel and pebble bars, whereas *Runoff* sites within the region generally have smaller portions of open-canopy areas adjacent to the stream with cobble and boulder substrate, providing fewer potential interstitial spaces for microbial activity. Soil respiration rates were predicted to be greatest in spring due to greater soil moisture and warming temperatures, but lowest in winter due to cold soil temperatures. Soil respiration, temperature, and moisture were all expected to decrease from the stream bank moving away from the channel (Figure 1).

METHODS

This field study was conducted within the riparian area of six minimally impacted streams with forested land cover ranging from 84.8 to 97.0% of total watershed area (CAST

2007). Three streams were located within the Boston Mountains ecoregion (Woods et al. 2004) and were classified as *Runoff* systems according to Leasure et al. (2016). *Runoff* streams are fed primarily from overland flow and subsurface runoff and dry for up to several weeks each year. The land surrounding the *Runoff* streams was dominated by sycamore (*Platanus occidentalis*) trees. Three additional streams were located within the Ozark Highlands ecoregion (Woods et al. 2004) and were classified as *Groundwater* flow (Leasure et al. 2016), which is characterized by groundwater-dominated and perennial flows. Dominant vegetation in the riparian zone adjacent to *Groundwater* streams was oak (*Quercus* spp.) trees. Site locations and soil taxonomy can be found in Table 1.

Lateral transects across both banks of each stream reach were established at the bottom, middle, and top an approximate 200-m reach representing each flow-regime site. Plastic collars, 10 cm in diameter, were inserted directly adjacent to the stream channel to a depth of approximately 2 cm, then 10 and 20 m away from the channel. There was a total of nine sample collars on the bottom left, middle left, and top left banks and nine additional collars on the bottom right, middle right, and top right banks (n= 18 per stream) to allow for analysis of respiration trends along the stream continuum, as well as from the channel into the riparian area. Live vegetation was removed from inside each collar and allowed to equilibrate for approximately one hour before measurements were conducted. Soil measurements were made inside collars at each stream once per season for a total of four sampling events associated with each stream. Sampling events took place in October of 2015 (autumn) and January (winter), March (spring), and June (summer) of 2016 (Table 2). Soil surface CO_2 flux was measured across three lateral transects within each riparian area using a Li-Cor LI-6400 XT Portable Photosynthesis System (Lincoln, NE) equipped with a 10-cm-diameter soil respiration chamber (model LI-6400-09, Li-Cor), similar to procedures described in Brye et al. (2006). Soil temperature was measured at the 2-cm depth with a standard probe thermometer adjacent to each collar during respiration measurements. Soil moisture in the top 6 cm was recorded inside each collar after the respiration measurement was conducted using a Dynamax SM 150 soil moisture probe (Houston, TX).

Stream-surface CO_2 flux was measured in June 2017 (Table 3). Measurements of CO_2 exchange with the atmosphere were collected using a floating chamber constructed from an inverted bucket attached to a sheet of foam approximately 4 centimeters from the edge of the bucket so as to ensure the chamber was completely sealed to the stream surface. Tubing was inserted into the top of the chamber and was clamped shut between samples. The chamber was attached to small weights to anchor it in place in the stream channel. Discrete gas samples were collected with a syringe from the sealed chamber every ten minutes from zero to thirty minutes (i.e. four samples in each location) at the top and bottom of each 200-meter stream reach. Air samples were injected into pre-evacuated gas bags. Carbon dioxide gas concentrations in each sample were determined by running gas samples into a Picarro G2201-i carbon isotope analyzer (Picarro Inc., Santa Clara, CA). CO_2 fluxes were calculated based on the gas concentration change within the sealed chamber over time (i.e. over ten minutes) according to the equation

$$J_{CO2} = dc/dt * h \quad [1]$$

where dc/dt is the change in gas concentration in the chamber air in parts per million (ppm) over time in minutes multiplied by the height (h) of the chamber in meters minus the submerged portion (Crawford et al. 2013). Fluxes were converted using the Ideal Gas Law and are reported in µmillimoles $meter^{-2}$ $second^{-1}$ to allow for comparison of soil and stream surface C fluxes.

Two-way repeated-measures analysis of variance (RM-ANOVA) was used to test for differences in soil respiration, temperature, and moisture across seasons by flow regime. *Post hoc* Tukey's HSD was used to determine significant pairwise differences within and across treatments and seasons. Pearson's and Spearman's rank correlations were conducted to explore relationships among soil temperature, soil moisture, and soil CO_2 flux to determine potential drivers of soil respiration at annual, seasonal, and single-measurement scales. The statistical significance threshold was $p \leq 0.05$. All statistics were performed in R version 3.4.3.

RESULTS

Differences in soil respiration between flow regimes emerged across seasons, but not by distance from the stream. There was no significant 3-way interaction between flow regime, distance from the stream, and season ($F_{(6,40)}= 1.02$, $p= 0.42$) (Table 4). There was a significant 2-way interaction between flow regime and season ($F_{(3,40)}= 3.95$, $p= 0.01$). There was also a significant interaction between season and transect distance from the stream ($F_{(6,40)}= 8.46$, $p<0.0001$). Measured soil CO_2 fluxes across flow regimes were greatest in the summer and lowest in winter across sites. Soil surface CO_2 fluxes tended to be most variable in the summer across flow regimes, while fluxes tended to be least variable in fall. Soil respiration appeared to be similar between flow regimes across seasons except during spring, when soil CO_2 flux was 47% greater ($p= 0.01$) at *Runoff* than at *Groundwater* sites. *Runoff* sites also tended to emit more CO_2 from the soil in the summer as well, but the magnitudes of soil CO_2 flux were much greater during the summer than during any other season for both flow regimes (Figure 2). Consequently, differences in soil CO_2 flux between flow regimes during summer were not statistically significant ($p= 0.24$).

Soil surface CO_2 fluxes ranged from 3.14 to 5.01 μmol CO_2 m^{-2} s^{-1} at *Runoff* sites and from 2.36 to 3.82 μmol CO_2 m^{-2} s^{-1} at *Groundwater* sites over the sampling year. Soil temperature at the 2-cm depth ranged from 14.5 to 16.2 °C at *Runoff* sites and from 15.9 to 18.4 °C at *Groundwater* sites, whereas soil water content in the top 6 cm ranged from 8.0 to 10.0% (v/v) at *Runoff* sites and from 6.0 to 18.5% (v/v) at *Groundwater* sites over the sampling year. Near-surface soil moisture and temperature tended to be numerically greater and more variable among *Groundwater* than *Runoff* sites, though soil surface CO_2 flux tended to be greater at *Runoff* sites over the year.

Soil surface CO_2 flux and soil temperature and moisture differences were examined across sites within the longitudinal transects. No differences in soil CO_2 flux, soil temperature, or soil moisture content occurred between flow regimes at any position along the longitudinal transects (i.e., immediate bank, 10 m from bank, and 20 m from bank) Though soil CO_2 flux was numerically largest at the immediate bank position for *Runoff* sites among across both flow regime and transect position combinations, the large variability at this measurement position along the transect resulted in no significant spatial trend in soil CO_2 flux along the transect for the *Runoff* sites. Similarly, there was no spatial trend in soil CO_2 flux across transects for the *Groundwater* sites. Trends were only significant across transects by season when flow regime measurements were considered together (Figure 4).

At the site-level, soil CO_2 fluxes across sampling dates were positively correlated with soil temperature at five of the six sites, while one *Runoff* site (Big Piney Creek) exhibited a negative correlation between soil CO_2 flux and soil temperature ($\rho = -0.63$, $p < 0.0001$). Soil CO_2 fluxes across sampling dates were negatively correlated with soil moisture at the other two *Runoff* sites, Little Piney Creek ($\rho = -0.31$, $p = 0.03$) and Murray Creek ($\rho = -0.35$, $p = 0.02$). Soil

CO_2 flux was negatively correlated with soil temperature (ρ= -0.58, p= 0.01). Soil CO_2 flux was also negatively correlated with soil moisture at Murray Creek on the summer sampling date (ρ= -0.66, p= 0.007 (Figure 5). No other sites yielded significant correlations between measured variables on other sampling dates. However, soil moisture content data during spring at Murray Creek were unobtainable due to equipment malfunction and soil fluxes were not measured at Roasting Ear Creek during spring.

Soil temperatures differed across flow regimes across seasons ($F_{(3,40)}$= 9.10, p= 0.0001), though not by distance across seasons ($F_{(6,40)}$= 0.04, p= 0.99) (Table 4). There was no interaction between season, flow regime, and distance from the stream ($F_{(6,40)}$= 0.04, p= 0.99). Soil temperatures during spring across flow classes tended to be numerically greater in *Groundwater* sites, while soil temperatures were more similar between flow regimes during the other three seasons and tended to be numerically lowest during winter (Figure 3). Summer soil temperatures at *Runoff* sites averaged 25.8 (± 0.5) °C, while average soil temperature at *Groundwater* sites was only slightly greater at 26.9 (± 1.3) °C. Winter soil temperatures averaged 5.0 (± 2.2) °C at *Runoff* sites and 3.3 (± 0.3) °C at *Groundwater* sites. Soil temperatures across *Groundwater* sites tended to be more variable in the autumn and least variable in winter, while soil temperatures across *Runoff* sites tended to vary the most during winter and were most similar during spring.

2-way RM-ANOVA revealed that soil moisture content differed across seasons ($F_{(3,37)}$= 24.82, p< 0.0001) as well as between flow classes ($F_{(1,37)}$= 4.44, p= 0.04) (Table 4) (Figure 5b). There was a marginally significant interaction between flow regime and season ($F_{(3,37)}$= 2.92, p= 0.05). No three-way interaction was observed between flow regime, distance from the stream, and season ($F_{(6, 37)}$= 0.69, p= 0.66). No interactions were observed between flow regime and distance from the stream ($F_{(2,37)}$= 0.34, p= 0.71) nor between distance from the stream and season

($F_{(6,37)}$= 0.66, p= 0.68). Soil water content was two to four orders of magnitude lower in the autumn than during any other season. During autumn, soil moisture content was less than 0.5% (v/v) across both flow regimes. Soil moisture content among sites for the rest of year was similar across flow regimes as well as seasons. Soil moisture content at *Runoff* sites ranged from 10.5 (± 0.4) % (v/v) in summer to 15.0 (± 1.9) % (v/v) in spring. Similar to *Runoff* sites, soil moisture content at *Groundwater* sites peaked in spring at 17.2 (± 7.2) % (v/v) and remained relatively consistent throughout the remainder of the year.

There was no association between soil temperature and soil moisture content across sites at annual or seasonal temporal scales. Carbon dioxide fluxes tended to be numerically greater from the soil than from the stream surface at *Runoff* (p= 0.07) and *Groundwater* sites (p= 0.08). Overall, the riparian soils emitted more CO_2 than the streams themselves (p= 0.005), where, averaged across sites, soil CO_2 fluxes ranged from 4.2 to 13.7 μmol m^{-2} s^{-1} and averaged 7.82 μmol m^{-2} s^{-1}, while stream CO_2 fluxes ranged from 0.7 to 4.8 μmol m^{-2} s^{-1} and averaged 1.74 μmol m^{-2} s^{-1} (Figure 6).

DISCUSSION

Soil respiration rates averaged over the study year did not support the prediction that *Groundwater* sites would have greater soil CO_2 emissions than *Runoff* sites. This result was particularly interesting given that *Groundwater* sites tended to exhibit numerically greater soil temperatures and water contents. While soil CO_2 fluxes tended to respond positively to increasing soil temperature and moisture, it may be that site-specific factors aside from soil temperature and moisture variations alone may have elevated soil respiration at *Runoff* sites. For example, it may have been that there was little difference in overall respiration rates over the year due to similarities in canopy cover and vegetation at the sites, as all sites were heavily forested in the riparian zone. However, *Runoff* streams may have encompassed soil conditions that favored greater transport of subsurface soil CO_2 to the soil surface. Work in agricultural systems has revealed that vegetation at a site can be an important factor in driving differences in soil C efflux (Wagai et al. 1998).

Previous research has indicated soil temperature to be the main influence on soil respiration rates, with warmer soil temperatures enhancing soil microbial activity (Mellilo et al. 2002). Soil water content exerts a secondary influence on soil respiration at intermediate levels, where extremes in soil moisture conditions (i.e., extremely wet and extremely dry) attenuate CO_2 production by limiting aerobic microbial oxidation of soil organic matter. In this study, the annual data exhibited these overall trends, where- soil moisture averaged over the year tended to increase soil respiration and soil respiration tended to increase when the soil water content ranged from 5 to 10% (v/v), but soil respiration tended to decline at approximately 20% volumetric water content.

When evaluated by season, soil CO_2 fluxes were significantly greater at *Runoff* sites during the spring and this trend continued into the summer, though differences were non-significant. Soil CO_2 fluxes were otherwise similar across flow regimes and were low during the autumn and winter seasons. Differences in surrounding riparian soil respiration based on ecoregion or hydroecological classification appear to be a seasonal phenomenon that can have direct consequences on C transport between the riparian zone and stream channel, as well as the processing and fate of terrestrial C. Soil CO_2 fluxes, temperature, and moisture all exhibited a pronounced seasonal trend across sites. The seasonality observed in this study should not be overlooked, as streams of different flow regimes may receive more or less C depending on landscape characteristics that also contribute to determining flow regime. Additionally, the interactive effect of flow regime and season on soil temperature and soil moisture is very likely playing a significant role in C fluxes. Seasonal differences in C cycling have potential consequences for stream processes, such primary and secondary production, that may be exhibiting previously overlooked patterns in riparian processes or a combination of riparian and stream characteristics unique to a flow regime. Soil CO_2 fluxes were greatest during summer, concurrent with peak primary production, while soil CO_2 fluxes were lowest during winter when plants were not actively growing and soil temperatures were low (Raich and Potter 1995, Hogberg et al. 2001). However, while *Runoff* site soil respiration rates successively increased over the spring and summer seasons, soil respiration at the *Groundwater* sites was similar across winter and spring and only increasing during summer. Even then, the increase in soil respiration among *Groundwater* sites was not as great as was observed at *Runoff* sites. Soil respiration at *Groundwater* sites appeared to vary less than at *Runoff* sites across seasons. Others have reported that, even when soil temperature is similar across treatments, seasonal differences in soil water content can affect variation in soil CO_2 flux (Epron et al. 1999, Davidson et al. 2002). However, both soil moisture and temperature varied in similar ways regardless of site flow regime classification, so it was unlikely that soil moisture and temperature alone were responsible for variation in soil CO_2 flux.

Spring soil respiration responses to increased soil temperature and moisture tended to be negative, contrary to what others have reported (Mellilo et al. 2001), but this trend continued into the summer with respect to soil temperature, but not soil moisture. Within each site during each season, soil temperature and soil CO_2 flux exhibited positive exponential functions across all sites. These two differing responses merit consideration. Overall, sites with lower soil temperatures tended to exhibit greater soil respiration, particularly during spring and summer seasons when soil temperatures were overall greater and soil respiration was greatest among all seasons. Soil microbial communities are likely heterogeneous enough across sites, such that, while microbes respond positively to warmer soil temperatures at a particular site, other soil conditions (e.g. waterlogged or excessively dry soil) at other sites that happen to have warmer soil temperatures are less optimal for microbial activity.

Soil moisture positively influenced soil respiration during the autumn, and soil respiration rates across sites tended to respond positively during the winter and summer seasons, but negatively during spring. Soil moisture conditions during the autumn were the lowest of the entire study period, likely leaving parched microbes to scavenge for whatever little soil moisture was available to support minimal metabolism and basic functioning. Conversely, wet spring soils may have reduced soil diffusivity and depressed soil respiration, particularly at the *Groundwater* sites.

There was no interactive effect of flow regime, transect distance from stream, and season on soil respiration. However, clear trends were observed across seasons. Others have shown a clear shift in soil respiration rates when transitioning from riparian to hillslopes positions, suggesting that greater soil respiration typically occurs in the riparian zone and respiration tends to decrease upslope away from the channel (Pacific et al. 2008). While the transects in this study were not great enough to move into more upland hillslope positions surrounding the riparian zones, respiration rates did tend to decrease moving away from the stream in winter and summer. Similar to summer measurements in this study, Pacific et al. (2008) reported a large amount of variability in CO_2 gas fluxes measured in the riparian zone, while the variability in soil respiration during the other seasons was low. The low variation in soil respiration may be due to more homogeneous conditions in the measurement areas during those seasons. With respect to the 2-way interaction between season and transect distance it is clear that *across* flow regimes, trends are more evident. While respiration was similar across transects in autumn and spring, there were interesting trends in winter and summer. Specifically, respiration decreased moving away from the stream in winter. In summer, respiration tended to also be greatest closest to the stream. Then, there was a marked decrease then a slight increase at the farthest transect. Summer measurements were also the most variable (especially close to streams).

Correlations between soil CO_2 flux and soil temperature and moisture indicated that, while the environmental factors measured did not vary much themselves, they also did not typically covary with soil CO_2 flux across sites and transects for much of the study, suggesting that perhaps other soil characteristics might have greater explanatory power than soil temperature and moisture. Soil characteristics not addressed in this study may be more influential espcially in the area closest to the wetted channel, where no clear relationships were observed. However, soil temperature and moisture both covaried with soil CO_2 flux on two occasions at the 10- and 20-m transect locations during respective summer and spring sampling events. Soil conditions are certainly subject to seasonal changes and, in turn, variables related to soil respiration may shift over the year, but results of this study also underscore the spatial heterogeneity of soil conditions that influence soil surface CO_2 flux. Additional research is needed in the soil and gravel bar areas directly adjacent to stream channels to more fully understand the drivers of CO_2 fluxes where terrestrial and aquatic systems intersect.

While few significant relationships occurred between soil respiration and soil temperature and moisture at the annual or seasonal scale across sites, more relationships between environmental factors and soil respiration occurred at the site level. Some sites had different or no relationships between variables at various times throughout the sampling year. For example, a positive correlation between soil respiration and soil temperature occurred at Big Piney Creek in the autumn, while soil respiration was inversely correlated with soil moisture at Murray Creek. Since some measurements were unable to be made at Roasting Ear Creek during spring and soil moisture was lacking for Murray Creek during spring, it was not possible to ascertain a definite relationship among soil CO_2 flux and soil temperature and/or moisture during the spring season. However, when plotted across seasons at each site, soil temperature clearly covaried with soil CO_2 flux at each site. All sites revealed positive exponential relationships between soil temperature and soil respiration across seasons. Soil respiration and moisture were only related at *Runoff* sites, and the direction of these relationships was roughly positive. These results warrant further study, as it appears site-level differences may be demarcated by hydroecological classification given that none of the *Groundwater* streams exhibited the dual relationship between soil CO_2 flux and soil temperature and moisture at the site level. The observed differences in relationships between soil temperature, moisture, and CO_2 flux based on hydroecological regime also underscores the often site-specific nature of conditions that control biogeochemical cycles.

Ecoregion and stream size have been shown to affect organic matter decomposition, C-use efficiency, and aquatic microbial respiration rates (Hill et al. 2017), underscoring the need to develop more integrated models of C transport and use from terrestrial through riparian systems into adjacent waterways. Understanding the interplay between soil and stream C fluxes will be important for determining C mitigation and sequestration strategies in the face of climate change and ecosystem disturbance. While soils are a greater source of atmospheric CO_2 relative to small headwater streams (Hope et al. 2004), waterways represent an important and, until recently, largely overlooked conduit of C flux from the terrestrial sink to the atmosphere. Studies of C dynamics need, where possible, a multi-system approach to fully understand and elucidate patterns of C uptake, release, and transport within local and regional land-stream-atmosphere continua.

It is important to note that this study only addressed C as CO_2, while streams and soils are also conduits of methane (CH_4) and nitrous oxide (N_2O) to the atmosphere, which have 34 and 298 times the 100-year global warming potential of CO_2, respectively (Myhre et al. 2013). Similar efforts undertaken in Arkansas agricultural systems have shown clear trends in CH_4 production in response to differing hydrologic regimes (Brye et al. 2013, Rogers et al. 2014), but, similar to the results of this study with CO_2, fluxes of N_2O showed few consistent patterns over the growing season (Rector 2018). More work is needed to adequately characterize how hydrology and land cover influence greenhouse gas dynamics in areas where land is subject to variations in flood regime and water-table depth.

This study was designed to address a gap in soil respiration studies with respect to surface water flow regime/ecoregion, as well as proximity to stream channel, though several caveats must be acknowledged. While soil temperature and moisture are known to account for a large amount of the variation in soil respiration rates, other factors may interact with or confound the known relationships. Soil diffusivity (Davidson and Trumbore 1995) as well as the assimilation rate of C into soil (Trumbore et al. 1995) can also affect soil CO_2 fluxes. However, these additional soil properties and processes were not measured in this study and they may very well have accounted directly for additional variations in C fluxes or, perhaps, influenced the other soil characteristics that were measured. Additionally, the frequency of soil respiration measurement was only once per season; more measurement events would likely have captured greater variations in soil surface CO_2 fluxes and more accurately portrayed relationships that may have been obscured by the low temporal resolution of the dataset generated in this study (Pacific et al. 2008). Nonetheless, the results of this study clearly point to a need to further study stream-associated C dynamics by expanding spatial measurements to adjacent riparian zones.

Results of this study support and extend the current understanding of stream-corridor C fluxes by drawing upon hydroecological classifications that are likely influenced in some way by riparian soil processes. Conducted entirely within minimally disturbed sites, this study provides a basis for comparison among riparian CO_2 fluxes at disturbed sites, which is especially important given that stream corridors and riparian buffers are particularly susceptible to anthropogenic landscape alteration. These results suggest that riparian CO_2 fluxes are highly temporally and spatially variable, highlighting the need for more studies of riparian soils within and across ecoregions and flow regimes to enhance predictions of changes in soil CO_2 dynamics as climate change and human landscape alteration modify soil conditions on a global scale.

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APPENDIX

TABLES

Table 1. Site information with reach UTM coordinates and soil taxonomic (Order, Subgroup, Family, and Series) information by bank. Bank side corresponds to direction when facing upstream. Flow regime is defined based on Leasure et al. (2016). Soil data from Web Soil Survey (Soil Survey Staff 2018).

Site	Flow Regime	Coordinates	Bank	Soil Order	Soil Subgroup	Soil Family	Soil Series
Big Piney	Runoff Flashy	35.770726, -93.394299	Left	Ultisols	Typic Hapludults	Loam	Spadra
			Right	Entisols	Typic Udifluvents	Cobbly Fine Sandy Loam	Ceda
Little Piney	Runoff Flashy	35.669063, -93.372059	Left and Right	Entisols	Typic Udifluvents	Cobbly Fine Sandy Loam	Ceda
Murray	Runoff Flashy	35.616035, -93.367468	Left and Right	Ultisols	Typic Hapludults	Fine Sandy Loam	Spadra
Roasting Ear	Groundwater Flashy	35.951412, -92.287885	Left and Right	Entisols	Typic Udifluvents	Gravelly Loam	Elsah
Spring	Groundwater Flashy	36.018415, -92.586139		Alfisols	Typic Paleudalfs	Fine Sandy Loam	Portia
Sylamore	Groundwater Flashy	36.014068, -92.247944	Left	Alfisols	Mollic Hapludalfs	Fine Sandy Loam	Razort
			Right	Mollisols	Lithic Hapludolls	Moko: Stony Loam	Moko-Estate Complex
				Alfisols	Typic Hapludalfs	Estate: Fine Sandy Loam	

Table 2. Sampling dates within each season at each site and flow regime (Leasure et al. 2016) over the study duration.

Flow Regime	Site	Fall	Winter	Spring	Summer
Runoff	Big Piney	10/20/2015	1/11/2016	3/24/2016	6/9/2016
Runoff	Little Piney	10/12/2015	1/8/2016	3/24/2016	6/9/2016
Runoff	Murray	10/18/2015	1/6/2016	3/24/2016	6/9/2016
Groundwater	Roasting Ear	10/19/2015	1/4/2016	No Sample	6/6/2016
Groundwater	Spring	10/19/2015	1/5/2016	3/21/2016	6/7/2016
Groundwater	Sylamore	10/20/2015	1/4/2016	3/20/2016	6/6/2016

Table 3. Stream and soil summer sampling dates at each site and flow regime (Leasure et al. 2016).

Flow Regime	**Site**	**Soil Sampling Date**	**Stream Sampling Date**
Runoff Flashy	Big Piney	6/9/2016	6/15/2017
Runoff Flashy	Little Piney	6/9/2016	6/15/2017
Runoff Flashy	Murray	6/9/2016	6/15/2017
Groundwater Flashy	Roasting Ear	6/6/2016	6/13/2017
Groundwater Flashy	Spring	6/7/2016	6/13/2017
Groundwater Flashy	Sylamore	6/6/2016	6/13/2017

Table 4. Results of Repeated-Measures ANOVA analyses for riparian soil respiration, soil temperature, and soil moisture.

Dependent Variable	**Source of Variation (Between Groups)**	**F-statistic**	***p*-value**	**Source of Varation (Within Groups)**	**F-statistic**	***p*-value**
Soil Respiration	Flow Regime (Flow)	11.87	0.001	Season	62.35	<0.0001
	Distance From Stream (Distance)	1.50	0.23	Season * Flow	3.95	0.01
	Flow * Distance	2.80	0.07	Season * Distance	8.46	<0.0001
				Season * Flow * Distance	1.02	0.42
Soil Temperature	Flow Regime (Flow)	0.61	0.44	Season	292.52	<0.0001
	Distance From Stream (Distance)	0.01	0.99	Season * Flow	9.10	0.0001
	Flow * Distance	0.055	0.95	Season * Distance	0.04	0.99
				Season * Flow * Distance	0.016	0.99
Soil Moisture	Flow Regime (Flow)	4.44	0.04	Season	24.82	<0.0001
	Distance From Stream (Distance)	1.03	0.37	Season * Flow	2.92	0.05
	Flow * Distance	0.34	0.71	Season * Distance	0.66	0.68
				Season * Flow * Distance	0.69	0.66

FIGURES

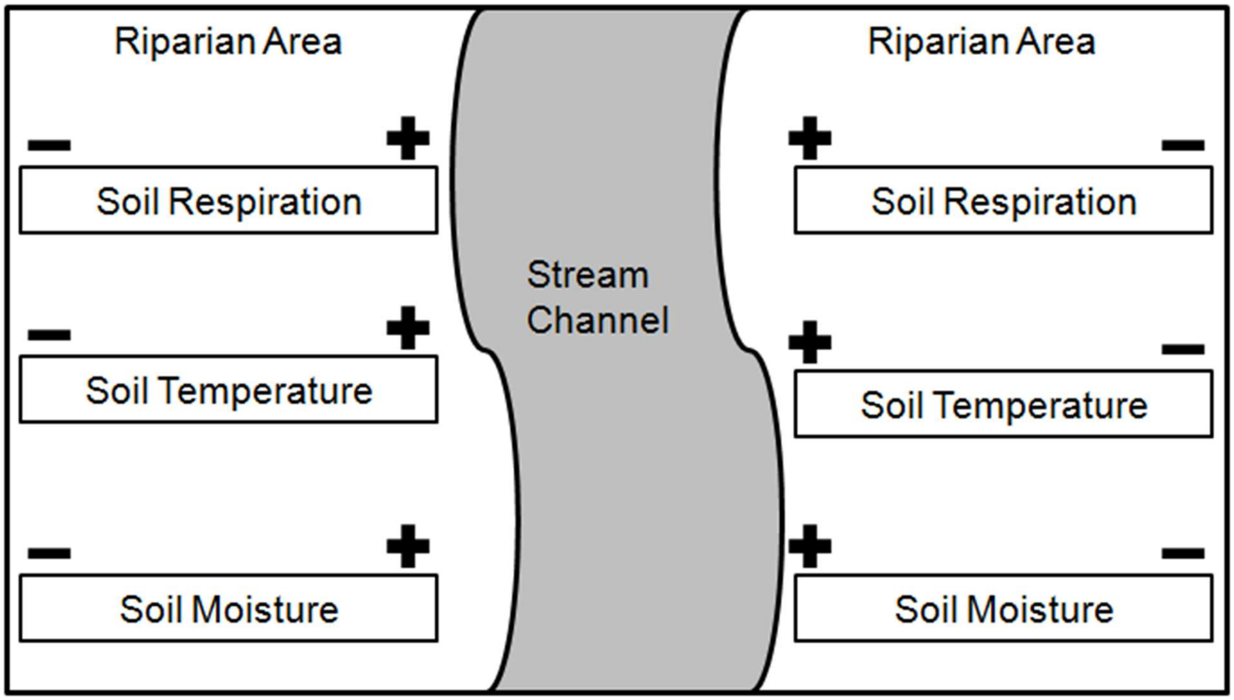

Figure 1. Conceptual model showing predicted trends in soil respiration, temperature, and moisture moving from the immediate stream bank into the riparian area.

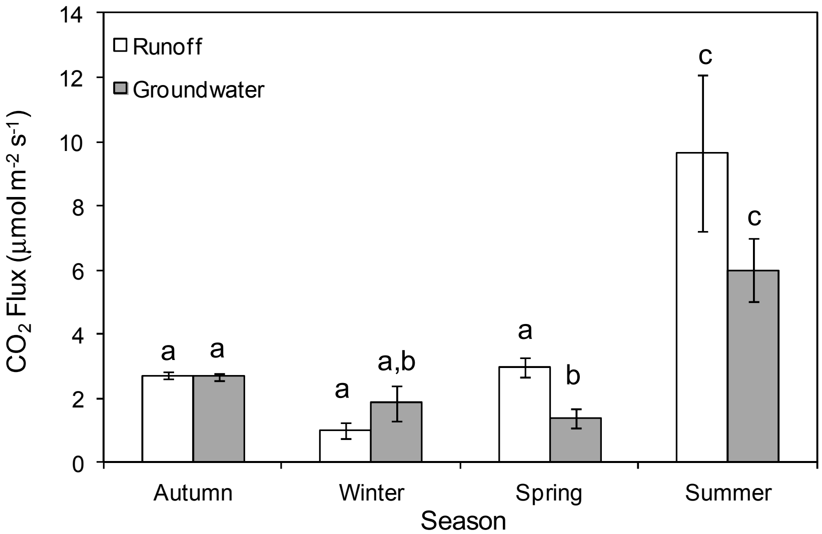

Figure 2. Soil surface carbon dioxide (CO_2) fluxes from longitudinal transects parallel to *Runoff* and *Groundwater* sites by season over the study year. Error bars represent ± 1 standard error. Letters indicate significant differences based on Tukey's HSD test comparing seasons; asterisk denotes significant difference between flow regimes within one season.

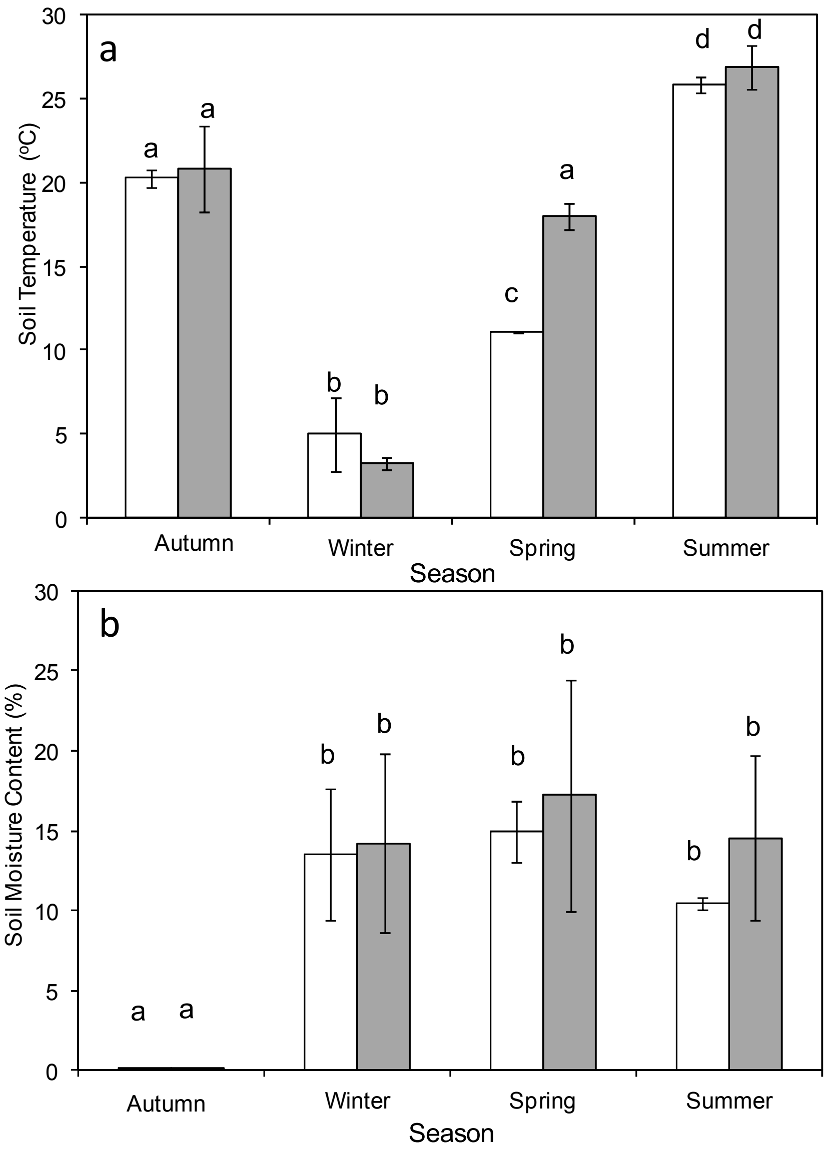

Figure 3. Soil temperature (2 cm; a) and moisture content (0-6 cm; b) from longitudinal transects parallel to *Runoff* and *Groundwater* sites by season . Error bars represent ± 1 standard error. Letters denote significant differences in values across seasons.

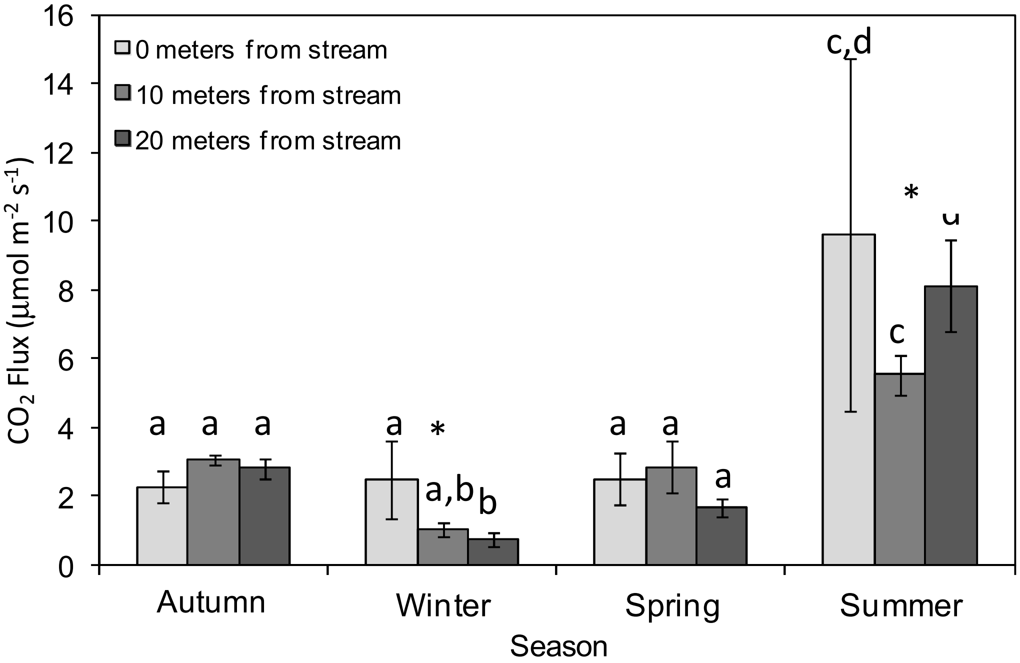

Figure 4. Soil surface carbon dioxide (CO_2) fluxes from longitudinal transects parallel to *Runoff* and *Groundwater* sites by distance along transect away from the stream bank across seasons. Error bars represent ± 1 standard error. Letters indicate significant differences based on *post hoc* tests comparing transects across seasons; asterisk denotes significant difference between transects within one season.

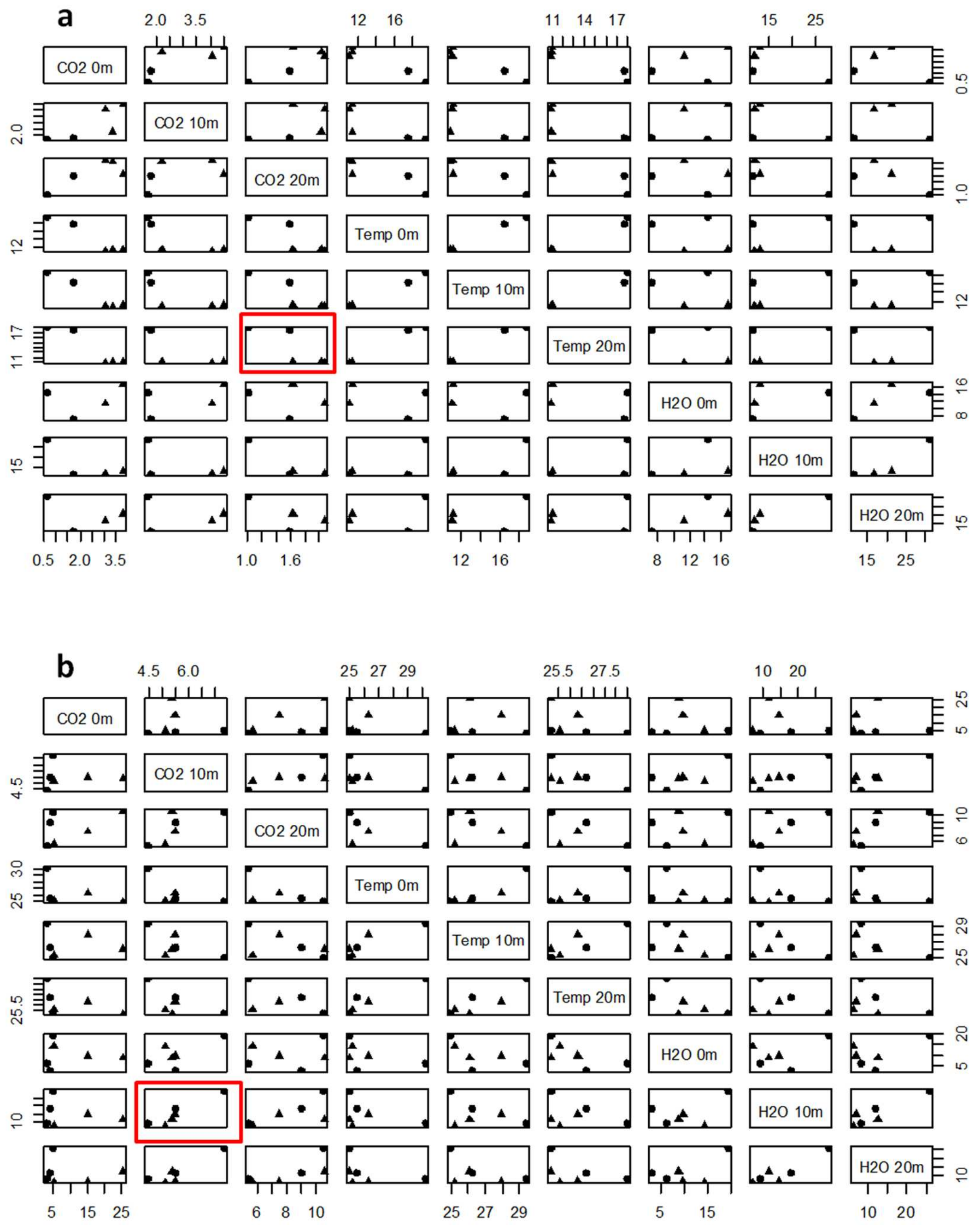

Figure 5. Correlation matrices between measured soil variables at three longitudinal transects in spring (a) and summer (b). Significant correlations ($p< 0.05$) are highlighted in red boxes around correlation plot. CO2 0m, CO2 10m, CO2 20m= CO_2 flux at transects zero, ten, and twenty meters from stream channel, respectively. Temp 0m, Temp 10m, Temp 20m= soil temperature at transects zero, ten, and twenty meters from stream channel, respectively. H2O 0m, H2O 10m, and H2O 20m= Soil moisture at transects zero, ten, and twenty meters from stream channel, respectively. All transects were parallel to stream channel on left and right banks.

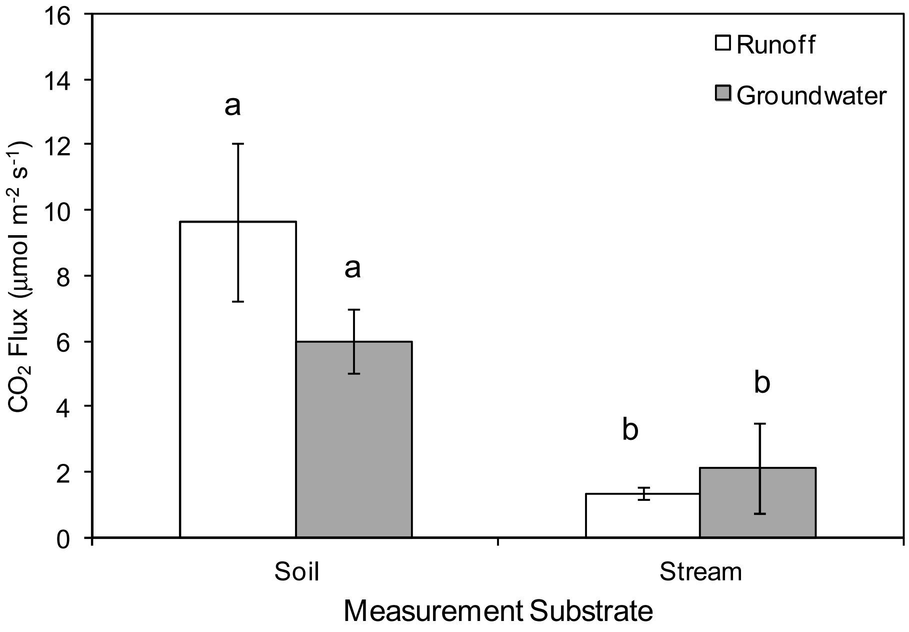

Figure 6. Comparison of soil and stream carbon dioxide (CO_2) fluxes during June at *Runoff* and *Groundwater* sites. Error bars represent ± 1 standard error.

CONCLUSIONS

Understanding the dynamic sources, patterns, and processes driving local, regional, and global C dynamics is an urgent task given that consequences of anthropogenic climate change and human perturbation of the C cycle are already impacting terrestrial and aquatic ecosystems (Parmesan and Yohe 2003, IPCC 2013, McNutt 2013). Ascertaining natural sources of variation in C fixation, release, and transport in forested, minimally-impacted systems is an important first step in evaluating how and by what mechanisms climate change and landscape alteration are modifying C balances. In lotic systems, climate-driven changes in precipitation patterns and reductions in groundwater intrusion from human extraction will alter the natural flow regime, which is the major conduit through which C fixed and dissolved into the stream is transported downstream, to the riparian, and to the atmosphere (Newbold et al. 1982, Wallin et al. 2013). In this dissertation, I utilized field studies and data simulation models to evaluate C dynamics in streams classified into the two dominant hydroecological types in this region based on a spatial model (Leasure et al. 2016), Groundwater Flashy compared to Runoff Flashy streams, to confirm flow class characteristics and explore ecosystem-level flow-ecology responses both within and adjacent to six forested headwater streams.

The impacts of natural variation in flow characteristics were investigated with respect to instream C fluxes as well as C gas fluxes between the stream surface and the atmosphere as well as riparian soil surface and the atmosphere. Flow regimes differed in a number of parameters that may have implications for community and ecosystem dynamics and, in turn, management applications. Metrics such as dissolved inorganic carbon (DIC), alkalinity, and conductivity were greater in *Groundwater* streams owing to the ion-rich groundwater that is predicted to make up the bulk of base flow in those systems. *Groundwater* streams were also colder and less turbid than *Runoff* streams. Unexpectedly, *Runoff* streams dried for a portion of each year, but they also experienced fewer large flood events, likely due to the lack of groundwater influence. While there were no significant differences in annual GPP and ER between categories, both tended to be greater in *Groundwater* streams. Importantly, data simulations revealed that flow regime may mediate the responses of production and respiration to high flows. Additionally, *Groundwater* streams emitted nearly twice as much CH_4 as *Runoff* streams, though the riparian soils of *Runoff* streams tended to emit more CO_2. Overall, field measurements and data simulations suggested that hydrology plays a role in stream and riparian C fluxes and exerts control on factors driving C fluxes within streams as well as between streams, soils, and the atmosphere.

Stream metabolism is sensitive to both natural and anthropogenic disturbances, revealing ecosystem-level responses to changes in hydrology and geomorphology (Izagirre et al. 2008, Williamson et al. 2008). While no significant differences in GPP and ER were found between flow regimes, there were clear trends showing greater biological activity (e.g. GPP and ER) in *Groundwater* streams. Importantly, significant differences may have been undetectable due to low sample size; simulations to increase statistical power showed significantly greater GPP and ER in *Groundwater* streams. Across streams, results showed that drying negatively influenced annual GPP and more heterotrophic streams exhibited greater annual discharge. Simulations also revealed differential impacts of high flows on metabolism based on flow regime. *Groundwater* stream GPP responded positively to high flow, but simulated *Runoff* stream GPP responded negatively to high flows. These results suggest that as climate change and human impacts alter flows, GPP may become more variable across flow regimes and, perhaps, *Groundwater* and *Runoff* streams may exhibit distinct metabolic regimes.

In Chapter 2, I showed that both *Runoff* and *Groundwater* streams are appreciable sources of CO_2 and CH_4 to the atmosphere and that DOC may reduce stream CO_2 gas fluxes.

Importantly, my data suggest that the role of flow regime in greenhouse gas evasion may be dependent on the gas species under consideration. Field and simulation data revealed that CO_2 fluxes, sources, and drivers were similar between *Runoff* and *Groundwater* flow regimes. However, flow classifications had differing sources and drivers of CH_4 fluxes, which are being produced by anaerobes either within or immediately adjacent to *Groundwater* streams. This may be linked to the dominant flow source and other characteristics that define *Runoff* and *Groundwater* streams, such as substrate size. The streambeds of *Groundwater* systems are comprised of gravel and pebble through most of the benthos, and *Groundwater* streams have observable hyporheic zones, whereas *Runoff* streams are bedrock-bottomed with cobble and boulder resting over the consolidated rock. Though all streams were highly aerated, it may be that anaerobic pockets occur within *Groundwater* stream hyporheic zones, especially during late summer when base flow was reduced to its annual minimum. *Groundwater* streams also house upwellings that provide a potential pathway for deep soil-derived CH_4 to enter the stream.

Field data in Chapter 3 provided evidence that riparian areas merit consideration and inclusion when establishing flow-ecology relationships and environmental flow standards. Riparian soil respiration in this study varied more over the year than by flow regime. However, *Runoff* sites emitted significantly more CO_2 than *Groundwater* sites during the spring. Additionally, factors related to respiration rates differed between flow regimes. Specifically, soil respiration at each *Runoff* site was related to both soil temperature and moisture, but fluxes were only related to soil temperature at each *Groundwater* site. During the summer, soils emitted more CO_2 to the atmosphere than adjacent streams, emphasizing the importance of riparian soils to watershed C cycles. Further, the greater soil C flux compared to stream C fluxes underscores the importance of protecting riparian buffers around streams.

Given that several of the streams within this study deviated from model-predicted characteristics- specifically, streams were expected to be perennial- variation in hydrology within a flow regime and the potential influence of this variation within my study designs and statistical analyses is worth considering. One way to address this in future work would be to attempt to select study sites that would behave more similarly within a flow class over a defined time frame. It is certainly likely that on broad temporal scales, the streams in this study are hydrologically similar, as long-term data were used to model flow classifications (Leasure et al. 2016). Addressing ecosystem functions at shorter temporal scales- such as annually or seasonally- may require refining hydrologic characteristics even within a flow classification to reduce variation. Additionally, future studies designs may need to weigh the benefits of grouped versus continuous explanatory variables. In this dissertation, regression and correlation results between flow metrics and ecosystem functions tended to provide a better explanation of what specifically is influencing C dynamics than t-test/ANOVA designs that simply grouped flow regimes together. That is, regression and correlation approaches tended to yield a greater number of significant trends that explained variation in C fluxes. My contribution here is twofold: I presented evidence of the importance of hydrology to stream and adjacent riparian C fluxes, and provided an example of multiple approaches (i.e. continuous flow variables and grouped flow classes) for exploring flow-ecology relationships. The mapped natural flow regimes of northern Arkansas have certainly proven useful for exploring flow-ecology relationships in the region (Bruckerhoff and Magoulick 2017, Yarra and Magoulick 2018, Lynch et al. 2018), but studies of ecosystem processes may require a more careful approach with respect to characterizing flow and flow-dependent variables driving variation in function . Flow regime is an important determinant of community and ecosystem processes and functions (Bunn and Arthington 2002, Poff et al. 2009, Poff and Zimmerman 2010, Lynch et al. 2018). This dissertation presents evidence that natural variation in hydrology within a temperate forested biome can influence whole-stream metabolism, stream greenhouse gas fluxes, sources, and drivers, and riparian soil respiration.

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