ARKANSAS WATER RESOURCES CENTER | UNIVERSITY OF ARKANSAS
FUNDED BY THE US GEOLOGICAL SURVEY 104B PROGRAM

Arkansas Water Resources Center Annual Technical Report FY2016

2017 June

AWRC

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RESEARCH & EXTENSION
University of Arkansas System

Arkansas Water Resources Center Annual Technical Report FY2016

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Abstract

This publication serves as the annual report to the U.S. Geological Survey regarding the 104B program projects and activities of the Arkansas Water Resources Center (AWRC) for FY2016. This document provides summary information for each of the projects funded through the 104B base grant. This year, the AWRC funded 3 faculty research proposals and 4 student centered proposals with faculty advisors. Faculty projects include: 1) “Partitioning rice field evapotranspiration into evaporation and transpiration components”, Benjamin Runkle, University of Arkansas, Department of Biological and Agricultural Engineering; 2) “Comparative Microbial Community Dynamics in a Karst Aquifer System and Proximal Surface Stream in Northwest Arkansas”, Matthew Covington, University of Arkansas, Department of Geological Sciences; and 3) “Biological and ecological consequences of sub-lethal ion concentrations on microbial and macroinvertebrate detritivores”, Sally Entrekin, University of Central Arkansas, Department of Biology. Student projects with a faculty advisor that were funded include: 1) “Investigating Fate of Engineered Nanoparticles in Wastewater Biofilms”, Wen Zhang and Connie Walden, University of Arkansas, Department Civil Engineering; 2) “Tracking the growth of on-site irrigation infrastructure in the Arkansas Delta with remote sensing analysis”, Kent Kovacs and Grant West, University of Arkansas, Department of Agricultural Economics and Agribusiness; 3) “Characterization of nutrient sources, transport pathways, and transformations using stable isotope and geochemical tools in the Big Creek watershed of northwest Arkansas”, Phil D. Hays and Kelly Sokolosky, University of Arkansas, Department of Geosciences; 4) “Does macrograzer activity drive seasonal variations in algal biomass in Ozark streams?”, Michelle Evans-White and Kayla Sayre, University of Arkansas, Department of Biological Sciences.

Keywords: Arkansas Water Resources Center, 104B Program Funding, Information Transfer, Water Quality

Report Introduction

The Arkansas Water Resources Center (AWRC or Center) is part of the network of 54 water institutes established by the Water Resources Research Act of 1964 and is located at the University of Arkansas in Fayetteville. Since its formation, the AWRC in cooperation with the US Geological Survey and the National Institutes for Water Resources has focused on helping local, state and federal agencies understand, manage, and protect water resources within Arkansas.

The Center has contributed substantially to the State's understanding of its water resources through scientific research and volunteer monitoring efforts. Additionally, the training of students – the future generations of scientists and engineers – is a top priority for the Center. The AWRC directs its research funding priorities toward providing local, state and federal agencies with scientific data necessary to make informed decisions that enhance their ability to protect and manage water resources throughout the State and region. AWRC helps to fund and coordinate research to ensure good water quality and adequate quantity to meet the needs of Arkansas today and into the future.

Another priority mission of the Center is the transfer of water resources information to stakeholders within Arkansas and around the country. The AWRC holds an annual water conference to address current water issues and solutions. The Center also publishes numerous types of publications including technical reports, peer-reviewed journal articles, and monthly electronic water newsletters. The use of social media has allowed the Center to reach more people, with a growing number of interested individuals from state agencies, water organizations, and the greater public.

The AWRC continues to enhance its activities to successfully implement its core missions – to generate competent research, train future water scientists and engineers, and actively disseminate information to water stakeholders throughout Arkansas. This report details the activities of the Center during the past project year (March 1, 2016-February 29, 2017).

Research Management Introduction

Since its formation, the Arkansas Water Resources Center (AWRC or Center) has focused on helping local, state and federal agencies manage and protect Arkansas' water resources. The Center has contributed substantially to the State's understanding of its water resources through scientific research and volunteer monitoring efforts. Training students – the future generations of scientists and engineers – is also a top priority for the Center.

Scientific Research

Each year, several researchers across the state submit proposals for research grants from the AWRC through the USGS 104B program. The AWRC directs its research funding priorities toward providing local, state and federal agencies with scientific data necessary to make informed decisions that enhance their ability to protect and manage water resources throughout the State. Center projects generally focus on topics concerned with the quality and quantity of surface water and ground water, especially regarding non-point source pollution, land use and climate change, agricultural water use, and sensitive ecosystems.

When soliciting research proposals for funding through the USGS 104B program, the Center emphasized the following objectives:

- Arrange for applied research that addresses water supply and water quality problems
- Train the next generation of water scientists and engineers
- Support early career faculty in water research and preliminary data
- Support faculty changing focus or addressing emerging water issues
- Transfer research results to stakeholders and the public
- Publish 104B funded research in peer-reviewed scientific literature
- Cooperate with other colleges, universities and organizations in Arkansas to create a coordinated statewide effort to address state and regional water problems.

Each of the proposals selected for funding this past year addressed the priority research topics and the objectives of the Center. The Center also encourages research proposals that support the USGS national water mission in one of its broad areas, including:

- Increase knowledge of water quality and quantity
- Improve understanding of water availability
- Evaluate how climate, hydrology and landscape changes influence water resources
- Create and deliver decision-making tools that support water management
- Improve the country's response to water-related emergencies

To formulate a research program relevant to current water issues in Arkansas, the Center worked closely with its technical advisory committee (TAC). The TAC is composed of representatives from state and federal water resources agencies, academia, industry and private groups. Members of the advisory committee reviewed and ranked proposals submitted to the AWRC, which helped ensure that funds addressed a variety of current and regional water resource issues.

In FY2016, the AWRC, with the guidance of the TAC, funded 3 faculty research proposals totaling $60,000 and 4 student research proposals with a faculty advisor totaling $20,000. Faculty projects that were funded include:

1) "Partitioning rice field evapotranspiration into evaporation and transpiration components", Benjamin Runkle, University of Arkansas, Department of Biological and Agricultural Engineering;
2) "Comparative microbial community dynamics in a karst aquifer system and proximal surface stream in northwest Arkansas", Matthew Covington, University of Arkansas, Department Geosciences;
3) "Biological and ecological consequences of sub-lethal ion concentrations on microbial and macroinvertebrate detritivores", Sally Entrekin, University of Central Arkansas, Department of Biology.

Student projects with a faculty advisor that were funded include:

1) "Investigating fate of engineered nanoparticles in wastewater biofilms", Connie Walden and Wen Zhang, University of Arkansas, Department of Civil Engineering;
2) "Tracking the growth of on-site irrigation infrastructure in the Arkansas Delta with remote sensing analysis", Grant West and Kent Kovacs, University of Arkansas, Department of Agricultural Economics and Agribusiness;
3) "Characterization of nutrient sources, transport pathways, and transformations using stable isotope and geochemical tools in the Big Creek watershed of northwest Arkansas", Kelly Sokolosky and Phillip Hays, University of Arkansas, Department of Geosciences;
4) "Does macrograzer activity drive seasonal variations in algal biomass in Ozark streams?", Kayla Sayre and Michelle Evans-White, University of Arkansas, Department of Biological Sciences.

During this past year, the AWRC research program successfully promoted the dissemination and application of research results to stakeholders through publications, conferences and workshops. The research program also emphasized the training of future scientists and engineers who are focused on water resources and watershed management, and supported undergraduate, Masters, and Ph.D. level students. The "seed" grants provided to research faculty through this program have led to the development of larger research proposals submitted to other funding agencies and also have provided research opportunities to new faculty and more senior faculty investigating new areas in water resources.

Once these scientists were funded, the Center coordinated and administered the grants, allowing the researchers to concentrate on providing a quality project. Support was provided to researchers in the form of accounting, reporting and water sample analysis (through the AWRC Water Quality Laboratory).

Volunteer Water Quality Monitoring

The Center supported and worked closely with Ozarks Water Watch, a non-profit water resources organization. AWRC provided guidance and support to StreamSmart, a program of Ozarks Water Watch. AWRC personnel conducted a formal training workshop related to sample collection and site assessment to volunteers. The Center also supported this program by funding the laboratory analysis of water samples collected by volunteer citizen scientists. AWRC supported another program of Ozarks Water Watch called Beaver LakeSmart by participating on the advisory board and providing guidance to the program director.

Student Training

The AWRC facilitates the training of students. For example, funding priorities are given to research proposals that emphasize student support and training. The Center also provides several training opportunities directly. This direct student support included:

- The AWRC participated in the Ecosystems Services Research Experience for Undergraduates (EcoREU) program, funded by the National Science Foundation, by advising students through the scientific research process.
- The AWRC helped train undergraduate students by mentoring them through their freshman engineering research projects at the University of Arkansas.
- The Center supported paid student work where the student gained experience in the water quality laboratory and in data organization and analysis.
- The AWRC continued with its third annual paid summer internship. The student intern was trained in graphic design and successfully completed several products associated with a variety of Center-related projects.

During this past year, 21 students and postdoctoral researchers were trained through participation in research projects and through the AWRC directly.

Project Title: Partitioning rice field evapotranspiration into evaporation and transpiration components
Project Number: 2016AR383B
Start Date: 3/1/2016
End Date: 2/28/2017
Funding Source: 104B
Congressional District: 003
Research Category: NA
Focus Category: Agriculture, water quantity, irrigation
Principal Investigator: Benjamin R.K. Runkle

Publications and Presentations:

Suvočarev K, Reba M, Runkle BRK (2016) for presentation at the 32nd Conference on Agricultural and Forest Meteorology, 22nd Symposium on Boundary Layers and Turbulence, and Third Conference on Biogeosciences, Salt Lake City, Utah, 20-24 June 2016.

Reba ML, Fong B, Runkle BRK, Suvocarev K, Adviento-Borbe A: Winter fluxes from Eastern Arkansas Rice-Waterfowl Habitats, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA

Suvocarev K, Reba M, Runkle, BRK: Surface renewal: micrometeorological measurements avoiding the sonic anemometer, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA

Runkle BRK, Suvocarev K, Reba ML, Novick KA, White P, Anapalli S, Locke MA, Rigby J, Bhattacharjee J, Variation in agricultural CO2 fluxes during the growing season, collected from more than ten eddy covariance towers in the Mississippi Delta Region, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA

Roby M, Reavis C, Reba M, Suvocarev K, Runkle BRK, Testing the reduction of methane emissions from alternate wetting and drying in rice fields: two years of eddy covariance measurements from Arkansas, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA

Reavis C, Suvočarev K, Runkle B, Reba M, Evaluating Methods for Quantifying Evapotranspiration in Commercial Rice Fields, awarded Best Poster, 2017 Arkansas Soil and Water Education Conference, Jonesboro, AR, January 25, 2017. *** student attendance at conference

Runkle BRK, Paddy Rice Research Group, Global Research Alliance talk, July 14, 2016, at Stuttgart meeting, and field site presentation. Talk titled: “Alternate Wetting and Drying as an Effective Management Practice to Reduce Methane in Arkansas Rice Production”

Runkle BRK, Presentation at Ameriflux PI meeting, Golden Colorado, September, 2016: Neighboring fields, neighboring towers: Testing climate-smart irrigation strategies to reduce methane emissions from rice fields

Runkle B., J. Rigby, M. Reba, S. Anapalli, J. Bhattacharjee, K. Krauss, L. Liang, M. Locke, K. Novick, R. Sui, K. Suvočarev, P. White, 2017, Delta-Flux: an eddy covariance network for a climate-smart Lower Mississippi Basin, Agricultural & Environmental Letters, 2:170003. doi:10.2134/ael2017.01.0003.

Reavis C., Advisor B. Runkle, 2017 expected, Evapotranspiration in Mid-South Rice Fields, MS Thesis, Department of Biological & Agricultural Engineering, University of Arkansas, Fayetteville, AR.

Partitioning rice field evapotranspiration into evaporation and transpiration components

Benjamin R.K. Runkle, Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville

Core ideas

- **Evapotranspiration (ET) is largely composed of transpiration during the growing season (74% over the season; up to 95% in the mid-summer)**
- **The transpiration signal is strong such that drying periods do not seem to show significant reductions in ET.**
- **The project team has expanded its spatial reach by developing a regional network of ET observation sites and will work with a USGS team to help constrain regional groundwater models.**

Executive Summary:

This project aimed to resolve uncertainties in the evapotranspiration (ET) portion of the water balance as rice farms transition from conventional to alternate wetting-drying (AWD) irrigation strategies. As 64% of regional precipitation is converted to ET, it is a dominant part of the surface water balance, and understanding its behavior is a key priority to determine the state's water resources situation. Our project's research work is performed at several scales. First, we directly monitor ET rates with the eddy covariance method at several rice production fields in Arkansas in concert with biometeorological measurements to detect underlying, predictive mechanisms. We interpret these measurements in a number of ways, including the Food and Agricultural Organization's implementation of the Penman-Monteith equation to partition ET into its transpiration and evaporation components. Here we find that AWD management does not significantly alter the surface water balance due to the high rates of transpiration during the growing season. Second, we have generated a regional network of research scientists focused on ET and related fluxes (e.g., land-atmosphere exchange of CO_2, which plays a major, interacting role in controlling plant water use). Further, we have connected to a USGS groundwater modeling team to enhance their representation of ET in their projections. Our local and regional results lay the groundwork for more nuanced experimental research in both ground observations and modeling strategies. The initial results will help to constrain the rate of ET in the region so that USGS-driven models more accurately anticipate changes in the region's water resources.

Introduction:

Rice agriculture uses 35% of Arkansas's irrigation water and contributes to the unsustainable depletion of the state's water resources (Reba et al., 2013; ANRC, 2014). A variety of new irrigation methods have been proposed to reduce water use, including alternate wetting and drying (AWD), which floods the soil and then allows a strategic dry down before reflooding to save water, reduce the risk of the straighthead disability on rice, and decrease field methane production. This method reduces greenhouse gas emissions by more than 70% (including from methane, which is produced under water-saturated conditions and is 20-30 times more potent as a greenhouse gas than CO_2) (Rogers et al., 2013; Linquist et al., 2015). Our 2015 project found that total evapotranspiration (ET) from an AWD field is similar or even slightly greater than a comparison, conventionally flooded field. This response may be due to the strong ability of rice roots to pull water from the soil matrix and from the relatively short length of the dry down period (approximately 11 days).

Therefore this project aimed to investigate further the relationships between evaporation and transpiration and to quantify a second growing season of ET rates in Arkansas rice production to test whether the initial results were robust over time. This project also aimed to generate broader interest through the creation of a regional network of measurement sites. While our eddy covariance datasets are still being developed, we have been able to compare initial findings with the Food and Agricultural Organization's Penman-Monteith method of reference ET (known as FAO56; Allen et al., 1998). The FAO56 method is also used to partition the total ET into contributing portions of evaporation and transpiration by applying a dual crop coefficient method.

Additionally, we recognize a need for a more regional perspective, and so sought out strategic partners who both collect and interpret ET observations. We generated the regional Delta-Flux observation network, established ties to South Korean researchers, and have begun working with a USGS team dedicated to improving groundwater modeling of the Mississippi Alluvial Aquifer. These efforts are described in more detail in the Results and Conclusions sections.

Methods:

This research is situated within a larger project aimed to measure year-round land-atmosphere fluxes of energy, water vapor, CO_2 and CH_4 from two side-by-side pairs of rice fields near Humnoke and Burdette, AR, respectively (Figure 1). This larger project provides meteorological instrumentation, eddy covariance equipment to measure the fluxes, and associated environmental monitoring devices to capture terms such as the water level and soil temperature. Presented here are the water vapor fluxes measured by the eddy covariance method, for the Humnoke fields in 2015.

Water vapor fluxes are both measured by the eddy covariance method to determine turbulent transport between the surface and atmosphere (Baldocchi, 2003) and they are modeled by the Penman-Monteith equation (Monteith, 1981). The eddy covariance measurements are generated from observations of vertical wind and water vapor recorded 20 Hz by using the EddyPro software, version 6.2 (Li-cor, USA), and are carefully quality controlled following standard protocols and an additional screen for outliers in the scalar statistics. The eddy covariance observations are gap-filled using an artificial neural network approach (Knox et al., 2015, 2016). These models use data equally apportioned into training, testing, and validating groups from natural data clustered identified using a k-means method. The procedure was replicated across 20 resampling runs and the median prediction was used for gap-filling. To estimate conservative uncertainty bounds from this procedure for the seasonal budget, we use the 95% confidence interval from the 20 extractions used to fill each gap. The ANN model for ET was created with explanatory variables including decimal day since the start of the study period, leaf area index (LAI)

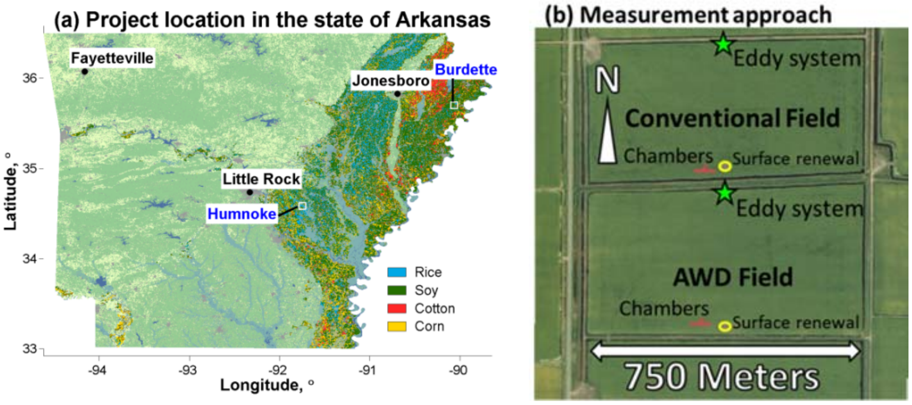

Figure 1: Two project field locations in Humnoke and Burdette, Arkansas, mapped upon a 2013 crop cover dataset (Han et al., 2014) with selected crops in legend. (b) Representative paired field site (Humnoke, AR farm) with measurement sites for the eddy covariance system (which includes soil and biometeorological measurements, closed chambers, and surface renewal system indicated). Gap-filling from northern winds – more common in the winter period – will be performed and validated with data from the surface renewal towers using a simpler, cheaper micrometeorological technique (Suvočarev et al., 2014).

and plant height interpolated using growing degree day, the friction velocity u*, air temperature, incoming solar radiation (Rg), vapor pressure deficit (VPD), and water table depth. The model also included representations of seasonality (spring, summer, and autumn) and the time of day (morning, afternoon, evening, and night), following the method of Papale and Valentini (2003).

Using observations of ET, meteorology, and assumptions about the roughness length and aerodynamic conductance, the Penman-Monteith equation can be inverted to estimate the canopy conductance g_c. The model is inverted to create estimates of g_c based on measured ET. This approach was previously used by the PI to determine canopy controls on ET in a Russian wetland (Runkle et al., 2014). The canopy conductance term is assessed during wet periods for both fields under the hypothesis that it should behave very similarly between fields under similar conditions. In the future, using the photosynthesis estimates derived from the simultaneous CO_2 flux measurements could enable a partition of ET into plant-controlled (transpiration) and water or soil controlled (evaporation) components. During dry down periods the hypothesis is that canopy conductance will become an increasingly important control on ET rates. The transpiration portion of ET should also increase during these periods even if the overall ET rate is similar to wetter periods.

The dual crop coefficient method requires biometeorological and phenological inputs in order to calculate two separate crop coefficients used to convert reference evapotranspiration (ET_{ref}) into transpiration and evaporation:

$$ET = \left(K_{trans.} + K_{evap.}\right) * ET_{ref}$$

where the part modified by K_{trans} is the estimated transpiration and the part modified by K_{evap} is the estimated evaporation. Each coefficient was calculated separately using guidelines presented in FAO 56, including recommendations and considerations for different crops, management practices, and climate. These coefficients are also adjusted for the higher relatively humidity conditions present in the US Mid-South. The reference evapotranspiration rate was calculated using methods also outlined in FAO 56 as part of the Penman-Monteith method for calculating reference evapotranspiration.

Site description: Two privately farmed, adjacent rice fields (34° 35' 8.58" N, 91° 44' 51.07" W) located just outside of Humnoke, Arkansas, were used for this research. Each field is approximately 350 m wide from north to south and 750 m long from east to west (i.e., 26 ha). One field was managed with continuous flooding (CF) during the rice growing season and the other with AWD management practice, facilitating a direct comparison of the two types of systems with minimal spatial separation. Both sites have been zero-graded and thus have approximately 0% slopes. Although only about 12.3% of total rice in Arkansas is grown on zero-graded land, this practice is growing due to the potential to save water in the fields (Hardke, 2015), to serve as a carbon-offset credit option (ACR, 2014) and to receive credit in the Natural Resources Conservation Service's Environmental Quality Incentives Program (EQIP). The sites are not tilled and are flooded for two months in winter for duck habitat and hunting. The dominant soil mapping unit in this area is a poorly-drained Perry silty clay. In 2015, the fields were drill-seed planted April 7 (AWD) and April 8 (CF), given an irrigation flush on May 3 (CF) and May 4 (AWD), and given a permanent flood on May 16 (CF) and May 18 (AWD). The AWD field dried on June 5 and received 3 more dry periods through the summer.

Results:

Evapotranspiration observations and partition into evaporation and transpiration: Observed ET in each field in 2015 was similar, regardless of water management (Figure 2). Even during periods when the AWD field had a water table below the surface and the CF field had a standing water table, the daily observed ET was very similar (the AWD field ET was 1.07 ± 0.06 times the CF field ET, n = 25 observed days; alternately, when both fields had a standing water table, the slope was 1.01 ± 0.03, n = 63). In 2015 the fields also had similar yields, though the field under AWD treatment had higher peak LAI (approx. 5 vs

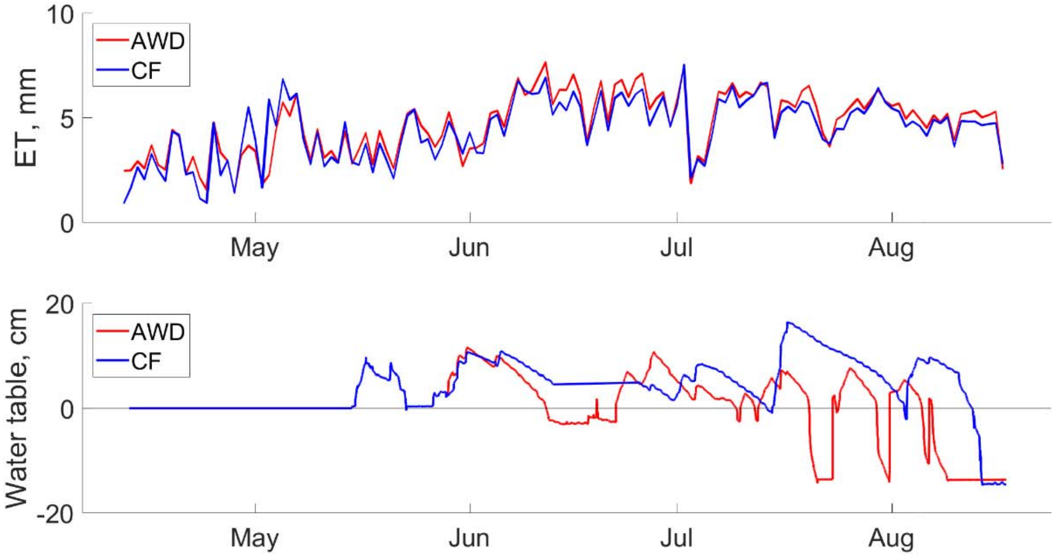

Figure 2: Daily ET estimates for both CF and AWD fields using eddy covariance, gap-filled with a neural network model, and presented with 30-min water table measurements throughout the 2015 growing season.

4.5). The contributions of modeled evaporation and transpiration to ET – both as observed and as modeled by the FAO56 method – for the entire 2015 growing season can be viewed in Figure 3. Transpiration was the highest contributing portion in both fields, composing 73-75% of total ET. Seasonal totals for each portion as well as eddy covariance observations can be found in Table 1. With these fields the modeled ET tended to overestimate the observed and gap-filled ET. Further work is being performed to test this finding by assessing the eddy covariance data for further corrections, including transducer shadowing on the sonic anemometer (Horst et al., 2015) and other possible causes for the well-known potential under-estimation bias of eddy covariance measurements (Foken et al., 2011).

Our initial investigation of surface conductance, looking at the noon-time value as representative of canopy characteristics, indicates that both fields were similar whether the two fields were under similar, ponded-water conditions or whether the AWD field was dry and the CF field was wet. In these cases the relationship between g_c of the AWD field and g_c of the CF field had a slope of 1.12 ± 0.01 (n = 18) or 1.17 ± 0.004 (n = 51), respectively (data not shown). Because these relationships look so similar, we cannot yet use surface conductance as a clear indicator of flooded or dried water flux source conditions, nor use it as a clear indicator by which to partition the flux into evaporation or transpiration components.

While we observed a second rice growing season, in 2016, and expanded our efforts to include measurements near Burdette, Arkansas, those results are not yet ready for release. They are being quality-controlled and checked for accuracy, and they were delayed in part through re-coding for the transducer shadowing effect as described above. An initial look at this data suggests that the findings are consistent with the 2015 growing season. These results will be published as soon as possible and then widely shared through the AmeriFlux website.

Network generation and project expansion: A major result of this project was an effort to generate several regional networks. Networked research sites are increasingly used to study regional land management impacts on carbon and water fluxes. However, key national networks lack contributions from the Lower Mississippi River Basin (LMRB), whose highly productive agricultural areas have potential for soil carbon sequestration through conservation practices. Therefore, we established the new Delta-

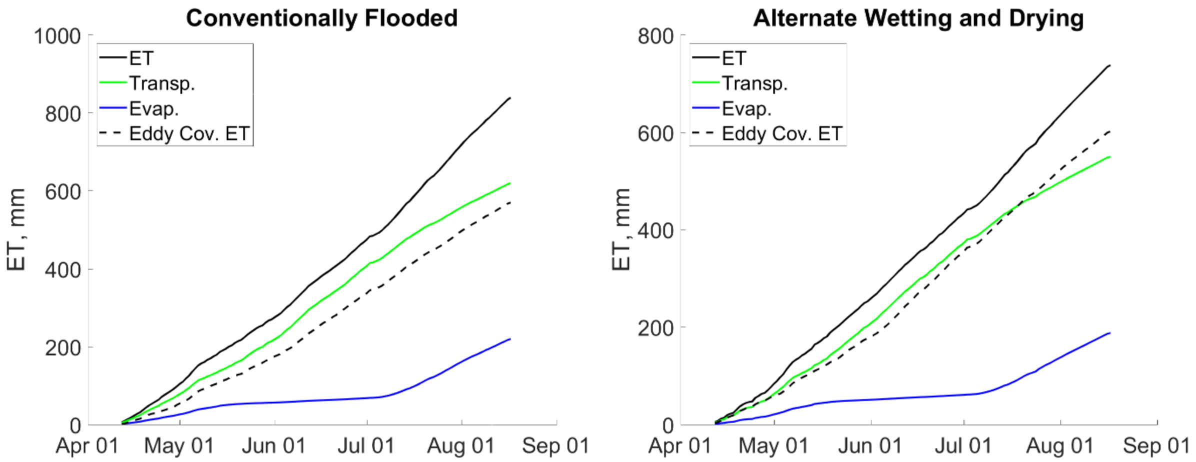

Figure 3. Cumulative transpiration (green) and evaporation (blue) for the 2015 growing season with both portions summing to total evapotranspiration (black) as predicted from the dual crop coefficient model. Eddy covariance observations (dashed) are also included for reference.

	Seasonal Total, mm	
	AWD	CF
Transpiration	550	619
Evaporation	188	220
Total ET	738	839

Table 1. Seasonal totals for each contributing portion of evapotranspiration for the 2015 growing season (April 13 to August 17) in Humnoke, Arkansas, based on the dual crop coefficient model.

Flux network to coordinate efforts to quantify carbon and water budgets and their interactions at seventeen eddy covariance flux tower sites in Arkansas, Mississippi, and Louisiana (Runkle et al., 2017). We are also working with USGS researchers to improve the water budget of the Mississippi Embayment Regional Aquifer System (MERAS) groundwater model (Clark and Hart, 2009) which is being used to provide projections on groundwater supply under various scenarios of climate and land use changes for the MAP. However, this modeling group lacks ground-based observations of ET, and we hope to integrate the MERAS model with the Delta-Flux network.

Beyond these regional networks, we also expanded our international network to build on work funded through the USGS 104(b) project. We leveraged the 104(b) project to seek funding from the AsiaRice Foundation for a travel grant for project graduate student Colby Reavis. In January, 2017, he visited Youngryel Ryu's research group at Seoul National University in South Korea. There, he learned how to use the Breathing Earth System Simulator (BESS) product, based on remote sensing products and ecophysiological relationships and built by Ryu's group (Ryu et al., 2011; Jiang and Ryu, 2016). The visit to Korea also involved a visit to a rice research site with an eddy covariance tower and discussions about how to better parameterize and clarify the role of rice phenology as an important factor in field ET. Together the site visit and rice phenology discussion highlighted the need to take advantage of cutting edge site-monitoring tools such as drone-based imagery and solar-induced fluorescence.

Conclusions, Recommendations and Benefits:

The project findings that ET is largely composed of transpiration during the peak growing season highlight that water savings from AWD are not derived from reduced ET. They are instead derived from a mixture of reduced over-application of water, AWD's ability to capture mid-summer rainfall that would otherwise have drained off the field edge, and reductions in other end-of-field drainage and soil percolation. The ET rates of the fields in this study are very similar to modeled ET using the Penman-Monteith method. This finding lends confidence to regional modeling initiatives that they can constrain this term's uncertainties and reduce uncertainty in projections of the region's full water balance, including its groundwater levels. To enhance partitioning efforts between evaporation and transpiration, we encourage more field-based techniques such as leaf photosynthesis measurements, analysis of water table fluctuations, or the use of lysimeters or isotopic methods. Coupling an analysis of ET rates with landscape CO_2 exchange may also prove fruitful for helping differentiate the two water flux pathways.

Local, regional, and national benefits: Local measurements of the ET terms will help in managing water demand and irrigation scheduling. Increased knowledge of how the components of rice field evapotranspiration respond to different weather conditions will enable two types of upscaling: (1) temporally, these relationships can be used to expand and improve on models of crop water use in different future climate scenarios, (2) spatially, changes in weather patterns across the state can generate a mosaic pattern of ET. The project outcome will therefore constrain estimates of groundwater recharge, the regional meteorological energy balance, and downstream water quality. We have begun collaborating with USGS partners on the MERAS groundwater model to contribute our ET datasets to their regional modeling initiatives. In addition to providing quantitative data on the magnitude of ET we also hope to generate locally-calibrated mechanistic relationships to place within their modeling framework.

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Project Title:	Comparative Microbial Community Dynamics in a Karst Aquifer System and Proximal Surface Stream in Northwest Arkansas
Project Number:	2016AR384B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	003
Research Category:	Water quality
Focus Category:	Groundwater, non point pollution, ecology
Principal Investigator:	Matthew D. Covington, Kristen E. Gibson

Publications and Presentations:

Rodriguez, J., Covington, M.D., Gibson, K.E., Almeida, G., and J.M. Jackson, Comparative microbial community dynamics in a karst aquifer system and proximal surface stream in Northwest Arkansas, South-Central Geological Society of America Meeting, 13-15 March 2017, San Antonio, TX.

Rodriguez, J.; advisors: M. Covington and K. Gibson, 2017 (expected), Comparative microbial community dynamics in a karst aquifer system and proximal surface stream in Northwest Arkansas, MS Thesis, Geosciences Department, Fulbright College, University of Arkansas, Fayetteville, AR.

Comparative Microbial Community Dynamics in a Karst Aquifer System and Proximal Surface Stream in Northwest Arkansas

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Core ideas:

- ***Escherichia coli* concentrations were significantly higher in Little Sugar Creek (median=120 MPN/100 mL) than in Blowing Spring Cave (median=56 MPN/100 mL).**
- ***E. coli* concentrations at Blowing Spring Cave were strongly correlated with discharge (Spearman's R=0.79, p<<0.05), whereas concentrations at Little Sugar Creek showed no statistically significant correlation with discharge.**
- **There was significant dissimilarity in microbial composition among water and sediment samples regardless of location or event type.**

Executive Summary: Northern Arkansas is underlain largely by carbonate bedrock, with relatively well-developed karst flow systems. Much of this region is rapidly urbanizing, leading to a variety of potential threats to groundwater, including increased, and redirected, runoff and the potential introduction of contaminants into the subsurface via septic systems, effluent wastewater discharge, and agricultural runoff. Because of the karst system, threats to groundwater quality are also threats to surface water quality, which is used widely in the region for both drinking water and recreation. Here, Blowing Springs Cave (BSC) and Little Sugar Creek (LSC) were selected to serve as a model for how non-point source pollution may move through the subsurface and subsequently impact springs as well as receiving streams via contaminated water and resuspension of contaminated sediments. The objectives of the study were to: 1) explore structure, diversity, and temporal variability of microbial communities in BSC and LSC; 2) differentiate allochthonous bacteria from land surface runoff with bacteria in the sediments and water of the karst aquifer; 3) determine impact of sediment movement from karst springs to LSC through comparison of microbial communities; and 4) delineate the recharge area of BSC and constrain potential sources of *E. coli*. Water and sediment samples were collected routinely once per month for 9 months and during 2 rain events in a 3-day time series (1, 2, 4 d). The following methods were applied: *E. coli* analysis of water samples by Colilert + Quantitray 2000 system; dye tracing tests to constrain recharge area of BSC; and 16s rRNA metagenomic analysis. During the study period, 92 water samples and 89 sediment samples were collected. Analysis of water samples for *E. coli* showed significantly higher median levels in LSC (120 MPN/100mL) when compared to BSC (56 MPN/100mL). Moreover, there was a strong correlation between discharge and levels of *E. coli* at BSC (Spearman's R=0.79, p<<0.05); however, this same relationship was not observed in LSC. Although microbial community analysis is ongoing, it is evident that there are significant differences in the microorganisms present in water and sediment samples regardless of event type and sampling location. Last, dye tracing indicated a connection between Blowing Spring and a sinkhole located ~1 km to the NE. The average flow velocity of the tracer between the injection point and spring was approximately 40 m/day. The results of the study suggest that sources of *E. coli*, and microbial diversity in general, are different between the karst system and surface stream, even though LSC is under the influence of BSC.

Introduction: Northern Arkansas is underlain largely by carbonate bedrock, with relatively well-developed karst flow systems. Much of this region is rapidly urbanizing, leading to a variety of potential threats to groundwater including increased and redirected runoff and the potential introduction of contaminants into the subsurface via septic systems, effluent wastewater discharge, and agricultural runoff (Heinz et al.. 2009; Katz et al. 2010). Impacts to groundwater can harm fragile karst ecosystems, but also pose direct threats to the public utilizing groundwater (Johnson et al. 2011). The karst systems within the Ozark Plateaus contain numerous linkages to surface water, with water often repeatedly entering and leaving the subsurface through karst sinking streams and springs. A large percentage of the population of Northern Arkansas utilizes decentralized wastewater treatment systems located within karst terrain. Consequently, threats to groundwater quality are also threats to surface water quality, which is used widely in the region for both drinking water and recreation.

The sites selected for the present study—Blowing Springs Cave (BSC) and downstream receiving surface water, Little Sugar Creek (LSC)—do not currently reside in an ANRC 319 Nonpoint Source Pollution Program priority watershed nor is the LSC or its tributaries listed on the ADEQ 303(d) list; however, there are several reasons for selecting these study sites. The Elk River Watershed (ERW) in which LSC resides, was identified in 1998 as impaired by the Missouri Department of Natural Resources due to excess nutrients primarily related to livestock and population growth. The ERW is bound in the east and west by the White River and Illinois River basins, respectively. Finally, Sugar Creek in MO has been listed on the 303(d) list for impairment related to low dissolved oxygen levels since 2006 though the source has yet to be identified.

Meanwhile, BSC is the site of several past and ongoing scientific studies. Specifically, Knierim et al. (2015) provided over six years of data on the presence of the *Escherichia coli* at the BSC discharge point as well as nitrate and chloride levels from 1992 to 2013. From 2007 to 2013, *E. coli* concentrations at BSC ranged from <1 to 2,420 most probable number (MPN) or colony forming units (CFU) per 100 mL. Median *E. coli* concentrations at base flow periods and during storm events were reported at 41 and 649 MPN or CFU per 100 mL, respectively, and storm event *E. coli* was significantly greater than base-flow concentrations. Based on the data, Knierim et al. (2015) hypothesized that septic tank effluents were a major contributor to chloride, nitrate, and *E. coli* levels in BSC. This hypothesis was largely based on the estimated recharge area for the spring, which was within a residential area that was known to have septic tanks present. Therefore, we selected the sites in the present study to serve as a possible model for how septic tank effluents may move through the subsurface and subsequently impact springs as well as receiving streams via contaminated water as well as resuspension of contaminated sediments.

The objectives of this study were to: 1) explore structure, diversity, and temporal variability of microbial communities in BSC and LSC; 2) differentiate allochthonous bacteria from land surface runoff with bacteria in the sediments and water of the karst aquifer; 3) determine impact of sediment movement from karst springs to LSC through comparison of microbial communities; and 4) delineate the recharge area of BS and constrain potential sources of *E. coli.*

Methods: ***Sample Collection*****.** Routine sampling was conducted in BSC and LSC once per month from March to November of 2016. Samples were collected from three sites along the main stream of BSC and from LSC at four sites,one rural and three within the town of Bella Vista (Figure 1). Water samples consisted of 500 mL grab samples. Sediment samples (10cm depth) were collected using a core sampler or scoop and placed in sterile Whirl-Pak® bags. Two storm events were also sampled at higher temporal resolution, with a threshold precipitation of 0.5 inch in a 24 hour period to trigger a storm sampling series. Storm sampling was conducted during the receding limb with samples taken approximately 1, 2, and 4 days following peak flow.

Dye tracing. A dye tracing test was conducted to better constrain the recharge area of BSC. The hypothesized recharge area for BSC (Knierim et al. 2015) was searched for potential injection sites, and a single prominent sinkhole was identified within the basin. Fluorescein dye was chosen for the tracing experiment to minimize adsorption onto sediment within the sinkhole. Before introduction of dye into the sinkhole approximately 50 gallons of BSC water were dumped into the sinkhole. This was followed by 55 grams of fluorescein dye dissolved in 500 mL of water, and then an additional 450 gallons of spring water. Dye was detected using activated charcoal packets, which were deployed in the field to

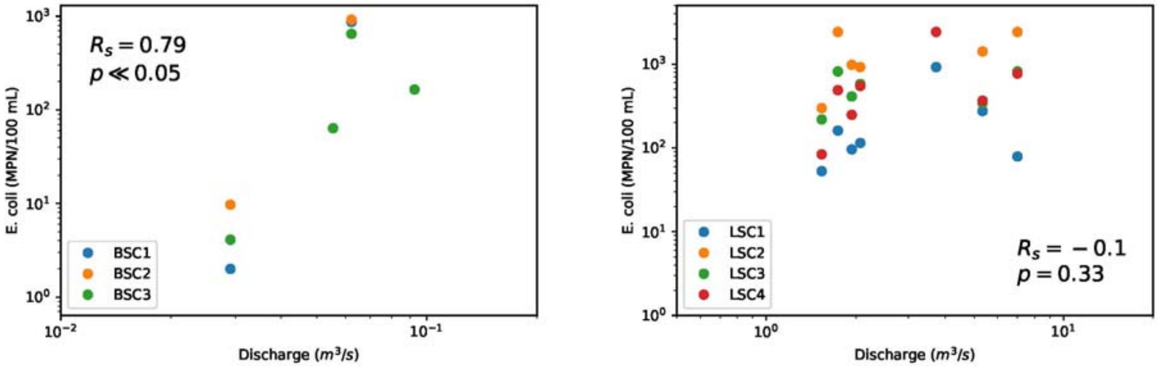

Figure 1. Locations of the sampling points, dDye injection, and charcoal packet deployment. A positive trace was detected from the sinkhole site to Blowing Spring Cave (indicated by arrow), but not at the other monitored sites.

cumulatively absorb dye. Dye was extracted from the charcoal packets in the lab using an alcohol-potassium hydroxide eluent. Elutant was analyzed on a Shimadzu RF-5301 Spectrofluorophotometer. Before injection of dye, charcoal packets were placed in the field to determine any background fluorescence. Charcoal packets were placed in BSC, LSC, and all other nearby springs that were identified. To better determine the timing of the dye pulse, a GGUN-FL24 field fluorometer was deployed in the cave stream.

***E. coli* Analysis.** For detection and enumeration of *E. coli* in water samples, Standard Method 9223B IDEXX Quanti-tray® 2000 system with Colilert™ reagent was used to determine the Most Probable Number (MPN) in each sample. A negative control containing 100 ml of 0.1% peptone was analyzed by Colilert™ for each batch of samples.

***DNA Extraction – Water and Sediments*.** For each sampling event, 200 ml of water from BSC and LSC was filtered through a 0.2-µm, 47mm Supor-200 filter membrane to capture total bacterial cells. Filter membranes were placed at −80°C in 500 µl of guanidine isothiocyanate buffer. The total genomic DNA (gDNA) was extracted from prepared filters using the Fast DNA Spin Kit for Soil (MP Biomedicals). Genomic DNA was extracted from sediment samples as described by Gomes et al. (2007). Total gDNA was quantified using a NanoDrop UV spectrophotometer.

***16S rRNA Metagenomic Analysis*.** Extracted gDNA from water and sediment samples was used as template DNA for amplification of 16S ribosomal RNA (rRNA) gene by polymerase chain reaction (PCR) as described by Kozich (2013). The PCR analysis was completed through the service center at the University of Arkansas under the direction of Program Associate Dr. Si Hong Park. Briefly, forward and reverse primers targeting the 16s rRNA gene including the partial adapter overhang sequence, PCR master mix, and templated DNA were combined in a single PCR reaction well for each sample. The resulting PCR amplicons were verified by gel electrophoresis. 16S rRNA metagenomics for determination of bacterial community structures in water and sediment samples collected from the karst aquifer system (BSC) and receiving surface stream (LSC) over a 9-month period was completed at the University of Arkansas . The high quality sequence reads have been assembled. For data analysis, bioinformatics procedures using QIIME for operational taxonomic unit (OTU) assignment was applied as described by Kozich et al. (2013). Data are currently being analyzed to answer research questions.

Results: Both monthly and rain event water samples were collected at BSC (n=42) and LSC (n=56) (Tables 1 and 2). *E. coli* MPN/100mL ranged from 0.9 to 921 at BSC and 4 to >2419.6 at LSC. *E coli.* concentrations were compared against discharge at both sites (Figure 2). Similar to Knierim et al. (2015), the highest *E. coli* concentrations at BSC in the present study were seen during and following high flow events. The correlation between discharge and *E Coli.* was strong at BSC as quantified using Spearman's rank correlation coefficient ($R_s=0.79$, $p \ll 0.05$). In contrast, LSC showed no statistically significant correlation between discharge and *E coli.* concentrations ($R_s=-0.1$, $p=0.33$). *E. coli* concentrations were statistically higher in LSC than in BSC as indicated by a nonparametric Mann-Whitney U test ($p=0.0013$). The median *E. coli* concentration at BSC was 56 MPN/100 mL, whereas the median at LSC was 120 MPN/100 mL. While *E. coli* concentrations were typically similar at all of the cave sites (Figure 3a), the LSC site located just downstream from Bella Vista Lake (LSC2) frequently had higher concentrations (Figure 3b), with a median value of 380 MPN/100 mL.

Table 1. *E. coli* concentrations (MPN/100 mL) and stream discharge at the Blowing Spring Cave sites.

Date	*E. coli*$_{BSC1}$	*E. coli*$_{BSC2}$	*E. coli*$_{BSC3}$	Q_{bs} (cms)
3/7/2016	1.0	0.9	0.9	0.038
4/4/2016	10.9	12.2	23.3	0.04
5/2/2016	435.2	285.1	290.9	0.097
5/25/2016	63.7	63.7	63.7	0.055
5/26/2016	165.0	165.0	165.0	0.093
5/27/2016	866.4	920.8	648.8	0.062
6/6/2016	143.0	165.8	117.8	0.041
7/11/2016	224.7	209.8	325.5	0.052
8/8/2016	161.6	88.2	88.0	0.052
9/8/2016	4.1	4.1	4.1	0.032
10/5/2016	48.7	48.7	48.7	0.015
10/6/2016	34.1	44.8	35.5	------
10/7/2016	18.3	18.9	24.3	------
11/10/2016	2.0	9.7	4.1	0.029

Table 2. *E. coli* concentrations (MPN/100 mL) and stream discharge at the Little Sugar Creek sites.

Date	*E. coli*$_{LSC1}$	*E. coli*$_{LSC2}$	*E. coli*$_{LSC3}$	*E. coli*$_{LSC3}$	Q_{lsc} (cms)
3/7/2016	22.7	45.3	15.4	22.7	2.41
4/4/2016	22.8	116.2	4.1	12.2	4.08
5/2/2016	137.6	86.0	100.8	93.2	7.40
5/25/2016	920.8	2419.6	2419.6	2419.6	3.73
5/26/2016	78.9	2419.6	816.4	770.1	7.00
5/27/2016	275.5	1413.6	344.8	365.4	5.34
6/6/2016	61.3	23.5	73.8	124.6	4.79
7/11/2016	36.4	461.1	113.7	41.4	7.84
8/8/2016	30.5	58.3	75.4	13.0	4.34
9/8/2016	1413.6	106.1	125.9	31.5	1.06
10/5/2016	160.7	2419.6	816.4	488.4	1.74
10/6/2016	95.9	980.4	410.6	248.1	1.94
10/7/2016	114.5	920.8	579.4	547.5	2.07
11/10/2016	52.8	298.7	218.7	83.9	1.54

Although metagenomic data analysis is still ongoing, there are some observations that can be reported. Figures 4a and 4b show the genus level results for water and sediment samples from the different sampling sites in BSC and LSC during a routine sampling event on 5/2/2016. The most abundant bacterial genus in water samples was *Acinetobacter*--a Gram negative bacteria commonly found in soil and water--followed by *Pseudomonas* and *Flavobacterium*, again both common to the soil and freshwater environments (Figure 4a). The family *Enterobacteriaceae* which includes *E. coli* is also represented at most water sampling locations though at lower percentages. With respect to sediment collected during the same routine sampling event, the microbial make up is quite different than paired water samples across all sampling sites (Figure 4b). The major bacterial families identified in sediment were *Bacillaceae* and *Enterobacteriaceae*, and one of the primary genera detected was *Clostridium.* The family *Bacillaceae* includes *Bacillus*, a microbe ubiquitous in nature. Meanwhile, *Clostridium* is also a soil microbe as well as an inhabitant of the intestinal tract of animals, including humans.

Samples were also analyzed by sample type for beta diversity which is the diversity of microbes between samples within a specific group. The weighted principal coordinate analysis (PCoA) UniFrac plot

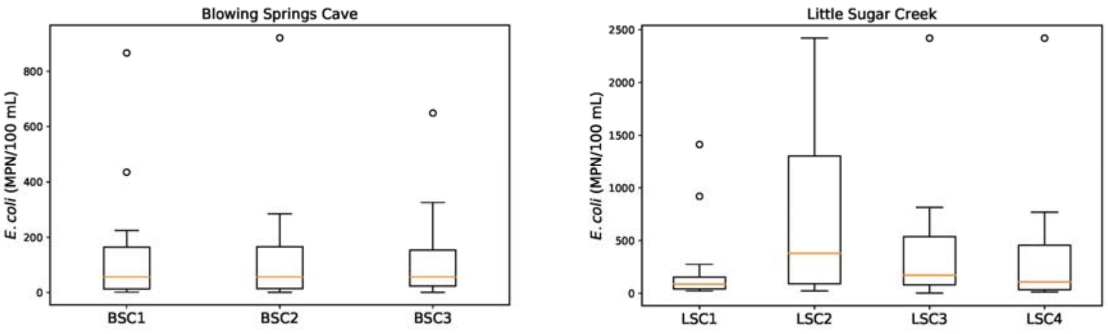

Figure 2. Discharge versus *E. coli* concentrations in Blowing Spring Cave (a) and Little Sugar Creek (b) during the study period. BSC1 is the site that is furthest downstream within the cave, and BSC3 is furthest upstream. LSC1 is the site that is furthest upstream, and LSC4 is furthest downstream. Spearman rank correlation coefficients (R_s) indicate that there is a strong positive correlation between *E coli.* and discharge at BSC, but there is no statistically significant correlation at LSC.

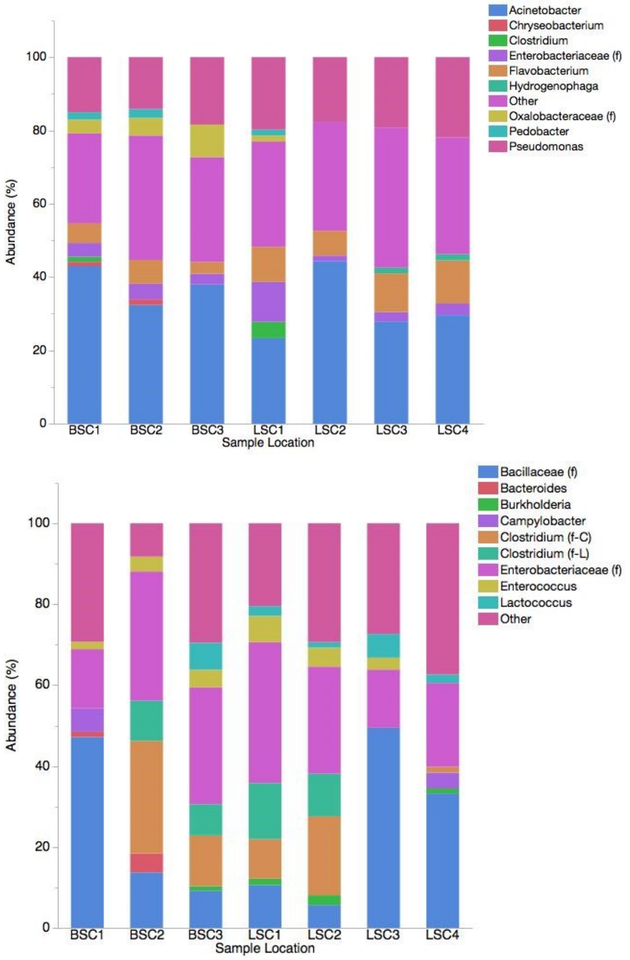

Figure 3. Boxplots of *E. coli* concentrations at: a) the three sites within Blowing Spring Cave from downstream (BSC1) to upstream (BSC3), and b) the four sites within Little Sugar Creek. Boxes indicate the median and quartile values and whiskers represent the range. Circles depict outliers, which are data points that lie outside of the box by more than 1.5 times the interquartile range. Note that the y-axis range on the Little Sugar Creek plot is much larger than on the Blowing Spring plot.

shown in Figure 5 illustrates the level of abundance of operational taxonomic units (OTUs) among sample types and their respective phylogenetic distances. In Figure 5, each data point representing an individual sample was aligned in parallel on the PC1 axis with 38.68%. A R value close to 1 was used to indicate that there was dissimilarity among sample type while an R value near 0 meant no separation. An R value from the weighted PCoA plot was 0.71 which implied a significant dissimilarity among water and sediment samples regardless of location or event type.

Fluorescein dye (55 grams) was injected into the sinkhole site on February 27, 2017 during a relatively dry period. Following heavy rains, dye was detected at Blowing Spring within a charcoal packet that was deployed from March 13-27, 2017. Additionally, a fluorescein pulse was detected on the field fluorometer on March 25, 2017. This suggests a travel time of approximately 26 days over a straight-line distance of 1100 m, giving an average velocity of roughly 40 m/day. There were no positive detections at the other monitored sites. This trace confirms a positive connection between BSC and a portion of the recharge area hypothesized by Knierim et al. (2015) that lies within a residential area that contains some remaining septic tanks.

Conclusions, Recommendations and Benefits: Even though Little Sugar Creek (LSC) receives

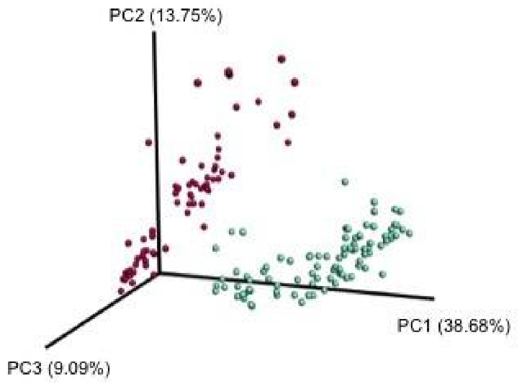

Figure 4. Relative abundance of major bacteria across the various sampling locations at the genus level in water (a) and sediment (b) collected on 5/2/2016. f in parenthesis indicates family, while f-C indicates family *Clostridiaceae* and f-L indicates family *Lachnospiraceae*--two families containing the genus *Clostridium*.

contributions from numerous karst springs, such as Blowing Spring, the *E. coli* dynamics at the two sites are quite different, with concentrations at BSC displaying a strong positive correlation with discharge, and LSC showing no statistically significant correlation. LSC frequently shows *E. coli* concentrations above the primary contact limit (410 CFU/100 mL) and sometimes above the secondary contact limit (2050 CFU/100 mL), indicating potential concerns for recreational users of the stream. The lack of correlation with discharge suggests that introduction of *E. coli* into the stream is not strongly linked with runoff, and that the sources are different than in BSC, where the contamination is hypothesized to result from septic tanks in the recharge area (Knierim et al. 2015). Concentrations just downstream of Bella Vista Lake (at LSC2) are particularly high, suggesting a source near that reach of the stream. The analysis of the microbial data is ongoing, but it is clear that the microbial communities within the water and sediment are significantly different. Further analysis will explore differences between BSC and LSC as well as changes in communities over time. This study provides insight into the microbial dynamics of karst spring and surface waters within a mixed urban and agricultural setting, where much of the population relies on decentralized wastewater treatment. This combination of geology and land use is common throughout the Ozark Plateaus and more widely throughout the southern and eastern United States. Therefore, insight gained here is likely to apply widely across the region.

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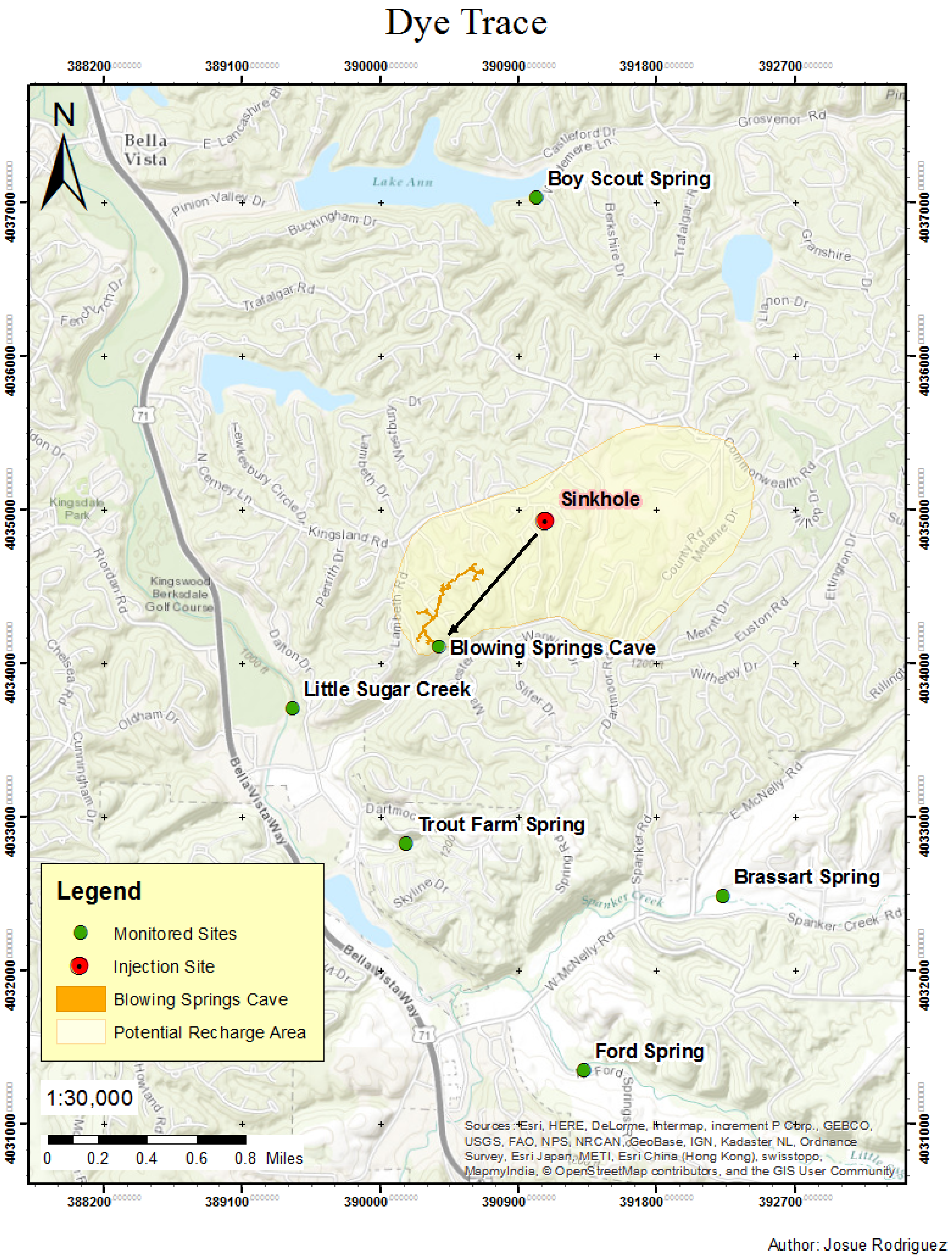

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Figure 5. Beta diversity analysis among sample type, water (mint green) and sediment (cranberry red). Weighted principal coordinate analysis (PCoA) Unifrac plot of individual samples for each sample type.

Project Title: Investigating Fate of Engineered Nanoparticles in Wastewater Biofilms
Project Number: 2016AR385B
Start Date: 3/1/2016
End Date: 2/28/2017
Funding Source: 104B
Congressional District: 003
Research Category: Engineering
Focus Category: Wastewater, water quality, treatment
Principal Investigator: Wen Zhang

Publications and Presentations:

Walden, Connie and Zhang, Wen. Investigating Fate of Silver Nanoparticles in Model Wastewater Biofilm. Poster presented at Arkansas Water Resources Center Annual Conference. Fayetteville, AR. 2016.

Walden, Connie and Zhang, Wen. Investigating Fate of Engineered Nanoparticles in Wastewater Biofilm. Department of Civil Engineering Seminar. University of Arkansas. 2016.

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Connie Walden; Advisor: Wen Zhang, PhD expected in August, 2017; Civil Engineering, College of Engineering, University of Arkansas, Fayetteville, AR.

Investigating Fate of Engineered Nanoparticles in Wastewater Biofilms

Connie Moloney Walden, Department of Civil Engineering, University of Arkansas
Wen Zhang, Department of Civil Engineering, University of Arkansas

Core Ideas:

- Silver nanoparticles can attach to model wastewater biofilm without significantly impacting biofilm biomass.
- Wastewater biofilm can become stressed under exposure to 1 mg L^{-1} of silver nanoparticles.
- By applying a mass balance, model biofilm *Comamonas testosteroni* was observed to accumulate 0.172 ng mm^{-2} of silver nanoparticles.

Executive Summary:

Engineered nanoparticles incorporated into consumer products have shown to negatively impact vital ecosystems once released into the environment. As wastewater reuse practices become increasingly necessary in areas of water scarcity, innovative wastewater treatment applications will be required. Attached growth (*i.e.* biofilm) processes for wastewater treatment generate less waste and are easier to operate compared to activated sludge. This study examines the interaction between silver nanoparticles (Ag-NPs) and wastewater biofilms. Two bench scale reactors were used to examine the impact of Ag-NPs on model biofilm, as well as the attachment of Ag-NPs to biofilm. The insights provided offer a basis for understanding the removal capabilities of Ag-NPs from wastewater through biofilm processes.

Introduction:

The application of silver nanoparticles (Ag-NPs) has expanded exponentially within manufactured products such as food packaging, cosmetics, and textiles (Boxall, Chaudry et al. 2009). Reuse of treated wastewater for various purposes such as drinking water, irrigation water, and/or cooling water is now a reality and will continue to increase as traditional freshwater sources become progressively stressed. Although Ag-NPs have previously been referred to as emerging contaminants, their presence is now a long-term issue that might have damaged vital microbiological ecosystems (de Faria, de Moraes et al. 2014). By modeling the fate and transport of Ag-NPs, environmentally relevant quantities will vary depending on location type. These concentrations are predicted generally in the range of 0.003 – 100 ng L^{-1} (Mitrano, Barber et al. 2012). Wastewater treatment plants, an important barrier between consumers and their surroundings, are not designed specifically for the removal of Ag-NPs (Walden and Zhang 2016). As wastewater influent complexity increases, treatment plants should be re-evaluated for their processing efficiency. Likewise, as competing demands increase upon limited freshwater resources, reuse practices of treated wastewater will increase across the United States, including Arkansas. Consequently, there is a pressing need for economical yet effective regionalized wastewater treatment. Biofilm systems (Figure 1) are easy to maintain and convenient for small communities. Here, we investigated the role of wastewater biofilms in the removal of Ag-NPs from waste streams. The goal of this proposal investigated the following hypotheses: *(1) ENPs within wastewater can attach to biofilms without significantly altering nutrient reduction capacity; (2) under certain steady-state parameters, biofilms can become an environmental sink for*

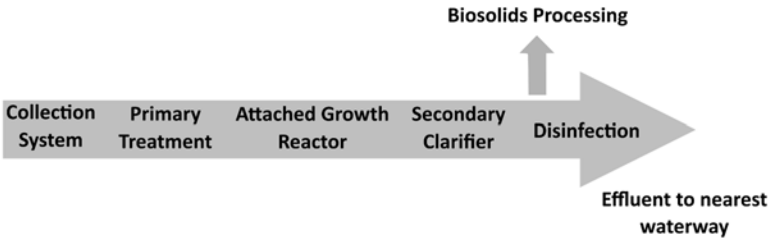

Figure 1. Representative schematic of a typical attached growth wastewater treatment plant.

ENP to accumulate within the extracellular polymeric substances (EPS); and (3) biofilm will become a potential source for ENP release as the wastewater environment fluctuates over time. Ag-NPs were exposed to model wastewater bacteria *Comamonas testosteroni* in two differently sized bench scale reactors for Ag-NP impact on biomass and removal from suspension. Ongoing work will explore dual and mixed species combinations with additional bacteria *Acinetobacter calcoaceticus* and *Delftia acidovorans* (Andersson, Kuttuva Rajarao et al. 2008).

Methods:

Experimental design.

The three species were first tested for biofilm forming capacity. A biofilm formation assay was conducted in a clear 96 well plate with 2% crystal violet as previously described (Djordjevic, Wiedmann et al. 2002, O'Toole 2011). A control experiment was conducted for 28 days to observe the time for a mature biofilm to form within the CDC biofilm reactor (BioSurface Technologies, Bozeman, MT), and to monitor biological reduction capacity in the absence of Ag-NPs. A non-limiting synthetic wastewater inoculated with *D. acidovorans* was fed and recycled through the CBR as nitrate, phosphate, sulfate, chlorides, COD, and pH were monitored. Shorter experiments with *C. testosteroni* used as a feed into the CBR and the custom flow cell were also performed for 48 hours. For the shorter experiments, the feed was switched to sterile synthetic wastewater to remove planktonic cells from the system. Then, biofilm was exposed to a spike of about 1 mg L^{-1} Ag-NPs (CBR) and 2 mg L^{-1} (flow cell) for 30 minutes.

Reactor descriptions and setup.

The CBR is a 1 liter glass beaker with a polyethylene lid which holds 8 polyethylene rods, each with three removable polyethylene coupons serving as an attachment site for biofilm growth. The working volume is about 350 mL. The custom flow cell holds three removable polyethylene coupons, and has a working volume of about 2 mL. The synthetic wastewater consisted of nutrient broth (300 mg L^{-1}), KH_2PO_4 (44 mg L^{-1}), NaOH (16.7 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (132.4 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (100 mg L^{-1}), $C_6H_{12}O_6$ (140 mg L^{-1}), KNO_3 (3 mg L^{-1}), $NaHCO_3$ (175 mg L^{-1}), $MnSO_4{\cdot}7H_2O$ (12.8 mg L^{-1}), $(NH_4)_2SO_4$ (118 mg L^{-1}), and $FeCl_3{\cdot}6H_2O$ (5 mg L^{-1}). The CDC biofilm reactor (CBR), flow cell, connectors/tubing, and synthetic wastewater solution were autoclaved at 121°C for 30 minutes prior to each experiment (Model 522LS Gravity Steam Sterilizer, Getinge, New York). The experimental setup (Figure 2) included the CBR or flow cell connected to a peristaltic pump set at 10 and 1 mL min^{-1} flow rate, respectively.

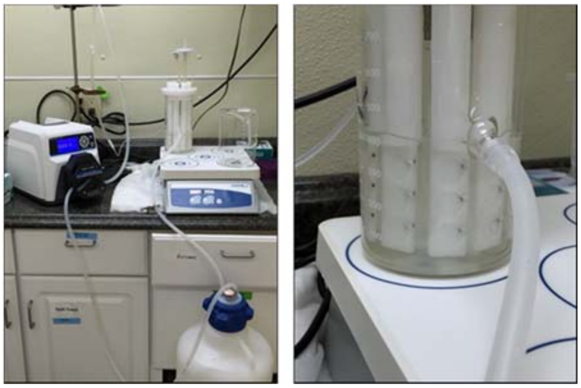

Figure 2. (left) The experimental setup included a peristaltic pump and autoclavable tubing to circulate synthetic wastewater through the CDC biofilm reactor (CBR). **(right)** A close up shows the detachable polyethylene sampling coupons suspended in the CBR for biofilm testing.

Biofilm analysis with CBR.

Biofilm amount was determined from Hoescht 33342 cell stain with an upright confocal fluorescence microscope (Nikon Eclipse Ni-E upright microscope, Nikon Instruments, Melville, New York). For biofilm stress, a modified dichlorofluorescein (DCF) assay was used as previously described in black-sided clear bottomed 96-well plates (Corning 3603, Corning, MA) and analyzed on a microplate reader (Synergy H1 Multi-Mode Microplate Reader, Biotek Instruments, Inc., VT) (Wang and Joseph 1999).

Biofilm analysis with flow cell.

The flow cell system has the advantage of a smaller working volume than the CBR, allowing for quick biofilm formation and simple mass balance measurements. *C. testosteroni* was recycled through the flow cell for 48 hours to establish a mature biofilm. Then, sterile synthetic wastewater was pumped through for 10 minutes to eliminate any planktonic cells. 2 mg L^{-1} Ag-NPs were aseptically injected into the cell. After 30 minutes, sterile wastewater was used to flush the flow cell of any unattached Ag-NPs for 10 minutes. All effluent was retained and analyzed for total volume and total silver concentrations. All effluent was collected in sterile centrifuge tubes for mass balance measurements. To remove biofilm from the coupon for ICP-MS, each coupon was aseptically removed from the flow cell and inserted into a sterile tube with 5 mL of DDI water. The tubes were vortexed for 5 minutes. The coupon was removed, and the total volume was brought up to 10 mL total volume and acidified with 2.5% nitric acid for ICP-MS. The concentration of silver ion was measured by centrifugal filtration and ICP-MS.

Silver synthesis.

Silver nanoparticles were formed using sodium borohydride to reduce silver nitrate with sodium citrate as a capping agent (Mulfinger, Solomon et al. 2007). All glassware was washed with phosphorus free detergent, rinsed three times with tap water, then rinsed three times with deionized water (Elga Process Water System (18.2 $M\Omega \cdot cm^{-1}$) Purelab flex, Veolia, Ireland). The reduction of silver nitrate occurred as follows:

$$AgNO_3 + NaBH_4 \rightarrow Ag + 0.5H_2 + 0.5B_2H_6 + NaNO_3$$

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^o + C_6H_5O_7H_3 + 3Na^+ + H^+ + O_2$$

The formation of Ag-NPs was confirmed by scanning the absorbance from 300 – 700 nm with a UV-vis spectrophotometer (Beckman Coulter, CA, USA.). The concentration of Ag-NPs was measured with ICP-MS. Particle size was verified with TEM (Jeol, USA) and DelsaNano (Beckman Coulter, Life Sciences, USA).

Statistical analysis.

All statistics and plots were generated in SigmaPlot (Systat Software, Inc., version 12.5) where statistic p values less than 0.05 were considered significant.

Results:

Biofilm formation assay. The capability to form biofilm was investigated for the bacteria combinations discussed using a crystal violet microtiter 96-well plate assay. For all single and multiple combinations with these species a strong biofilm was formed. Of the three single assays, *A. calcoaceticus* forms a significantly stronger biofilm than *C. testosteroni* or *Delftia acidovorans* (Figure 3, $p < 0.05$). There was no significant difference between the assay of all three mixed and the assay of *A. calcoaceticus* & *D. acidovorans*. (Stepanovic, Vukovic et al. 2000, O'Toole 2011).

Nutrient reduction capacity. The CBR setup as a closed system with recycle was inoculated with *D. acidovorans;* nitrate, phosphate, sulfate, chlorides, COD, and pH were monitored to test for nutrient changes without Ag-NPs present. Minimal or no change was observed for nitrate, phosphate, sulfate, chlorides and pH. COD was reduced to approximately 18.8 mg L^{-1} from above detection limit after 10 days. We concluded that the quantity of biofilm formed within this reactor type with single species *D. acidovorans* is not sufficient for nutrient reduction testing.

Silver nanoparticle formation.

The Ag-NPs exhibited the expected UV-vis peak at 395-400 nm for nano-sized silver. The average particle size from photon correlation spectroscopy was 7.9 nm, and confirmed with TEM (Figure 4). ICP-

MS measured a stock solution concentration of 76 mg L^{-1}, with less than 10% ionic silver present. This stock was stored in the dark and verified as unchanged with UV-vis at each use.

CBR experiment.

In the CBR system, *C. testosteroni* exhibited insignificant change in biomass after Ag-NP exposure (*p*=0.1323). This is consistent with previous conclusions that wastewater biofilms are tolerant to toxic loadings. However, reactive oxygen species present reflected significant cell stress after the 30-minute treatment (Figure 5, p = 0.0132). The CBR experiment addresses the first hypothesis that Ag-NPs can attach without significantly altering biomass.

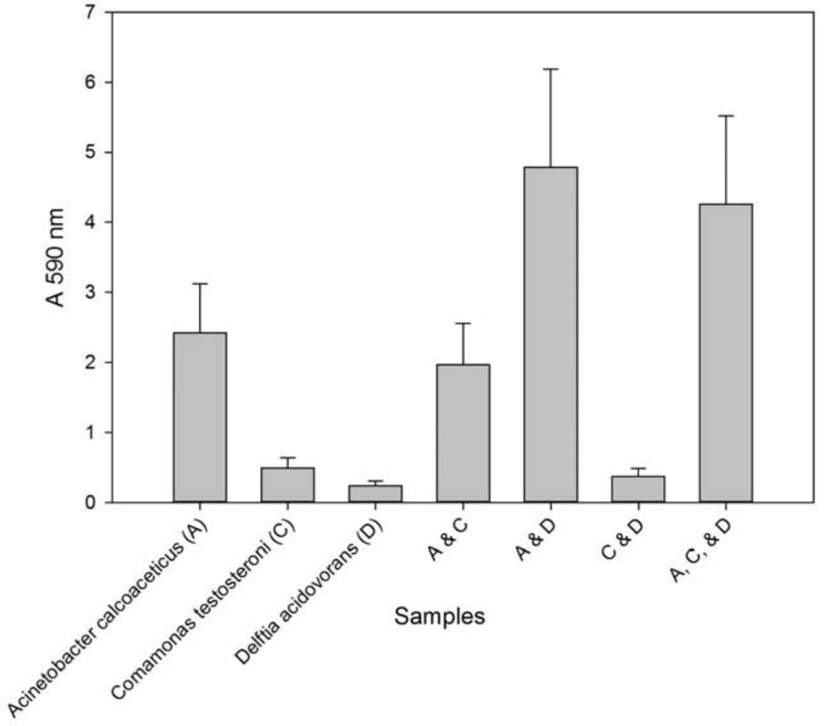

Figure 3. Biofilm formation assay results from crystal violet staining with standard error (n=3) for each species singly, duel, and mixed. A greater absorbance reflects increased ability to form biofilm.

Flow cell experiment.

The amounts of Ag-NPs per coupon (Table 1) were all less than 0.1 ng mm^{-2}. The total silver recovered from biofilms was 0.172 ng mm^{-2}. This is a first step toward proving the second hypothesis that biofilms can become a sink for Ag-NPs.

Future work. To complete the proposed investigation, several tasks are anticipated for completion by August 2017. The CBR nutrient reduction experiment will be completed with the remaining single, duel, and mixed species combinations. The differences in biofilm formation ability may impact the nutrient reduction capacity in control experiments. We anticipate different results for strong biofilm formers.

Flow cell experiments will be completed with the remaining species *A. calcoaceticus and D. acidovorans* as pure culture. The dual and mixed combinations will follow. These results will complete the second hypothesis in our investigation.

To measure accurately the attachment and release of Ag-NPs to each of these species, we will be using a quartz crystal microbalance with dissipation (QCM-D). Unlike fluorescent microscopy, QCM will allow quantitative measurement of changes in mass (ng) on a quartz crystal sensor. QCM-D measures the addition of mass on the surface by changes in the resonance frequency and the viscoelastic properties of liquid in contact with the sensor. This sensitivity will provide essential quantification of attachment, accumulation, and released quantities of Ag-NPs with each species combination. This will address our final question of biofilm being a source of Ag-NP release.

Conclusions, Recommendations and Benefits:

Model wastewater biofilm shows potential to resist acute exposure to environmentally relevant quantities of Ag-NPs. Further, this model biofilm can accumulate Ag-NPs into its biofilm structure. This fundamental look at the Ag-NP – biofilm interactions shows minimal potential for Ag-NP accumulation. However, the resistance to detachment in the presence of Ag-NPs shows the capability of even a single wastewater type species to tolerate toxic loadings. We recommend continuing this work with other model species and a more complex biofilm community.

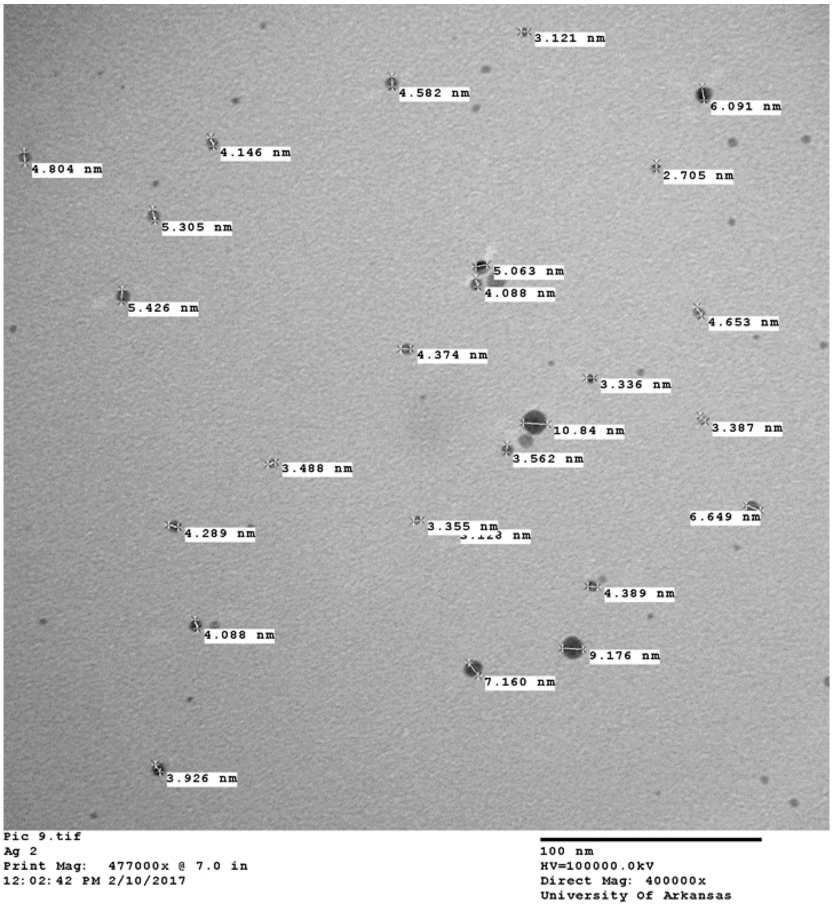

Figure 4. Transmission electron microscope image of silver nanoparticles (Ag-NPs) verifying the formation of nano-sized particles. Embedded within the image are

Although ENPs have been commonly referred to as 'emerging' contaminants, the presence of ENPs is now a persistent and long term issue that may have already damaged vital microbiological ecosystems. The goal is to explore realistic environmental conditions in wastewater biofilm systems that control the removal and release of potentially toxic ENPs (silver nanoparticles, Ag-NPs), thereby establishing the fundamental groundwork that will enable innovative use of biofilm processes in wastewater treatment for water reuse and recycling in areas of water scarcity. By investigating water supply and quality problems, this research directly addresses the goals of the AWRC. Likewise, by exploring issues that are of immediate concern in arid and semi-arid climates, this research furthers the U.S. Geological Survey's national water mission to increase knowledge of water quality and quantity. The United States Environmental Protection Agency (EPA) published many examples of current water reuse practice in Region 9 district (serving Arizona, California, Hawaii, Nevada, Pacific Islands and Tribal Nations), and reuse will continue to increase as traditional fresh water sources become increasingly stressed (Fachvereinigung Betriebs- und Regenwassernutzung e 2005).

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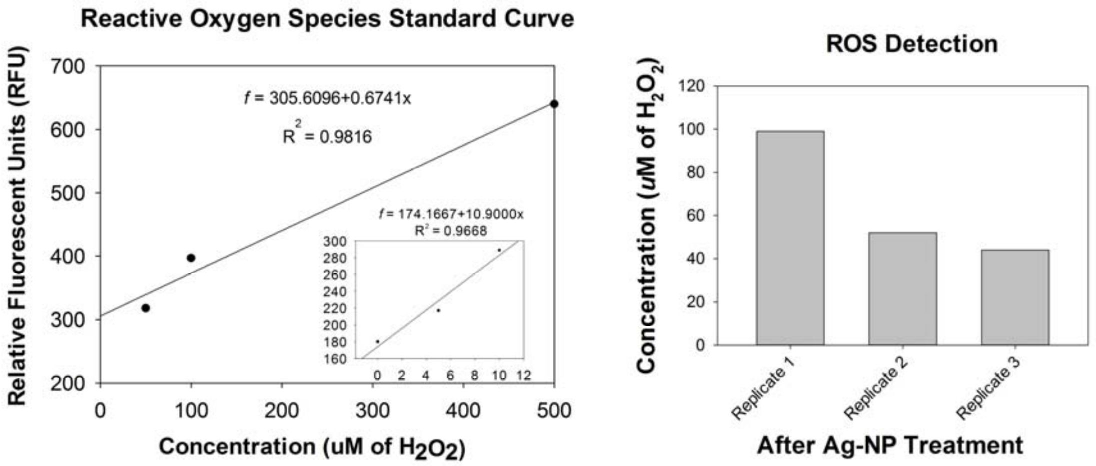

Figure 5. **(a)** RFU measurements were converted to concentration from the standard curve. **(b)** Reactive oxygen species measurements after a 30-minute exposure to 1 mg L^{-1} Ag-NPs.

Table 1. The amount of silver retained on each coupon removed from the flow cell measured by ICP-MS.

Silver Mass Balance

	Ag-NPs per coupon (ng/mm^2)	Ag-NPs per coupon (ng/mm^2)	Ag-NPs per coupon (ng/mm^2)	Total Silver in Biofilms (ng)	Percent Recovered	Total Silver Accumulation (ng/mm^2)
Control (No Biofilm)	-	-	-		104.0	-
Comamonas testosteroni	0.0906	0.0531	0.0281	21.8	91.7	0.172

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Project Title:	Tracking the growth of on-site irrigation infrastructure in the Arkansas Delta with remote sensing analysis
Project Number:	2016AR386B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	003
Research Category:	Social sciences
Focus Category:	Water quantity, irrigation, economics
Principal Investigator:	Kent Kovacs

Publications and Presentations:

West, Grant H., Rachael M. Moyer. 2016. "The Impact of Agency Actors' Value Predispositions on the Perceived Effectiveness of Water Conservation Policies in Arkansas." In 2016 Annual Meeting, Nov. 3-5, 2016, Washington D.C. Association of Public Policy Analysis and Management.

West, Grant H., Rachael M. Moyer. 2016. "The Impact of Agency Actors' Value Predispositions on the Perceived Effectiveness of Water Conservation Policies." In 2017 Annual Meeting, April 6-9, 2017, Chicago, IL, Midwest Political Science Association.

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Tracking the Growth of On-site Irrigation Infrastructure in the Arkansas Delta with Remote Sensing Analysis

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Core Ideas:

- Publicly available imagery can identify on-farm surface water storage built in Eastern Arkansas.
- The algorithm developed to identify the facilities for surface water storage identifies more than 97% of verified reservoirs.

Executive Summary:

Surface water impoundments built on farms to store water in the wet season for irrigation later in the year are one approach to reduce groundwater pumping and to sustain aquifers. However, there is limited information on where and how many of these reservoirs are present in Eastern Arkansas. This information would be useful to formulate effective policies to encourage the construction of more surface water systems. Analysis of Landsat imagery from 1995 to 2015 provides evidence for where and when reservoirs and tail-water recovery systems are present, doing so with annual resolution. Comparing our analysis – which extends the Dynamic Surface Water Extent (DSWE) algorithm for Landsat to identify irrigation storage reservoirs in Arkansas County – to the verified locations of these surface water impoundments, the analysis identifies nearly 98% of all reservoirs in the verified study area.

Introduction:

The sustainability of the Mississippi River Valley Alluvial Aquifer (MRVA) is vital to maintaining long-term agricultural profitability in Arkansas (Maupin and Barber, 2005; Konikow, 2013). The extent of the aquifer includes seven states, and Arkansas is the largest consumer of water from the aquifer (Maupin and Barber, 2005). Although Arkansas has often been considered an area rich in water resources with annual precipitation amounts ranging from approximately 50 to 57 inches (NOAA, 2014), there are several key constraints to maintaining agricultural profitability in the region. The first is lack of timely rainfall, and the second is the increasing need for irrigation. The number of irrigated acres continues to increase in Arkansas in order to maintain and increase yields and mitigate risk as a result of recurring drought conditions (Vories and Evett, 2010). Moreover, most irrigated acres result from producers privately funding the installation of irrigation wells that draw groundwater from the MRVA. It is known that the current rate of withdrawals from the aquifer is not sustainable, especially as the number of irrigated acres continues to increase each year (Barlow and Clark, 2011; ANRC, 2012; Evett et al., 2003).

The Agricultural Act of 2014 (or 2014 U.S. Farm Bill) introduced the Regional Conservation Partnership Program (RCPP) which consolidated several programs including the Mississippi River Basin Healthy Watersheds Initiative, Environmental Quality Incentives Program (EQIP) and the Conservation Stewardship Program (CSP), in order to promote coordination between Natural Resources Conservation Service (NRCS) and its partners and provide technical and financial assistance to producers and landowners. These federal and state programs encourage more efficient and effective irrigation and have contributed to the voluntary implementation of water conservation practices such as tail-water recovery ditches, on-farm storage reservoirs, and use of sensor technologies, to name a few. Despite the prevalence of programs that are targeted to help farmers sustainably manage agro-ecosystems in Arkansas, the level of information about the use of these management practices and technologies is less than ideal and can be improved significantly. We do not yet know how much adoption of water conservation measures has already occurred and to what extent these various water conservation measures reduce pumping pressure on the MRVA. This lack of knowledge is a pressing problem, especially as federal incentive programs face increased public scrutiny. We need to determine if conservation practices are effective at reducing groundwater declines in the MRVA and also which practices are most frequently adopted and retained by farmers.

While the National Agricultural Statistic Service (NASS) does collect some data on water conservation practices, they depend on problematic sampling techniques when only a small proportion of producers use a practice, which is the case for on-site water storage and tail-water recovery. Further, NASS data do not disclose the location of the producer adopting a practice, and this prevents a full assessment of available surface water and what spatial features of the landscape might have caused the producer to adopt the practice.

The objective of this research is to understand the construction of on-site water storage and tail-water recovery systems over time in the critical groundwater area of Arkansas County. Using various sources of multispectral imagery and aerial photography, we aim to identify and map the spatial extents of on-site water storage and tail-water recovery in the area and to attribute construction dates in a GIS database layer.

Methods:

Data

Because of its continuous operation over the last several decades and its frequent return times, Landsat satellite imagery was used to track the construction of on-site irrigation storage reservoirs. Using the United States Geological Survey (USGS) EarthExplorer tool, we acquired all Landsat scenes overlying a study area of Arkansas County, Arkansas between January 1995 and December 2015. Landsat data are multispectral images with a spatial resolution of 30 meters and a return time of 16 days. Landsat-based methods for identifying on-site water storage are cost-effective, time-efficient, reliable, and easily repeatable.

Water Identification

In order to make the initial classification of all surface water we use the Provisional Dynamic Surface Water Extent (DSWE) algorithm developed by USGS (Jones and Starbuck, 2015; Jones, 2015). The identified scenes were pre-processed using the provisional DSWE algorithm which classifies water and non-water pixels in the Landsat imagery according to their surface reflectance and slope characteristics. Primary inputs to the algorithm are a Digital Elevation Model (DEM) and the Landsat reflectance bands for Blue, Green, Red, NIR, SWIR1, and SWIR2, along with the CFMASK band used to filter cloud and cloud shadow (Jones and Starbuck, 2015).

Extending the Algorithm for Reservoir Identification

Using Python and the arcpy library, all non-water pixels, including cloud and shadow, were reclassified to a value of “0” while all pixels identified as water were assigned a value of “1”. This was done for each scene between 1995 and 2015. With only surface water pixels containing values, we use TerrSet Geospatial Monitoring and Modeling software in combination with Python to apply filters based upon size and shape characteristics. Using TerrSet’s *Group* function, clusters of water pixels were identified as bodies of water and all pixels in a water body were assigned an ID value for that body of water. The *Area* and *Perim* functions calculated the area and perimeter of each grouped and identified water body, assigning these values to each pixel in a group. We characterize shape using a measure for compactness ratio and TerrSet's *cratio* function. Using the area and perimeter layers as inputs, the *cratio* function calculates the square root of the ratio of the area of the polygon to the area of a circle having the same perimeter as that of the polygon. This value is assigned to each pixel in a group.

We use Python and the *arcpy* library to filter out bodies of water with size and shape traits that are uncharacteristic of on-site irrigation storage reservoirs. Data on the characteristic size of reservoirs were obtained from both a 2016 survey (Edwards, 2016) and communication with Charolette Bowie of the USDA Natural Resources Conservation Service (NRCS) in Lonoke, Arkansas. The USDA-NRCS administers the EQIP program and maintains records on the construction of irrigation reservoirs under the cost-share program. Based on the information obtained from these sources, bodies of water smaller than 2.5 acres and larger than 600 acres were removed from all scenes.

Features with a high compactness ratio have a high likelihood of being man-made (McKeown and Denlinger, 1984). Because some of the constructed reservoirs do have organic, natural, shape qualities, we apply a minimal level of filtering based upon compactness. We do this primarily to eliminate streams and rivers with the lowest compactness ratios. Bodies of water with a compactness ratio less than .005 were removed from all scenes. For each scene, we executed a *BooleanAnd* operation, keeping surface-water pixels that satisfied both the area and compactness criteria. The results of this operation represent potential reservoirs in each individual scene.

The three-month period of March, April, and May is the wettest period of the year, and being prior to the growing season, irrigation storage reservoirs are likely to be most full. Interpreting Landsat scenes in these months is complicated by the presence of cloud cover (Kaufman, 1987; Ju and Roy, 2008). Due to this, we created a composite of probable reservoirs for the period (March – May) by taking the *union* of all algorithm-processed scenes within the calendar period, doing this for each year (1995 – 2015). Compositing of Landsat images provides a method for addressing data gaps resulting from cloud cover (Roy et al., 2010; Wulder et al., 2011). Probable reservoirs missing in one scene due to cloud cover are likely to be captured in the composite by another scene. Figure 1 summarizes the extended algorithm, while supplemental material reports the Landsat scenes used in constructing each of the annual composites.

Verification and Construction of Annualized Reservoir Data Layer

High-resolution imagery from the National Agriculture Imagery Program (NAIP) and Google Earth were necessary to identify tail-water recovery ditches and verify the presence of irrigation storage reservoirs. Mary Yeager and Michele Reba with USDA Agricultural Research Service (USDA-ARS) recently used these imagery sources and manual methods to identify and map irrigation storage reservoirs and tail-water recovery ditches for 2015 in the Cache and Grand Prairie areas, including Arkansas County. Though Yeager and Reba were not able to produce an annualized data layer, they do use NAIP imagery and historical imagery from Google Earth to verify reservoirs for each of the years 1996, 2000, 2006, 2009, 2010, and 2013, in addition to 2015.

We use this layer to assess the accuracy of reservoir identification for our extension of the DSWE algorithm and to aid in verifying annual reservoir locations. For each year verified manually, reservoir extents were compared to annual composites from the matching year. We also construct an annualized reservoir data layer using the annual composites, verified years, and some cases of deductive reasoning. We create Boolean identifiers in a GIS data layer to indicate the presence of a reservoir in a given year from 1995 to 2015.

Results:

Table 1 reports the results of the algorithm accuracy assessment using manually verified years. The percentage of the manually verified reservoirs that were identified by matching annual composites ranged from 97.5% to 99.2% for the seven years included in the assessment. The most accurate composite was 2013 where 247 of 249 reservoirs were identified by the algorithm. The composite for 2015 failed to identify the largest number of reservoirs, missing six, though it was not the least accurate by percentage.

Between 2000 and 2006, the number of reservoirs increased by 30 which is the largest increase between verified years. It is also the longest period of time without available high-resolution imagery. Due to the ground-truth requirements that necessitated a time extension on the grant, the annualized reservoir data layer is not yet complete at the time of reporting. When complete, it will provide annual resolution to the growth in on-site irrigation storage infrastructure.

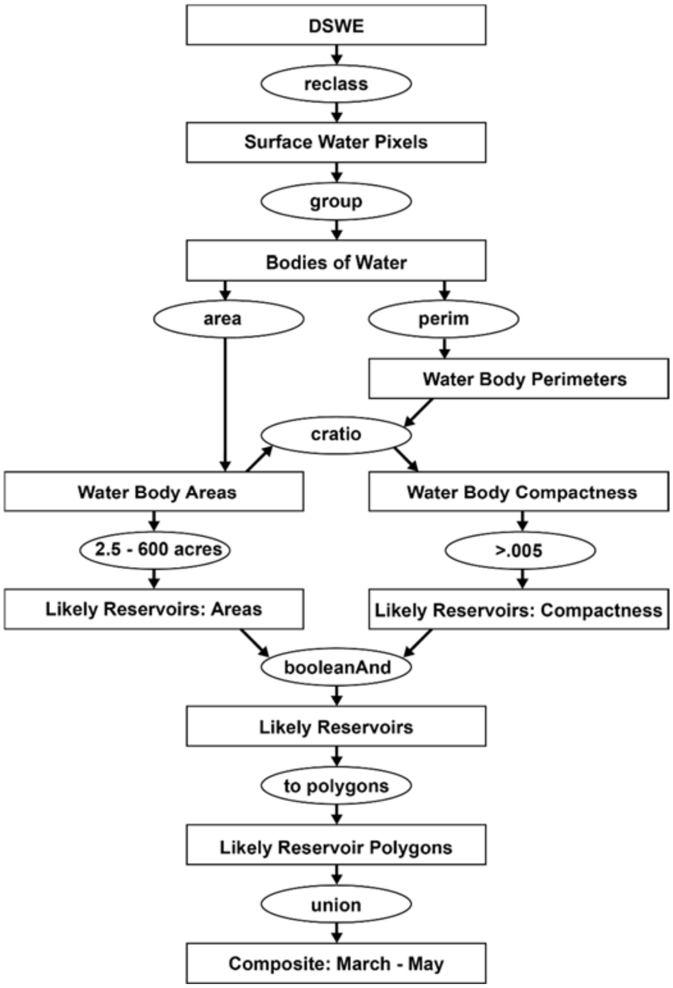

Figure 1. Reservoir Identification Algorithm. This summarizes the algorithm used to process Landsat scenes for identifying irrigation storage reservoirs. It takes scenes processed using the U.S. Geological Survey's Provisional Dynamic Surface Water Extent (DSWE) algorithm and extends that using spatial and temporal constraints (Jones and Starbuck, 2015; Jones, 2015). Rectangles in the figure represent data layers used or created in the algorithm, while ovals represent operations applied using Python and GIS.

Conclusions, Recommendations and Benefits:

We develop an algorithm using Landsat imagery that is more than 97% accurate at identifying verified surface water reservoirs. This algorithm is useful for application to future imagery without undertaking expensive travel to verify the presence of the reservoirs or to identify the presence of a reservoir not readily visible from public roadways. The ability to employ an accurate algorithm with Landsat imagery enables manual verification using high-resolution imagery to be much more feasible. In addition, the algorithm works with public Landsat imagery that is available at high frequencies. This could allow a temporally more granular investigation of the water levels at these storage systems to help irrigation specialists understand how these systems are in use throughout the year. The information gathered about the storage systems is useful for tailoring programs and policies to encourage more surface water use for irrigation and to help stabilize the aquifer levels in Eastern Arkansas.

Future research to complement the imagery information is to collect data on the groundwater levels, weather patterns, and producer characteristics near the farms where the storage systems are

Table 1. Accuracy Assessment. This summarizes the results of the accuracy assessment comparing annual composites to years with verified reservoir layers.

Manually verified years	Number of verified reservoirs	Number identified by matching composite	Percentage Identified by composite
1996	188	185	98.4%
2000	201	196	97.5%
2006	231	228	98.7%
2009	241	238	98.8%
2010	245	241	98.4%
2013	249	247	99.2%
2015	255	249	97.6%

present. This should help us to identify which of the factors that potentially drives the adoption of these systems plays the greatest role. A pilot survey or a series of focus groups might provide this information for the areas where clusters of the storage systems are present and built with greater frequency over the past few years.

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Project Title: Biological and ecological consequences of sub-lethal ion concentrations on microbial and macroinvertebrate detritivores.
Project Number: 2016AR387B
Start Date: 3/1/2016
End Date: 2/28/2017
Funding Source: 104B
Congressional District: 002
Research Category: Water quality
Focus Category: Surface water, water quality, ecology
Principal Investigator: Sally Entrekin, Michelle A. Evans-White

Publications and Presentations:

Mogilevski, A., B. Howard-Parker, M. Evans-White, S. Entrekin. The Effects of Low Level Salt Concentrations on Photosynthesis, Growth, and Community Composition of Periphyton. Arkansas Water Resource Center Annual Conference, Fayetteville, AR July 26-27, 2016.

Howard-Parker, B., M. Evans-White, S. Entrekin. Microbial Respiration Dose-Response to Low Level Salt Concentrations. Arkansas Water Resource Center Annual Conference, Fayetteville, AR July 26-27, 2016.

Entrekin, S. Biological and Ecological Consequences of Sub-Lethal Ion Concentrations on Microbial and Macroinvertebrate Detritivores. Arkansas Water Resource Center Annual Conference, Fayetteville, AR July 26-27, 2016.

Entrekin, S. Biological and ecological consequences of unconventional gas development and sub-lethal ion concentrations on microbial and macroinvertebrate detritivores. Louisiana Tech, Biology Seminar Series, October, 2016.

Howard-Parker, B. M. Evans-White, 2018, Effects of light, phosphorus, and salts on aquatic microbes and detrital processing in freshwater systems, PhD, Biology, Natural Sciences, University of Arkansas, Fayetteville, AR, 100.

Biological and ecological consequences of sub-lethal ion concentrations on microbial and macroinvertebrate detritivores.

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Executive Summary

Freshwater detritivores are essential to stream productivity, carbon cycling, and subsidies to terrestrial systems. Gradual low-level, sub-lethal increases in ion concentrations such as sodium (Na), chloride (Cl), and bicarbonate (HCO_3) are common, but their impacts on freshwater detritivores and stream processes are not well understood. However, these ions may impact leaf litter decomposition by 1) directly altering fungi and bacteria biomass and respiration, 2) directly altering macroinvertebrate detritivore consumption, respiration, and growth, and 3) indirectly altering litter quality. We tested each of the pathways in stream mesocosms by amending water with one of 3 NaCl and 3 $NaHCO_3$ treatments: natural (from a local stream), low (16 mg/L Na added), medium (32mg/L Na added), and high (64 mg/L Na added) and measuring stonefly growth, respiration, and consuption, and fungi and algal growth over 8 weeks. Similarly, we measured the same variables for isopods that were raised in stream water but fed leaf discs amended with Na as above. Salt treatments had little effect on microbial-mediated leaf litter decomposition and the associated fungal and algal community; however, microbial respiration tended to be elevated on the leaves incubated in $NaHCO_3$ throughout the 134-day study with the lowest $NaHCO_3$ concentration having the greatest stimulatory effect. Further, algal growth also showed a pattern of increase from HCO_3 that may have been an added food resource for macroinvertebrate detritivores in the previous studies but these changes in microbial activity did not change decomposition rates. The stonefly *Amphinemura* increased in biomass and respired more in Na- (both Cl and HCO_3) amended water without increased leaf consumption. Conservation of mass suggests that stoneflies may be feeding on an alternative resource like fungi or algae when Na is present. Na-incubated leaf discs resulted in decreased isopod *Lirceus* growth relative to stream water with little change in respiration and leaf consumption in Na-amended treatments. This suggests that salts negatively impact the quality of detritus. Together, these results demonstrate that low-level, non-lethal Na impacts detritivores both directly and indirectly even at concentrations that are near the chloride standard in Arkansas Regulation 2. Other ions, like HCO_3, have a similar effect on detritivores but are not currently considered in State regulations despite their prevalence in the environment from waste water.

Introduction

Ion increases in Arkansas streams are from a combination of agriculture, wastewater effluent and development associated with urbanization and resource extraction (Griffith, 2014; Musto, 2013). Small amounts of Na and Cl are essential for animals, bacteria, and fungi to maintain hormone signaling pathways, generate electrical cell potentials and regulate bodily fluids (Kaspari et al., 2009). However, increased Na and Cl concentrations have the potential to alter rates of leaf litter decomposition and subsequent carbon cycling in streams by three pathways: 1. directly altering heterotrophic fungi and bacteria consumption, respiration, and growth that colonize and decompose leaf litter from osmoregulatory changes, 2. directly altering macroinvertebrate detritivore consumption and respiration from osmoregualtory changes or 3. indirectly altering macroinvertebrate detritivore feeding rates via changes in litter quality. Greater fungal and bacterial biomass increases the nutritional value of detritus for macroinvertebrate detritivores and typically results in increased leaf litter decomposition rates.

Macro-detritivores both, directly and indirectly, increase leaf litter decomposition rates via leaf consumption and by increasing surface area for microbial colonization. Thus, changes in stream ions can have large impacts on freshwater ecosystems through these direct and indirect effects on detrital processing.

Sodium and chloride ions play a key role in osmoregulatory processes of freshwater organisms, and ion imbalances between organisms and their environment can negatively impact freshwater organisms and ecosystems through increased energy expenditure to maintain osmotic balance. Arkansas streams and rivers have among the lowest natural ion concentrations in the U.S. (Griffith, 2014). However, our past studies have documented small, but increased ion concentrations from sodium (Na: 0.7-7.0 mg/L) and chloride (Cl: 0.8-21.2 mg/L) in 20 wadeable streams. Additionally, Arkansas Department of Environmental Quality (ADEQ) has measured a range in Cl concentrations from 0.4 to over 150 mg/L in Arkansas Valley streams (ADEQ database accessed 27Oct15). Sodium bicarbonate ($NaHCO_3$) has also increased in streams in the Illinois River Basin (Scott et al., 2016). Our study will inform ecological impacts of rising ions that are below documented toxicity levels but are 1) below-, 2) near- and 3) more than-state-set chloride concentrations and quality standards detailed in Arkansas State Regulation 2 (ADEQ). We aim to investigate how detrital organisms and their associated processes change in response to sub-lethal increases in common ions; specifically, Na, Cl and bicarbonate (HCO_3). Changes in litter processing rates in combination with altered detritivore growth will support stream ecosystem responses to modified surface water quality.

Methods

Experiment 1 (micro-detritivores):

We tested low-level NaCl and $NaHCO_3$ additions on heterotrophic fungal biomass on leaf litter. First, sweet gum leaves were cut into standard-sized discs, leached, and incubated in one of 3 NaCl and 3 $NaHCO_3$ treatments: natural (from a local stream), low (16 mg/L Na added), medium (32mg/L Na added), and high (64 mg/L Na added). Each salt treatment was represented by 10 growth chambers, and each chamber had 10 leaf discs (N=70). Conductivity and total dissolved solids increase with mineral concentrations and they were measured and interpreted along with effects from salt additions. Chambers were aerated each day to prevent low oxygen conditions and kept in a greenhouse for normal day-night cycles. Leaf discs were incubated for about 4.5 months to allow for possible microbial adaptation. Respiration was measured at the end of weeks 1, 4, 7, 10, 13, 17, and 19 following at least 2 hours of dark incubation using a Membrane Inlet Mass Spectrophotometer (MIMS; Halvorson et al., 2016). Fungal biomass was measured by solid-phase extraction (SPE) of ergosterol followed by high pressure liquid chromatography (HPLC) (Gessner, 2005). Bacteria will be measured with the DAPI method in the coming months. We needed to assess mass loss and macro-detritivore feeding patterns before examining bacterial colonization of leaf litter. Leaf mass was measured before and after the experiment to estimate amount remaining. Finally, chlorophyll a was estimated after observing growth on leaf discs late in the experiment using ethanol extraction and standard spectrophotometric methods (Steinman, 1996).

Experiment 2 (macro-detritivore exposed to salts and fed naturally conditioned leaves):

We tested if experimental addition of salts reduce macro-detritivore growth and litter consumption from an increase in osmoregulatory stress. We used the same salt concentrations as in experiment 1. The common macro-detritivore, *Amphinemura*, was collected from a local stream that has low stream water conductivity ($<50\ \mu S\ cm^{-1}$), sorted into size class to the nearest 2 mm and placed in one of two salt types and one of the 4 treatments (natural, low, medium, and high). The detritivores were placed in their own growth chamber (10 chambers per treatment) and fed microbial conditioned leaf litter incubated for 30 days in natural stream water (2 salt types x 3 concentrations +1 stream water x 10 growth chambers, N=70). Leaf discs were replaced each week after 7 days to estimate consumption and to prevent starvation. Detritivores were weighed at the end of 4 weeks. Macro-detritivore growth was expressed as (final-initial mass)/final mass*100. Initial leaf mass was measured from subsampled leaf discs and final leaf mass was measured after the 7-day exposure to detritivores upon experiment termination. Leaf disc respiration and fungal biomass were measured as described in experiment 1.

Experiment 3 (macro-detritivore not exposed to salts but fed salt-incubated leaf discs):

We measured the effects of long-term, low-level salt additions used in the other two experiments on litter quality and macro-detritivore growth. First, we used the same common macro-detritivore, *Amphinemura*, as in experiment 2, collected from a local stream, separated by size class and placed in natural stream water with no added salts. Unfortunately, because of an unusually warm winter, the stoneflies emerged after a week into the experiment. We set-up a second experiment with the Isopod, *Lirceus*. The detritivores were then fed sweet gum discs from one of the above 2 salts and 3 salt concentrations after a 30-day incubation period. Detritivores were separated by size class as above and randomly placed in one chamber. Experimental design was as above except 5 isopods were placed in each chamber and their average growth was used as the unit of replication (2 salt types x 3 concentrations +1 stream water x 10 growth chambers, N=70). A sub-sample of detritivores that did not get placed in chambers were dried and weighed and their size class was recorded. Final detritivore dry mass water measured for all individuals. Macro-detritivore growth was measured as (final-initial mass)/final mass*100. Leaf mass lost was measured using the same methods as above.

Statistical Analysis

We used one-way analysis of variance to compare salt treatments effects on response variables (e.g. growth, biomass, leaf mass loss) for each of the proposed experiments and Student's t post-hoc pairwise comparison if main model $\alpha \leq 0.05$. Repeated measures ANOVA was used to test differences in leaf disc respiration with a Tukey's honest significance test. If data did not follow parametric assumptions, then Wilcoxon test was used with a follow-up Wilcoxon each pair post-hoc test when $\alpha \leq 0.05$.

Results:

Experiment 1 (micro-detritivore; Figures 1-4 & Tables 1-4).

Overall, salt treatments had little effect on leaf litter decomposition and the associated fungal and algal community; however, respiration tended to be greater on the leaves incubated in $NaHCO_3$ throughout the 134-day study with the lowest $NaHCO_3$ concentration having the greatest stimulatory effect. Both salt treatment and time had significant main effects on microbial respiration (*p*=<0.001, 0.013), but did not interact (*p*=>0.005, Table 1). Salt treatment appeared to be the primary driver of microbial respiration and respiration varied across time (Figure 1). During week 1, low $NaHCO_3$ and NaCl elicited greater respiration than moderate and high $NaHCO_3$ and high NaCl on discs compared to stream

Table 1. One-way repeated measures ANOVA (α=0.05) output for microbial respiration across time. Salt factor includes 7 levels: filtered stream water at ambient salinity (3 mg/L Na); filtered stream water amended to low, medium, and high sodium bicarbonate concentrations (16, 32, and 64 mg/L Na); and filtered stream water amended to low, medium, and high sodium chloride concentrations (16, 32, and 64 mg/L Na). Repeated measures were carried out on weeks 1, 4, 7, 10, 13, 17, and 19.

	Factor	df	F	*p*
Dry Mass	Salt	6	6.299	**<0.001**
	Time	6	2.738	**0.013**
	Salt*Time	36	1.159	0.247
AFDM	Salt	6	2.973	**0.007**
	Time	6	1.901	0.079
	Salt*Time	36	0.717	0.889

water (SW). Low NaCl also resulted in significantly greater respiration than moderate NaCl on leaf discs. During week 19, low and moderate $NaHCO_3$ elicited a significantly greater respiration response than SW, high NaHCO3, and all NaCl treatments; low $NaHCO_3$ respiration was significantly greater than moderate $NaHCO_3$.Despite differences in respiration, there were no statistically significant differences in dry mass remaining across salt treatments (Table 2). Percent dry mass remaining in $NaHCO_3$ treatments tended to be greater than in SW and peaked at the medium $NaHCO_3$, suggesting the least amount of microbial activity (Figure 2). In contrast, fungal biomass did not differ statistically across treatments (Table 3) but tended to increase with salt concentrations where it peaked in medium salt treatments and then decreased below fungal biomass on leaves incubated in SW (Figures 3). Algal biomass also did not differ across treatments statistically (Table 4) but NaCl treatments tended to have lower algal biomass than SW (Figure 4). Leaf discs incubated in $NaHCO_3$ treatments showed a pattern of increasing algal biomass where it was most variable at the greatest $NaHCO_3$ concentration that was likely from the more basic pH that supports optimal algal growth (Brock, 1973).

Table 2. One-way ANOVA (α=0.05) output for % leaf litter remaining at termination (week 19, day 134).

	df	F	*p*
Dry Mass	6	1.577	0.169
AFDM	6	0.389	0.884

Table 3. One-way ANOVA (α=0.05) output for fungal biomass at termination (week 19, day 134).

	df	F	*p*
Dry Mass	6	0.517	0.793
AFDM	6	1.115	0.364

Table 4. One-way ANOVA (α=0.05) output for algal biomass at termination (week 19, day 134).

	df	F	*p*
Dry Mass	6	1.167	0.336
AFDM	6	1.664	0.145

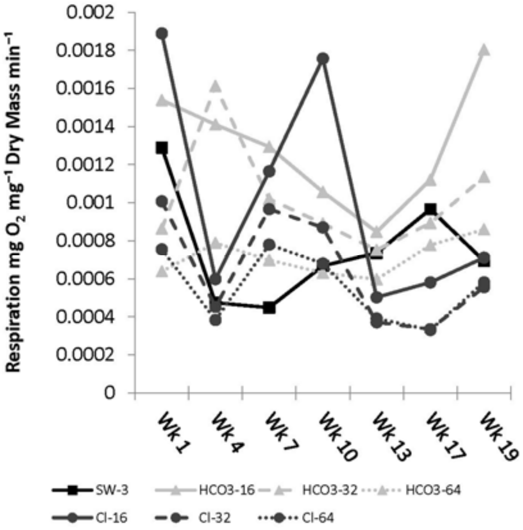

Figure 1. Mean microbial respiration expressed per unit dry mass over time. Salt treatments were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high $NaHCO_3$ treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Both salt treatment and time had significant main effects on microbial respiration (p=<0.001,0.013), but did not interact (p=>0.005). Salt treatment appeared to be the primary driver of microbial respiration responses and respiration varied across time.

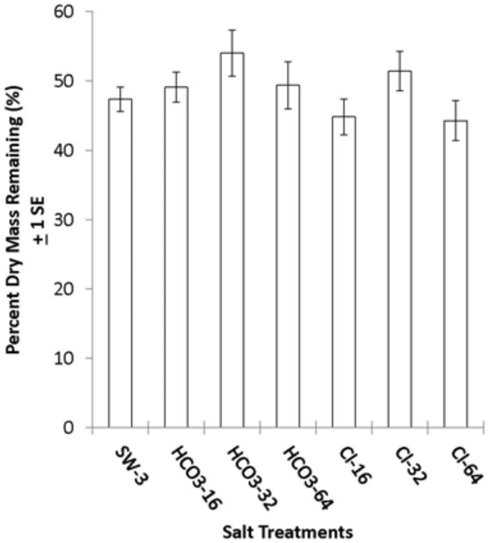

Figure 2. Mean (±1 SE) percent dry mass of litter remaining. There were no statistically significant differences in percent dry mass remaining across salt treatments, although percent dry mass remaining in $NaHCO_3$ treatments tended to be greater than in ambient (3mg/L) stream water. Additionally, percent dry mass remaining showed an increasing pattern with increasing salt concentration for $NaHCO_3$ treatments until peaking at median salt and then decreasing at the two greatest salt concentrations.

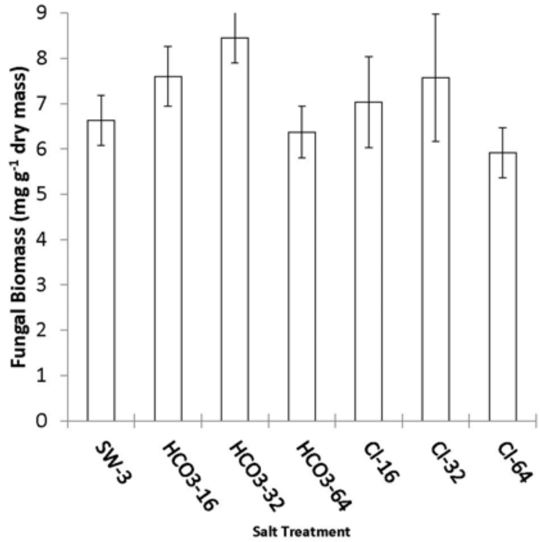

Figure 3. Mean fungal (±1 SE) expressed per unit litter dry mass across salt treatments. Fungal biomass tended to increase with salt concentrations peaking in moderate salt treatments (32mg/L) and then decreasing in the highest salt treatments (64mg/L) to levels below that found in ambient salinity controls for NaCl and $NaCO_3$ salts (p>0.05).

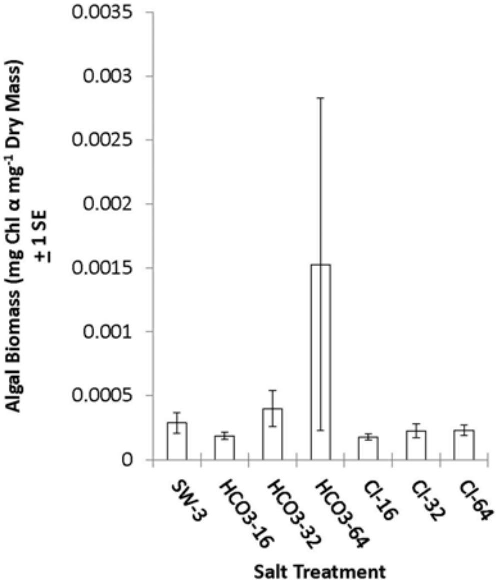

Figure 4. Mean algal biomass measured as chlorophyll a (chl a ± 1 SE) expressed per unit litter dry mass across salt treatments. NaCl treatments tended to have lower algal biomass than ambient stream water (p>0.05). $NaHCO_3$ treatments had increasing algal biomass with increasing salinity, but only moderate (32mg/L) to high (64mg/L) $NaHCO_3$ treatments had higher algal biomass than ambient (3mg/L) steam water (p>0.05). The greatest variation occurred in high (64mg/L) $NaHCO_3$ treatments.

Experiment 2 (macro-detritivore exposed to salts and fed naturally conditioned leaves; Figure 5-6).

Overall, salt amendments to SW tended to stimulate stonefly growth, respiration, and fungal biomass on leaf discs. Stoneflies in stream water gained about 50% mass over the month long experiment compared to ~60% increase for stoneflies in low and high NaCl and $NaHCO_3$ amended water (p=0.04). Stoneflies in the medium salt treatments gained about the same mass as those in SW (p>0.05). Added low and high salts resulted in ~10% increase in mass (Figure 5A). Stonefly respiration was measured on day 30 of the experiment. Stonefly respiration in salt-amended water was ≥ stonefly respiration for individuals in SW (p=0.02). Stonefly respiration was ~ 3 times faster for individuals in the highest $NaHCO_3$ treatments and the low and medium NaCl than for stoneflies in SW (Figure 5B). Leaf litter mass remaining after 7 days in stonefly chambers did not differ across treatments (p=0.73). Leaf discs lost 20-30% of their mass over the week-long feeding period (Figure 6A). Leaf discs placed in salt amended water with stoneflies gained fungal biomass particularly in NaCl amendments from 1 mg/g on leaves in SW up to an average of 9 mg/g on leaves in the lowest NaCl added treatment (p=0.04, Figure 6B). The increase in fungal biomass in the presence of the stoneflies may be from added nutrients provided by stonefly excretion and the overall positive stonefly growth response is probably from this added fungal biomass as a more nutritious food resource (Ferreira et al., 2014).

Experiment 3 (macro-detritivore not exposed to salts but fed salt-incubated leaf discs; Figures 7-8).

Overall, feeding isopods leaves incubated in salt had no effect or suppressed growth and respiration compared to isopods that were fed leaves incubated in SW. Isopods fed leaves incubated in SW increased their mass by 70%. In contrast, isopods fed leaves incubated in medium $NaHCO_3$ and NaCl grew 20% less. Isopods fed leaves incubated in 32mg/L NaCl amendments grew about 28% less than those fed SW-incubated leaves (Figure 7A). Isopod respiration was equal to or greater than respiration of isopods fed leaves incubated in SW compared to salts. Isopods that were fed leaves from low NaCl

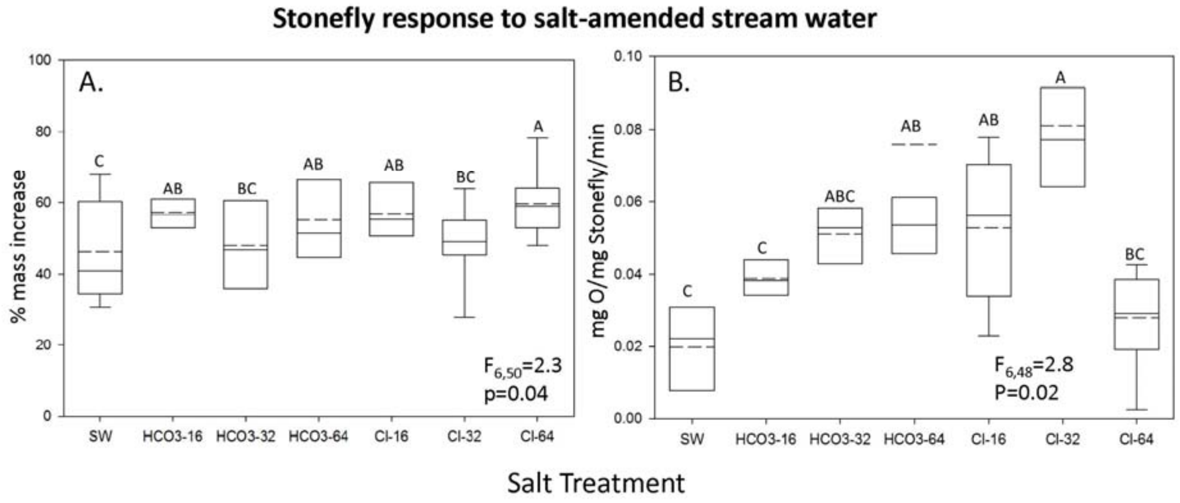

Figure 5. Stoneflies (*Amphinemura* sp.) were fed sweet gum leaves incubated in stream water and reared in chambers with stream water amended with salts. Salt treatments were: SW=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high $NaHCO_3$ treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A. is stonefly growth. Panel B. is stonefly respiration measured on the final day of the experimentDifferent letters represent statistical significance at α=0.05.

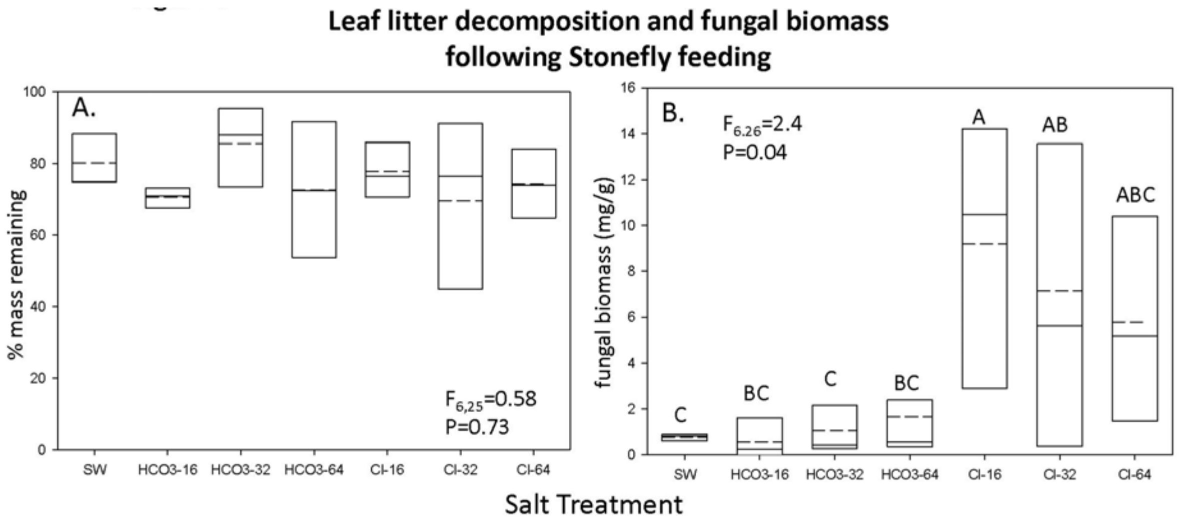

Figure 6. Stoneflies (*Amphinemura* sp.) were fed sweet gum leaves incubated in stream water and reared in chambers with stream water amended with salts. Salt treatments were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high $NaHCO_3$ treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A is leaf disc mass remaining on final discs. Panel B is fungal biomass on leaf discs following the final stonefly feeding period. Different letters represent statistical significance at α=0.05.

incubations respired the least (and gained the least amount of mass) with nearly 3x lower respiration than isopods fed leaves from SW and medium $NaHCO_3$ and NaCl (p=0.03, Figure 7B). There was no measurable difference in leaf mass remaining across salt treatments (p=0.13). All leaf discs lost 20-40% of their mass

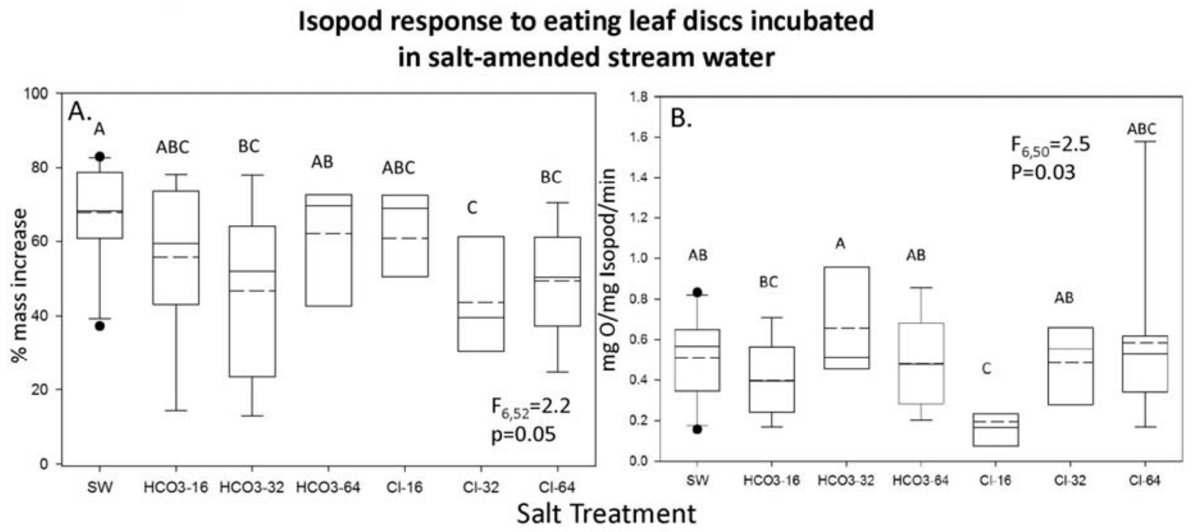

Figure 7. Isopods were fed leaves incubated in stream water amended with salts and chambers had only stream water. Salt treatments that leaves incubated in prior to being offered to isopods were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high $NaHCO_3$ treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show black circles as outliers, the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A is isopod growth about one month after being fed salt-incubated leaves. Panel B is isopod respiration per mg of their body mass (mg). Different letters represent statistical significance at α=0.05.

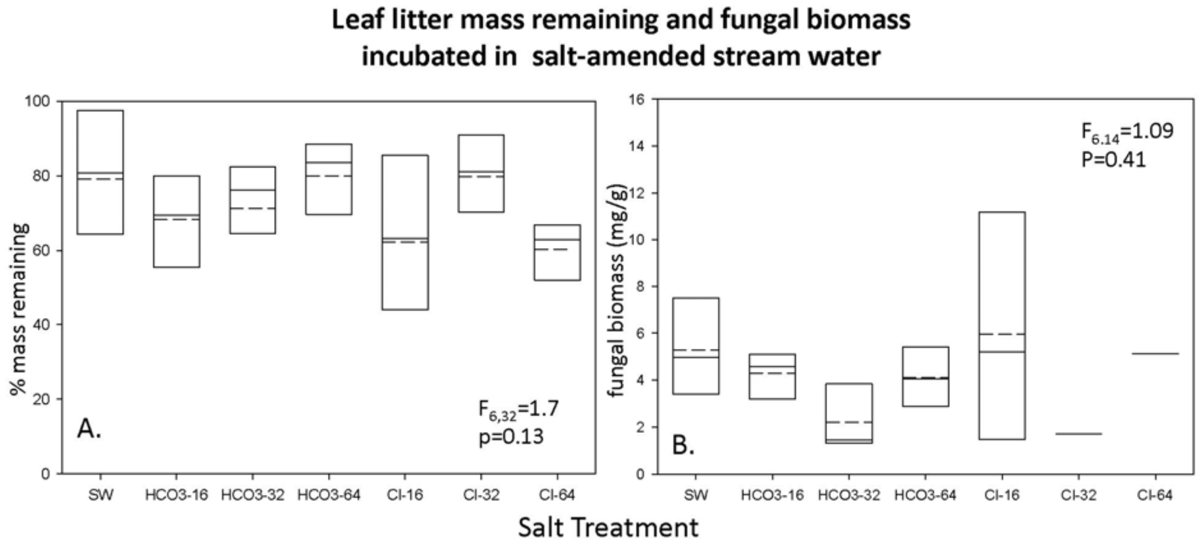

Figure 8. Salt-incubated leaf disc mass remaining and fungal biomass following the last isopod feeding period. Salt treatments that leaves incubated in prior to being offered to isopods were: SW-3=ambient stream water (3mg/L Na); HCO3-16,-32,-64=low, moderate, and high $NaHCO_3$ treatments (16, 32, 64mg/L, respectively); Cl-16,-32,-64=low, moderate, and high NaCl treatments (16,32,64mg/L, respectively). Box plots show black circles as outliers, the upper value as the top whisker that is not an outlier, upper quartile, then a dashed line represents the average and the solid line is the median. Lower box is the lower quartile and the lower whisker is the minimum value excluding outliers. When whiskers are not present it is because they equal the upper and lower quartile, respectively. Panel A. is average leaf disc mass remaining on final discs following isopod feeding. Panel B is fungal biomass on final discs.

over the week-long feeding period. Although not statistically significant, the trend was more leaf mass lost in the low NaCl incubated leaf discs where isopod growth and respiration were lowest (Figure 7A&B,

8A). Fungal biomass on discs incubated and then fed to isopods had variable biomass ranging from 2 to 6 mg/g and there was no treatment effect (p=0.41).

Conclusions

These results demonstrate the complexities of nutrient subsidies on stream processes. In spite of the lack of significance for fungal biomass estimates, low level salts, especially $NaHCO_3$, appear to stimulate microbial respiration. Considering there were no significant differences in percent dry mass remaining across treatments, higher microbial respiration rates may be indicative of microbial energy diverted toward osmoregulation in the presence of ionic stress instead of growth and consumption. Increased algal biomass and fungal biomass can provide added resources to detrital invertebrates, which may initially help mitigate macro-detritivore osmoregulatory stress due to increased ion concentrations. *Amphinemura* increased growth rates and respired more in Na- (both Cl and HCO_3) amended water without increased leaf consumption. Conservation of mass suggests that stoneflies may be feeding on an alternative resource like fungi or algae when Na is present. However, diet switching could have long term effects on resource availability (Brown et al., 2004). In addition to potential osmoregulatory stress caused by water ion concentrations, changes to detritus from salts resulted in decreased *Lirceus* growth relative to stream water with little change in respiration and leaf consumption in Na-amended treatments. This suggests that salts impact the quality of detritus. Although non-lethal, ion increases may impact stream ecosystem processes 1) directly via changes in fungi biomass and respiration, 2) directly by altering macroinvertebrate detritivore consumption, respiration, and growth, and 3) indirectly by altering litter quality. Together, these results demonstrate that low-level, non-lethal Na impacts detritivores both directly and indirectly even at concentrations that are near the existing chloride standard in Arkansas Regulation 2. Other ions, like HCO_3, have a similar effect on detritivores but are not currently considered in state and federal regulatory standards despite their prevalence in the environment from waste water treatment and release (Canedo-Arguelles et al., 2016).

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Project Title: Characterization of nutrient sources, transport pathways, and transformations using stable isotope and geochemical tools in the Big Creek watershed of northwest Arkansas
Project Number: 2016AR388B
Start Date: 3/1/2016
End Date: 2/28/2017
Funding Source: 104B
Congressional District: 003
Research Category: Water quality
Focus Category: Nutrients, surface runoff
Principal Investigator: Phil D. Hays

Publications and Presentations:

Sokolosky, Kelly. Characterization of Nutrient Sources, Transport Pathways, and Transformations Using Stable Isotope and Geochemical Tools in the Big Creek Watershed of Northwest Arkansas. Abstract #286754. Geological Society of America Annual Meeting 2016, Denver, CO. GSA Abstracts with Programs Vol. 48, No. 7.

'Brown Bag' talk to UA Geoscience Department members, Feb. 6, 2017

Sokolosky, K, 2017, MS Thesis, Department of Geosciences, J. William Fullbright College of Arts and Sciences, University of Arkansas, Fayetteville, AR, in progress.

Characterization of nutrient sources, transport pathways, and transformations using stable isotope and geochemical tools in the Big Creek Watershed of northwest Arkansas

Kelly Sokolosky, Department of Geosciences, University of Arkansas
Dr. Phil D. Hays, Department of Geosciences, University of Arkansas, U.S. Geological Survey
Erik Pollock, Department of Biological Sciences, University of Arkansas

- The CAFO's location on a tributary of the BNR raises concerns for the nutrient enrichment and degradation of water quality in the watershed
- The numerous possible nutrient sources in the Big Creek watershed requires a multifaceted approach which combines stable isotope analysis with conventional geochemical analysis of water quality

Executive Summary:

The recent establishment of a concentrated animal-feeding operation (CAFO) near Big Creek, a tributary of the Buffalo National River, has raised concern for degradation of water quality in the watershed. Agricultural land use, as well as residential and urban land use, has the potential to provide excess nutrients to watersheds in the form of phosphorus and nitrogen. This study aims to establish an isotopic reference library of nutrient sources, specifically, δ^{15}N-NO$_3$, δ^{18}O-NO$_3$, and δ^{18}O-PO$_4$ in the Big Creek watershed by sampling directly from possible sources, including septic system effluent, poultry, swine, and cattle manure, storm runoff, and agricultural runoff. Samples will also undergo basic water-quality analyses and analysis of selected trace elements used as feed additives. Isotopic signatures will be assessed and related to source signatures through consideration of fractionating processes and mixing relationships coupled with geochemical characteristics of samples. Preliminary results indicate that the nitrogen in the nutrient sources remains in its original form, ammonia, and that there is likely a small amount of nitrification and denitrification occurring in the waste lagoons. This research is critical to protect the water quality of the Buffalo National River and will assist future researchers in identifying nutrient inputs in other waterways.

Introduction:

Nutrient enrichment in surface waters is a common occurrence in many developed watersheds, and understanding nutrient sources, processing mechanisms, and transport pathways is critical to nutrient management and water-resource protection. Traditional methods of geochemical analysis often fall short of providing adequate characterization of watershed contamination. Stable isotope geochemical tools can augment traditional methods and improve our understanding of nutrient enrichment in aquatic environments and enable development of more effective management practices.

The production of poultry, beef, and swine, and the use of manure from these animals as fertilizer can contribute nutrients to streams and groundwater (Heathwaite and Johnes, 1996). Rural and suburban residential land use has the potential to provide excess nutrients due to the use of septic systems and the interconnectedness of surface water and groundwater, resulting in ultimate delivery of nutrients to streams (Kaushal et al., 2006). Storm-water runoff and waste-water treatment plant drains in urban land use areas can add nutrients (Anderson et al., 2002). These practices contribute primarily nitrogen, phosphorus, and dissolved organic carbon (DOC) to watersheds.

Nutrients are essential to the health of aquatic life in streams; however, elevated phosphorous and nitrogen concentrations are associated with eutrophication, which causes a reduction in biodiversity and the creation of harmful anaerobic conditions (Millennium Ecosystem Assessment, 2005). Nitrogen contamination can be extremely harmful to humans: it has been linked to blue baby syndrome, properly known as infant methemoglobinemia (Comly, 1945), and cancer of the digestive tract (National Academy of Sciences, 1981). Increased DOC concentrations in surface waters can negatively impact the availability of light, energy, and nutrients, as well as increase the mobility and toxicity of metals (Evans et al., 2005).

Increased agricultural use of land in the Big Creek watershed, particularly the recent establishment of a Concentrated Animal-Feeding Operation (CAFO) near Mt. Judea in Newton County, AR, has raised concern for the nutrient enrichment and degradation of water quality (Figure 1). In the Big Creek watershed, as for the larger Buffalo River watershed, the complex distribution of land use and nutrient sources, combined with the occurrence of karst terrain with rapid connection of groundwater and surface water, creates a challenging technical problem for understanding nutrient sources, transport pathways, and processing. However, such knowledge is critical to the development of effective, economically viable management practices.

This project has applied a combined approach of traditional water quality analysis and novel geochemical tools in characterizing nutrient concentrations, sources, transport pathways, and transformation processes in the Big Creek watershed. An isotopic reference database of nutrient sources in the Big Creek watershed being developed by sampling directly from possible nutrient sources. This database is essential for characterizing pollutant sources in this study as well as for future projects. The study also will analyze Big Creek water quality and relate source signatures to nutrient species, providing a preliminary characterization of nutrient dynamics in Big Creek. The specific objectives of the study are (1) to establish a database on isotopic compositions of potential nutrient sources; (2) to employ nitrate isotopes for characterizing sources, transport, and transformations; (3) to characterize stream phosphate oxygen isotopic compositions and identify sources and biological cycling. Isotopic tools will be combined with standard geochemical approaches to achieve our objectives.

Methods:

To develop a database on isotopic compositions of nutrient sources, nine samples have been taken from sites representative of potential sources. A sample has been taken from (1, 2) both of the CAFO's waste holding ponds. (3) A waste lagoon sample was collected from the University of Arkansas Swine Farm. (4, 5) Two cattle manure samples were taken from Fields 1 and 12. (6) Few poultry operations exist in the immediate area of Mt. Judea; therefore a poultry litter sample was collected from the University of Arkansas' Applied Broiler Research Unit. (7, 8, 9) Three runoff samples were collected by the Big Creek Research and Extension Team's (BCRET) auto-samplers from Fields 5a, 1, and 12 on 5/1/17. The UA Swine Farm waste lagoon sample was replicated due to sampling difficulties. The waste lagoons at the CAFO were sampled in May 2016, and the waste lagoon at Savoy was sampled in April 2016. Testing was not completed before the summer, so fresh samples were taken at Savoy in August 2016. The owner of the CAFO would not allow any fresh samples of the lagoons to be

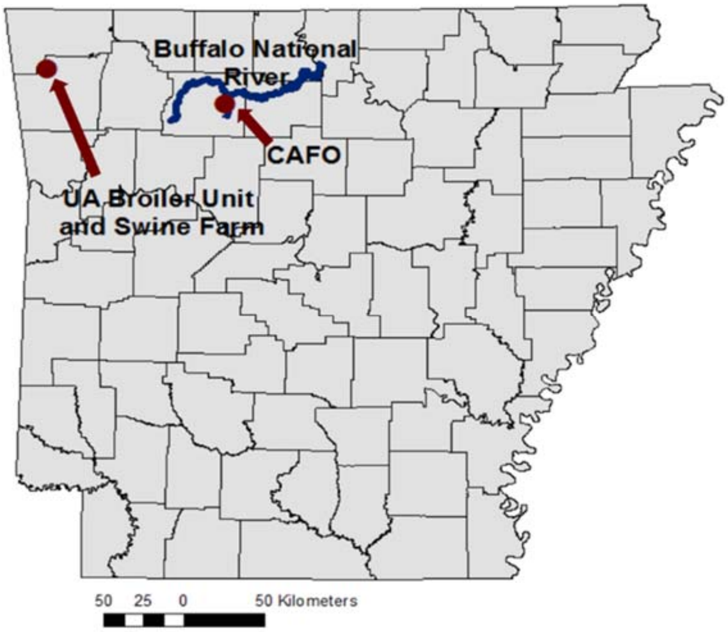

Figure 1. Location of the Buffalo National River, the Confined Animal Feeding Operation (CAFO) on Big Creek, and the University of Arkansas Broiler Unit and Swine Farm.

collected in August 2016. Both old and new Savoy lagoon samples were collected to determine how the geochemistry of the CAFO lagoon samples most likely changed over the long holding period. Two cow manure samples were included to quantify any geochemical change between fresh and old manure.

Four sites on Big Creek were selected for stream sample collection: the confluence of Big Creek with the Buffalo River, an ephemeral stream between the CAFO and Big Creek, a site upstream of the CAFO, and downstream of the CAFO. The location of the upstream and downstream sites along with the ephemeral stream is shown in Figure 2. The ephemeral stream was sampled on 5/2/16. On 3/30/17, all stream sites were sampled after a rainfall of 0.254 cm. On 4/17/17, stream samples were collected at all sites following a rainfall of 0.762 cm. All samples were kept on ice or refrigerated and filtered to 0.45 μm prior to analysis.

Nitrate $\delta^{15}N$ and $\delta^{18}O$ have been analyzed for the source samples (except for runoff samples) using the microbial denitrifier method at the University of Arkansas Stable Isotope Laboratory. Denitrifying bacteria (Pseudomonas auroeofaciens) convert NO_3 to gaseous N_2O: the denitrification process is cut short at this step in order to analyze both nitrogen and oxygen isotopes of nitrate. Samples must be completely converted from nitrate to nitrous oxide due to the inherent fractionation in the denitrification process. Complete conversion prevents this fractionation from influencing isotopic signatures due to the nitrogen mass balance between product and reactant. After conversion to N2O, samples were analyzed on a continuous flow Thermo Delta plus isotope ratio mass spectrometer (CF-IRMS). As for the precision of this method, Sigman and others yielded a standard deviation of 0.2‰ or better when analyzing the isotopic standard IAEA-N3 (Sigman et al., 2001).

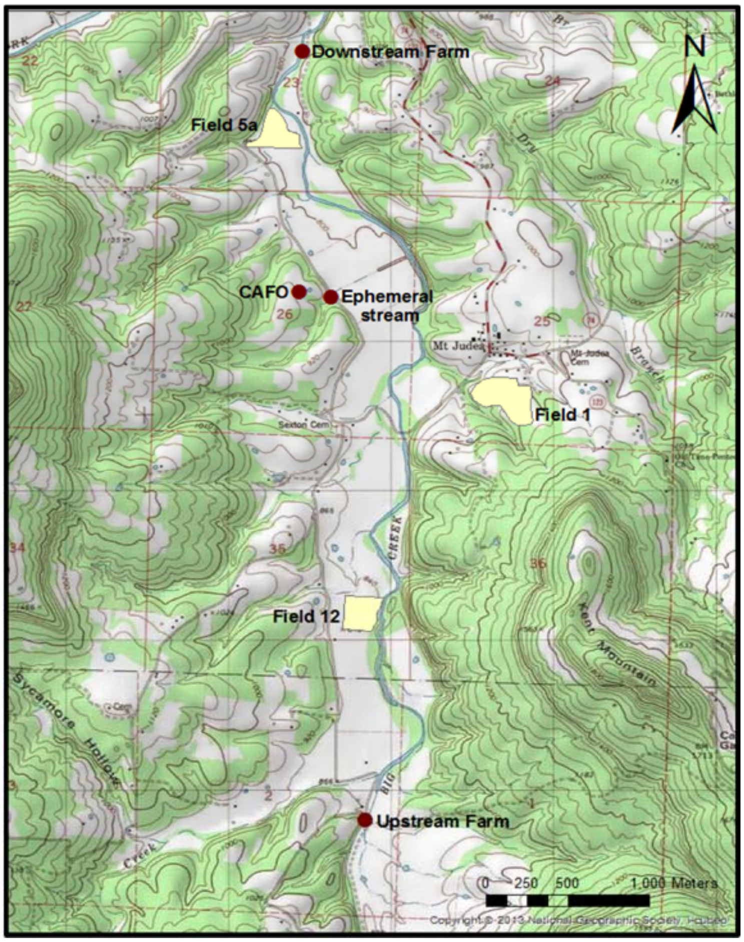

Figure 2. Big Creek stream sampling locations. The sampling location located at the confluence of Big Creek with the Buffalo River is not pictured. If possible, runoff samples will be collected from Fields 5a, 1, and 12.

The source samples have undergone a geochemical analysis, including pH, alkalinity, and major and minor anions. The anion analyses were conducted at the AWRC Water Quality Laboratory using ion chromatography. Total nitrogen and total organic carbon were analyzed at the University of Arkansas Stable Isotope Laboratory. Alkalinity, conductivity, and pH were analyzed by Kelly Sokolosky. Phosphate concentration was measured at the University of Nebraska Water Sciences Laboratory.

Results:

The geochemistry of the collected samples can be seen in Table 1. The nutrient sources all contain a large amount of ammonia but little to no nitrate. This indicates that the nitrogen in the manure has experienced minimal transformations and has remained in its original form, ammonia. The only source

Table 1. Geochemistry of collected samples.

Sample ID	Sample Description	NH4-N (mg/L as N)	Cl (mg/L)	Fl (mg/L)	NO3-N (mg/L as N)	SO4 (mg/L)	N+N (mg/L as N)	TP (mg/L as P)	SRP (mg/L)	pH	Cond. (μS/cm)	Alk. (mg/L as $CaCO_3$)
1A	Old Savoy Lagoon	354	444.149	0	0.105	24.704	0.16			7.77	6770	118.8
1B	New Savoy Lagoon	227	542.874	0	0	43.057	0.17	52.950	16.75			
2	Savoy Hog Manure	491	92.773	428.34	0	61.951	0.27	455.0	318.9	6.08	5260	10.1
3	Fresh Cow Manure	307	98.418	3.353	0	0	0.14	38.200	14.05	7.19	1732	49.0
4	Chicken Litter	716	1196.99	905.61	0	4103.68	1.45	86.200	347.4	6.28	7310	53.6
5	Ephemeral Stream	0.03	3.015	0.907	0.586	2.561	0.51		0	7.79	339	
6	Pond 1 Solids	1040	586.68	0	0	43.622	0.22	75.200	121.7	8.16	4581	413.5
7	Pond 2 Liquids	448	472.332	0.627	0.108	6.175	0.12	110.400	91.3	7.96	3314	298.7
8	Old Cow Manure	7.93	16.248	0.242	0	0	0.05	37.900	21.4	7.06	297.7	27.3

samples containing nitrate are the Savoy lagoon and the solids lagoon (Pond 1) at the CAFO. This suggests that only a small amount of denitrification is possibly occurring in these anaerobic environments. Total nitrogen, total organic carbon, and nitrate isotopes have also been analyzed, and the data is currently being processed.

Conclusions, Recommendations and Benefits:

The limited data collected to date are not adequate to address the nutrient inputs into Big Creek. At this time, nutrient levels in Big Creek are below maximum contaminant limits. Conclusions and recommendations will be developed as the remaining data are processed in the coming months. This research is imperative to protect the water quality of America's first National River. The Buffalo National River is one of Arkansas' most treasured waterways and an important tourist destination in the state, and preserving it for future generations is essential. The Buffalo National River supports a strong fish population with several species endemic to the Ozark Plateaus. Eutrophication would be disastrous for the health of the organisms in the stream, and this project aims to identify nutrient inputs in order to prevent this process from occurring. Agriculture is vital to the state of Arkansas. This research has the potential to benefit farmers and producers in the Big Creek area by identifying which nutrient sources are present in Big Creek, which will allow the farmers and producers to re-evaluate their practices and develop more efficient waste management plans to protect their operation from regulatory penalties. This project will also assist in addressing the national issue of water quality. Many watersheds across the country are agriculturally dominated, like Big Creek. Building a nutrient source library will help other state and USGS researchers with identifying nutrient inputs in other watersheds, as well as within other tributaries of the Buffalo National River.

Future Work

The objectives of this study were not completed in the grant time frame due to sampling and lab delays. The University of Nebraska Water Sciences Lab has a 3-6 month processing period which has delayed phosphate isotope data. Objective 4 (to characterize water sources and pathways through the application of water isotopes) was proposed but later eliminated. The necessary equipment to test water isotopes is not currently operational at the U of A Stable Isotope Lab, and the budget did not allow for testing at another facility.

Storm runoff will be collected from pavement in the town of Mt. Judea near the creek. Artificial fertilizer will be analyzed. Septic effluent will be taken from a residence in or near the Big Creek watershed. Two base-flow stream samples from Big Creek will be collected in the coming months. Samples of stream-bottom sediments will also be taken at all stream sites in order to analyze for phosphate and phosphate isotopes, as phosphate may be stored in stream sediments.

Nitrate isotope data for collected samples will be reported relative to atmospheric N_2 for $\delta^{15}N$ and Vienna Standard Mean Ocean Water (VSMOW) for $\delta^{18}O$. For analysis of phosphate oxygen, phosphate will be removed from solution by adding $MgCl_2$ and NaOH to samples to induce co-precipitation of $Mg(OH)_2$ and PO_4 (Karl and Tien, 1992). Phosphate will be further purified after the method of McLaughlin et al. (2004). Silver phosphate is the result of a series of dissolution and precipitation reactions. Phosphate in raw samples will be converted to silver phosphate and $\delta^{18}O$ of Ag_3PO_4 will be measured on a CF-IRMS after pyrolyzation at the University of Nebraska Water Sciences Laboratory.

Geochemical analyses remaining include major and minor cations and selected trace elements, such as chromium, zinc, and beryllium. These selected trace elements are used as livestock feed additives and may serve as a tracer for manure. Cations, trace elements, total nitrogen, and total organic carbon will be analyzed at the University of Arkansas Stable Isotope Laboratory. The data previously collected by the BCRET will be incorporated into the analyses. The project objectives will be completed as soon as possible and an updated final report with a complete dataset will be submitted.

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Project Title:	Does macrograzer activity drive seasonal variations in algal biomass in Ozark streams?
Project Number:	2016AR389B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	003
Research Category:	Biological sciences
Focus Category:	Ecology, water quality, nutrients
Principal Investigator:	Michelle A. Evans-White

Publications and Presentations:

Sayre, K.R. and M.A. Evans-White. 2016. Relationships among nutrient, algae, and macrograzer in the Ozark Highlands ecoregion. Speaker, Arkansas Water Resource conference. July 26-27 2016.

Sayre, K.R., Advisor: M. Evans-White. 2017. Relationships among nutrient, algae, macroinvertebrates, and macrograzers in the Ozark Highlands ecoregion. MS. Thesis. Department of Biological Sciences. University of Arkansas.

Stoneroller fish (*Campostoma spp.)* influence on dose-response relationship between nutrients and algae in summer 2016 and winter 2017

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Core Ideas:

- Increased nutrient concentrations associated with declining water quality can stimulate benthic algal biomass; grazers, like the stoneroller (*Campostoma* spp.), may dampen the effect of nutrients on benthic algal biomass, but they are often not considered when constructing nutrient-algal relationships for the development of numeric nutrient criteria.
- Grazers tended to reduce algal biomass measured as chlorophyll *a* (chl *a*) in each stream, but most of the differences between grazer excluded and grazer present treatments were not statistically significant at $p \leq 0.05$; grazer chl *a* effect sizes tended to be positively related to TP ($p>0.05$) and were greater in the summer compared to the winter (ANCOVA $F=59.85$, $p=0.0163$).
- Our results suggest that nutrient and grazer effects on benthic algae can be variable and seasonal.

Executive Summary:

Elevated nitrogen (N) and phosphorous (P) in streams can cause nutrient pollution leading to instream and downstream problems of excess algal growth which can constrain the recreational use of streams and reduce stream biodiversity (Dodds and Welch 2000). The United States Environmental Protection Agency (USEPA) provided national numeric nutrient criteria standards based on ecoregion and states and tribes can adopt these criteria or develop their own. Therefore, many states have decided to develop regional numeric nutrient criteria standards based on scientific methods, which can include assessment of algal biomass (USEPA, 2013).

Seasonal variations in algal density and associated determining factors, such as macrograzer activity, may cause some variation in dose-response relationship between nutrients and benthic algal biomass. Most studies examining the relationships between grazers, algae, and nutrients have used snails and caddisflies as the study organism. Less in known about the influence of algivorous fish, such as stonerollers on algal biomass responses to nutrient enrichment (Cattaneo and Mousseau 1995). Stonerollers (*Campostoma* spp.) are minnows that occur in high abundances in Ozark streams, and possess a sub-terminal mouth that makes them well-equipped grazers. *Campostoma spp.* grazing can be an important determining factor on algal biomass and community composition (Steward 1987; Power et al. 1988) and they are thought to be grazing most actively during the warm season since they are ectotherms.

The objective of this project was to examine how stonerollers (*Campostoma spp.)* may modify the dose-response relationship between nutrients and algal biomass in wadeable Ozark Highland streams seasonally. We hypothesized that stonerollers would have a significant negative effect on benthic algae within each stream during the summer (hypothesis 1; H1). Next, we thought that stonerollers would have a more negative effect on benthic algae with increasing total phosphorus (TP; hypothesis 2; H2). Finally, we expected that stoneroller effect will be greater in the summer than the winter (hypothesis 3; H3).

We completed a randomized block experiment, with three blocks per stream, in five stream reaches in summer of 2016 and 3 sites in winter of 2017. The TP concentration ranged from below detection to 0.06 mg/L across study reaches. Each block consisted of two rectangular frames 4 unglazed ceramic tiles zipped tied. One tile in one frame at each block were surrounded by a 12 gauge copper wire that was to a 6-volt solar charger. This sent an electrical pulse through the exclosure and deterred large-bodied organisms. Randomized block analysis of variance was used to address H1, regression was used to address H2, and analysis of covariance (ANCOVA) was used to address H3. Grazers tended to reduce algal biomass measured as chlorophyll *a* (chl *a*) in each stream, but most of the differences between grazer excluded and grazer present treatments were not statistically significant at $p \leq 0.05$; grazer chl *a* effect sizes tended to be positively related to TP ($p>0.05$) and were greater in the summer compared to the winter (ANCOVA F=59.85, p=0.0163). This suggests that seasonality plays a role in stoneroller's influence on stream algae and it should be considered when examining dose-response relationships between nutrients and algae.

Introduction:

Elevated nitrogen (N) and phosphorous (P) in streams can cause excess algal growth, which can constrain the recreational use of streams and reduce stream biodiversity (Dodds and Welch 2000). The United States Environmental Protection Agency (USEPA) provided national numeric nutrient criteria standards based on ecoregion and states and tribes can adopt these criteria or develop their own. Therefore, many states have decided to develop regional numeric nutrient criteria standards based on scientific methods, which can include assessment of algal biomass (USEPA, 2017). Arkansas department of environmental quality (ADEQ) is currently working toward federal TN and TP standards by assessing dose-response relationship between algae (chlorophyll *a* and ash-free dry mass), but does not currently have published federal total nitrogen (TN) or total phosphorus (TP) numeric nutrient criteria in accordance with the EPA (USEPA, 2017). Arkansas currently has algae narrative criteria for all water bodies and TP point source criteria for streams.

Currently Arkansas have narrative standard for algae in waterbodies, Arkansas Regulation No. 2, which states that "Materials stimulating algal growth shall not be present in concentrations sufficient to cause objectionable algal densities or other nuisance aquatic vegetation or otherwise impair any designated use of the waterbody." The state intends to develop numeric nutrient criteria from dose-response relationships between nutrient levels and stream benthic algae; Arkansas Department of Environmental Quality (ADEQ) is leading that effort. Relationships between nutrient concentrations and algae can be variable in Arkansas and Oklahoma (Stevenson et al. 2012, Haggard 2013) since other factors in addition to nutrient concentrations can affect benthic algal concentrations. Specifically, some of the variation in the relationship between nutrients and benthic algae may be explained by macrograzer activity (Stevenson et al. 2012).

Seasonal variations in algal density and associated determining factors, such as macrograzer activity, may cause some of the variation in dose-response relationship between nutrients and benthic algal biomass. Thus, these variations in dose-response relationships should be considered when developing numeric nutrient criteria for the Ozark Highland Ecoregion. Most studies examining the relationships between grazers, algae, and nutrients have used snails and caddisflies as the study organism while less in known about the influence of algivorous fish, such as stonerollers on algal biomass responses to nutrient enrichment (Cattaneo and Mousseau 1995). Stonerollers (*Campostoma* spp.) are minnows that occur in high abundances in Ozark streams, and possess a sub-terminal mouth that makes them well-equipped grazers. *Campostoma spp.* grazing can be an important determining factor on algal biomass and community composition (Steward 1987; Power et al. 1988) and they are thought to be grazing most actively during the warm season since they are ectotherms. During late summer, the standing stock of algae in pools can be nearly devoid of algae biomass due to grazing by *Campostoma spp.* (Mathews et al. 1987), but little is known about their potential to effect on algal biomass in the winter. Seasonal variation in *Campostoma* spp. grazing could explain variation in algal biomass across seasons and sites in Ozark streams with varying nutrient concentrations.

The proposed study examining the seasonality of *Campostoma* spp. effects on benthic algae across streams with a gradient of total phosphorus concentrations can help the state understand how and why seasonality may result in variation in the relationship between nutrients and algae. The objective of this project is to examine how stonerollers (*Campostoma spp.)* may modify the dose-response relationship between nutrients and algal biomass in wadeable Ozark Highland streams seasonally. We hypothesized that stonerollers would have a significant negative effect on benthic algae within each stream during the summer (hypothesis 1; H1). Our second hypothesis was that stoneroller effects on algae would increase with total phosphorus (TP; hypothesis 2; H2). Finally, we expected that the stoneroller effect would be greater in the summer than the winter due to greater activity at greater stream temperatures.

Methods:

Our experiment was conducted in five Ozark Highland wadeable streams during the summer of 2016 (18 July- 3 October) and three streams during the winter of 2017 (24 January-6 March). Sites with a gradient of TP were selected (Table 1). Three blocks were set up in runs in the upper, middle, and lower sections of each stream (reach≥200m) where each block was separated by at least one pool. Each block consisted of one treatment exclosure (stoneroller excluded) and one unelectrified control exclosure (stoneroller present) that were set up side-by side in equal flow conditions. Four unglazed tiles (121cm^2) were zip-tied into each quadrate exclosure (31 X 5-cm built from 19-mm polyvinyl chloride pipe) to measure benthic algae. Treatment enclosures were set up with a 12 gauge insulated copper wire surrounding tiles and connected to 6 volt ParMak solar fence charger (ParMak Precision Kansas City, MO) that sent an electrical pulse into the water deterring large-bodied organisms (>~1cm) which exclude most crayfish and fish (Pringle and Blake 1994). The charge extends about ~10cm outside the quadrate (Ludlam and Magoulick 2009). Tiles were inoculated for 14 days in treatment and control conditions before they were collected on days 14, 21, and 28 in summer and 14, 21, 28, and 35 in the winter. Algae was then measured for chlorophyll *a,* and ash-free dry mass (AFDM) was calculated using slurry from the whole tile. Water samples were taken throughout the experiment at each stream bi-weekly, placed in an iced cooler, and frozen upon returning to the laboratory to measure for total phosphorus (TP) and total nitrogen (TN). Total phosphorus was measured in water samples by using a persulfate digestion and colorimetric analysis using the ascorbic acid method (American Public Health Association, 2005). Total nitrogen was measured in water by using a sodium hydroxide digest to convert all nitrogen forms to nitrate and colorimetric (Hach DR 3900) analysis using Hach reagent powder pillows (Hach Permachem® Regant NitroVer© 5 nitrate reagent).

Statistical analysis was conducted in a hierarchal manner to understand the influence of grazers within each stream (H1), nutrients among streams (H2), and season among streams (H3). We addressed the grazing effect on benthic algal chlorophyll a and AFDM collected on day 28 within each stream during the summer and winter using a randomized-block analysis of variance (RB-ANOVA). Assumptions of

Table 1: Five streams were studied in the summer and three streams were studied in the winter. Water samples were measured for total phosphorus (TP) and total nitrogen (TN) on day 28 of study in summer of 2016 (Sept 27-Oct 3) and winter of 2017 (15-16 February). TP shows a gradient while TN does not. Land use data from King et al. 2016.

			Summer		Winter		
Stream	**State**	**Watershed**	**TP (mg/L)**	**TN (mg/L)**	**TP (mg/L)**	**TN (mg/L)**	**Land use**
Saline	OK	Eucha	0*	4.2	0*	0.7	60% Forest, 26% Pasture, 8% Grassland
Evansville	OK	Illinois	0.009	2.1	---	---	52% Forest, 40% Pasture, 3% Grassland
Beaty	OK	Eucha	0.027	1.9	0.029	1.7	30% Forest, 61% Pasture, 2% Grassland
Baron Fork	OK	Illinois	0.047	3.7	---	---	45% Forest, 48% Pasture, 2% Grassland
Flint	OK	Eucha	0.06	1.2	0.049	7.3	28% Forest, 58% Pasture, 3% Grassland

TP=Total Phosphorus, TN=Total Nitrogen

variance, covariance, and normality assessed visually using histograms and box plots. Interactions between environment and experiment were visually assessed using a line graph. The mean effect size was calculated per stream by averaging the effect size from each block (treatment: control, Grazer-excluded:Grazer-present) to address our second hypothesis. The mean effect size was regressed against nutrient concentrations (TP) to determine whether the grazer effect on benthic algae depended upon stream nutrient concentrations for the summer using all five stream reaches. Assumptions of normality and homogeneity of variance were assessed visually. Last, analysis of covariance (ANCOVA) was used on streams sampled in both winter and summer (Beaty, Saline, and Flint) to understand how effect of stonerollers differs between the two seasons. In the ANCOVA, the mean effect size (ratio Grazer-excluded:Grazer-present chlorophyll *a* and AFDM for each block averaged per stream) was the dependent variable, nutrient concentrations were the independent variable, and season was the covariate. Assumptions of linearity, homogeneity if variance, and relationship dependent and independent variable were assessed.

Results:

As expected, stream TP ranged from below detection to 0.06 mg/L (Table 1). The TN concentration was high at all sites and varied less than TP. Grazers reduced benthic chlorophyll a in Saline and Beaty Creek in the summer (Table 2; Figure 1), but not in the winter (Table 3; Figure 2; H1). Grazers reduced benthic AFDM in the summer in Saline Creek only (Table 1; Figure 1). There was no statistically significant difference between treatment and control for either chlorophyll *a* or AFDM in any stream during the winter (Figure 2). Campostoma spp. Abundance was measured in summer 2015, but we found that our abundance measurements did not influence the relationship between chlorophyll *a* and TP in this study (104b-Sayre and Eanvans-White 2016), and this data does not correlate with effect size for data taken in summer 2016 (p=0.82).

Chlorophyll *a* effect size and stream TP had a positive trend in the summer when all five study streams were included, but this trend was not statistically significant (Figure 3). However, there was no relationship between AFDM effect size and stream TP in the summer (Figure 3). The ANCOVA that included the three study sites sampled in both the summer and winter found no interaction between season and TP for either chlorophyll a or AFDM (Table 4; Figure 4). There was a season and a TP main effect for chlorophyll a (Table 4; Figure 4), but no interaction between those factors. Therefore, all six chlorophyll *a* effect sizes were combined into one regression, which was not statistically significant.

Conclusions, Recommendations and Benefits

Many studies have shown negative effects of stream grazers on benthic algae (Mathews et al. 1987; Steward 1987; Power et al. 1988). Although grazer-exclosures tended to have greater benthic algal biomass than grazer-present treatments in the present study, these differences were only statistically significant in two streams with low to moderate TP concentrations during the summer (Table 2; Figure 1). A large amount of variation was observed in response variables across sites and increasing the number of replicates would help improve the power to address the interactive effects of grazers and nutrients on benthic algal biomass (Figures 1 and 2). Additionally, electrical exclosures did not exclude smaller macroinvertebrate grazers, like snails, that can negatively affect benthic algal biomass (Steinman et al. 1996). The electrical treatment should not have affected their presence, but the abundance and biomass of smaller benthic macroinvertebrates were not measured in this study and they could have added to the variability in effect sizes.

Our results suggest that macrograzers, such as *Campostoma* spp., can be more active and effective at grazing in the summer relative to the winter. The mean and variation in grazer chl *a* effect sizes tended to increase with TP concentrations in the summer, but not in the winter season (Figures 3 and 4). In addition, the mean grazer chl *a* effect size was greater in the summer than in the winter. *Campostoma* spp. were not seen during winter months except on a few occasions when the temperature

Table 2: Five streams were sampled on day 28 in summer of 2016 (Sept 27-Oct 3). A Randomized block analysis of variance (ANOVA) was run on each stream understand the influence on algae that was under grazer excluded or grazer present condition. There was a treatment effect in Saline creek on both chlorophyll *a* and ash-free dry mass(AFDM). Beaty Creek also had a significant treatment effect but only for chlorophyll *a.* Refer to table 1 for nutrient concentrations in each stream.

Stream	**Variable**	**Factor**	**df**	**F-value**	**P-value**
Saline	Chlorophyll a	Treatment	2	6.497	0.056*
		Block	2	20.952	0.006*
	AFDM	Treatment	2	7.003	0.049*
		Block	2	26.47	0.004*
Evansville	Chlorophyll a	Treatment	2	0.750	0.529
		Block	2	0.874	0.484
	AFDM	Treatment	2	2.668	0.184
		Block	2	0.550	0.615
Baron Fork	Chlorophyll a	Treatment	2	0.885	0.481
		Block	2	2.126	0.235
	AFDM	Treatment	2	0.947	0.461
		Block	2	1.786	0.279
Beaty	Chlorophyll a	Treatment	2	11.545	0.022*
		Block	2	7.365	0.046*
	AFDM	Treatment	2	0.287	0.765
		Block	2	1.404	0.345
Flint	Chlorophyll a	Treatment	2	1.836	0.272
		Block	2	0.017	0.983
	AFDM	Treatment	2	1.012	0.441
		Block	2	0.107	0.901

AFDM=Ash-free dry mass

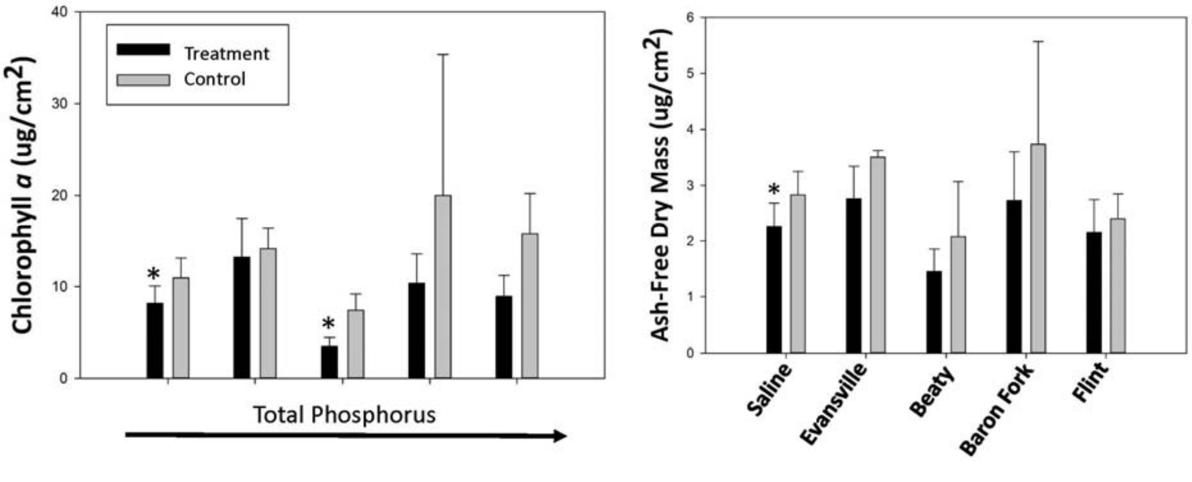

Figure 1: Algae collected from tiles on day 28 in late-summer of 2016, was measured for chlorophyll *a* and ash-free dry mass (AFDM, ug/cm^2) values under treatment and control conditions. Mean and standard error (SE) were calculated for each stream (n=3). Significant differences are indicated with an asterisk (*).

was high in sunny runs. Other studies in Ozark streams suggest that *Campostoma* spp. influence can vary spatially and temporally within a single stream (Ludlam and Magoulick 2009). The influence of grazers in these Ozark streams can depend on the presence of predators, stream conditions (e.g. drying), and depth (Ludlam and Magoulick 2009) and our study suggests that their effects may also vary across nutrient levels.

Table 3: Five streams were sampled on day 28 in winter 2017 (Feb 15-16). A Randomized block analysis of variance (ANOVA) was run on each stream understand the influence on algae that was under grazer excluded or grazer present conditions. There was no treatment or block effects.

Stream	Variable	Factor	df	F-value	P-value
Saline	Chlorophyll a	Treatment	2	1.98	0.252
		Block	2	0.33	0.735
	AFDM	Treatment	2	1.05	0.429
		Block	2	0.40	0.695
Beaty	Chlorophyll a	Treatment	2	2.96	0.234
		Block	2	3.85	0.117
	AFDM	Treatment	2	2.14	0.234
		Block	2	0.08	0.921
Flint	Chlorophyll a	Treatment	2	0.42	0.681
		Block	2	0.68	0.555
	AFDM	Treatment	2	0.88	0.482
		Block	2	0.22	0.810

AFDM=Ash-free dry mass

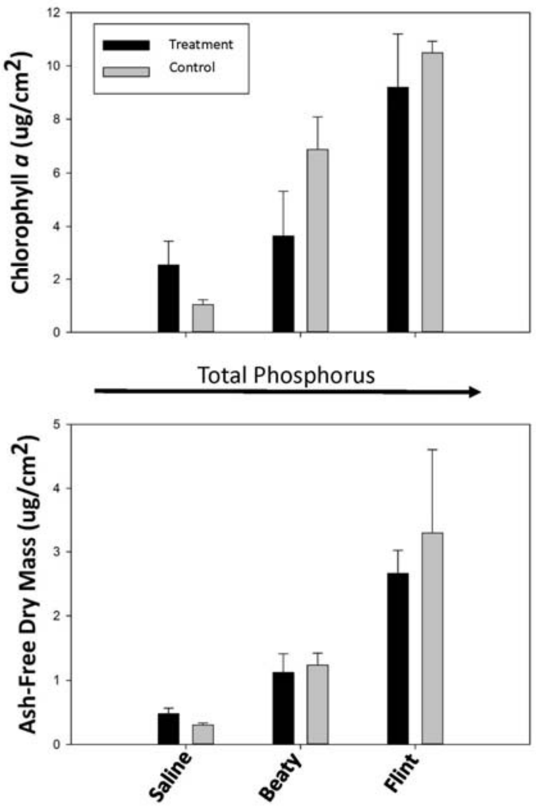

Figure 2: Algae collected from tiles on day 28 in winter of 2017, was measured for chlorophyll *a* and ash-free dry mass (AFDM, μg/cm^2) under treatment and control conditions. Mean and standard error (SE) were calculated for each stream (n=3). *RB-ANOVA indicated no statistically significant influence of grazer-exclusion for chlorophyll *a* or AFDM.

Grazer chl *a* and AFDM effect sizes were always greater than one suggesting that grazers tended to reduce benthic algal biomass across the stream TP gradient in the present study. A prior study that manipulated *Campostoma* and streamwater P levels in experimental streams found that stonerollers may stimulate benthic algal chl *a*, reduce benthic AFDM, and increase the autotrophic index even under P enriched conditions (Tayler et al. 2012). Taylor et al. (2012) focused on grazing effects in pools, included a greater P enrichment up to 0.1 mg/L, and was completed in outdoor experimental streams in the early spring (March-April). All of these factors could result in the differences observed between these two studies and future experiments could manipulate temperature as well as nutrient concentrations in experimental streams to get at relative effects.

Dodds et al. (1997) proposed an oligotrophic-mesotrophic boundary at 2.0 μg/cm^2, and a mesotrophic-eutrophic boundary at 7.0 μg/cm^2 of chlorophyll *a.* Chlorophyll *a* measurements in the present study indicate that all streams were within the oligotrophic to mesotrophic range during the summer months. However, Flint became eutrophic in the winter, with Beaty on the border of eutrophic (Dodds et al. 1997). Therefore, adding in-stream manipulations in reaches with greater TP and benthic algal biomass would improve our understanding of the effects of grazers across nutrient gradients.

Overall, our data suggest the importance of seasonality with respect to macrograzer resource acquisition, macrograzer effect size,

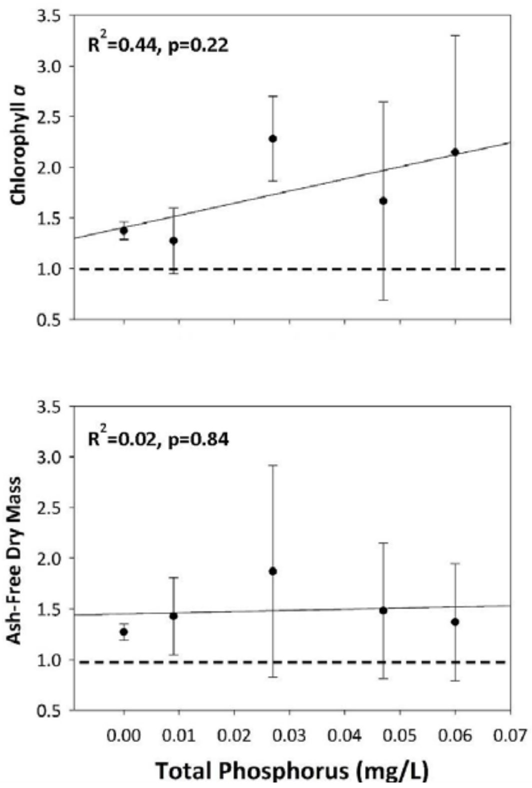

Figure 3: Mean effect size for algae collected from tiles on day 28 in late-summer of 2016, measured for chlorophyll *a* and ash-free dry mass (AFDM, ug/cm^2) values under treatment and control (grazer-excluded and grazed) conditions. Bars represent the standard error of the effect size, but are not used in calculating regression statistics. The dashed-line indicates the 1:1 ratio at which treatment is equal to control where grazers do not have an influence.

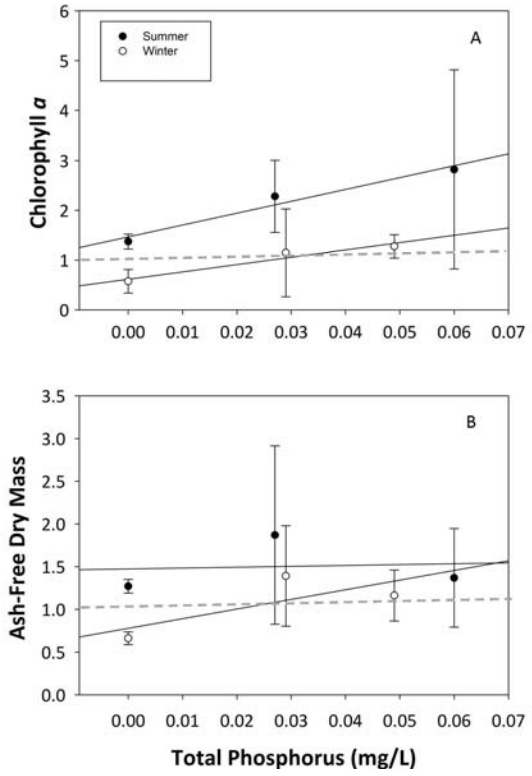

Figure 4: Mean effect size for algae collected from tiles on day 28 (27 September-3 October) in winter of 2017, measured for chlorophyll *a* and ash-free dry mass (AFDM, ug/cm^2) values under treatment and control (grazer-excluded and grazed) conditions. Bars represent the standard error of the effect size, but are not used in calculating regression statistics. The dashed-line indicates the 1:1 ratio at which treatment is equal to control where grazers do not have an influence. There was a season and a TP main effect for chlorophyll a, but not for AFDM.

Table 4: Three streams were sampled in both summer of 2016 and winter of 2017. Analysis of covariance (ANCOVA) was run where total phosphorus (TP) is the predictor, effect size chlorophyll *a* and ash-free dry mass (AFDM) was the response variables, and season (winter and summer) is the covariate. * Chlorophyll *a* effect size was significant for both TP and season. There was no interaction between TP and season.

Response	Predictor	df	F-Value	P-value
Effect Size Chlorophyll *a*	Total Phosphorus	1	47.84	0.0203*
	Season	1	59.85	0.0163*
	TP x Season	1	2.03	0.295
Effect Size AFDM	Total Phosphorus	1	0.10	0.101
	Season	1	0.26	0.258
	TP x Season	1	0.08	0.077
	Residuals	2	0.33	0.164

AFDM= Ash-free dry mass

and dose-response relationship between nutrients and algae. A prior study in the Illinois River basin found that nutrients explained more variation in benthic algal biomass in the spring compared to the summer

(Stevenson 2012). The present study suggests that grazer effects are also lower in winter season and they may play a role in the observed relationship between nutrients and benthic algae.   This seasonality effect on grazer influence should be considered when developing nutrient-algal dose response relationships and developing numeric nutrient criteria for the Ozark Highlands Ecoregion.

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Project Title: Arkansas Water Resources Center - Information Transfer
Project Number: 2016AR390B
Start Date: 3/1/2016
End Date: 2/28/2017
Funding Source: 104B
Congressional District: 003
Research Category: NA
Focus Category: Education, water supply, management and planning
Principal Investigator: Brian E. Haggard, Erin E. Scott

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Project Title: Information Transfer Program
Project Team: Brian E. Haggard, Arkansas Water Resources Center
Erin E. Scott, Arkansas Water Resources Center

Introduction:

An important component of the Arkansas Water Resources Center's (AWRC) mission is the transfer of water resources information to the user community within Arkansas and the region. This community of users includes researchers, resource planners and managers, environmental consultants, environmental advocacy entities, lawyers, and the general public. The transfer of information was accomplished through the following outlets:

1. Annual water research conference
2. Monthly electronic newsletters
3. Websites for the Center and to publish and archive newsletter stories
4. Reports and fact sheets
5. Social media
6. Infographics
7. Other news outlets
8. Peer-reviewed publications and presentations at scientific conferences

The dissemination of water resources information through the outlets listed above reaches a broad audience throughout Arkansas and neighboring states.

Annual Water Research Conference:

Over 150 people attended the annual water conference held in July 2016. The conference theme was "Nutrients, Water Quality, and Harmful Algal Blooms". The conference was geared toward a regional and even national audience, as speakers came from around the country to talk about the following topics:

- The complex nature of water quality management
- Nutrient sources and transport
- Watershed scale influences on water quality
- Biological thresholds
- Harmful algal blooms

The second day of the conference was devoted to harmful algal blooms (HABs). Sessions covered everything from the growing threat of blooms, to ways to monitor for HABs, to how to regulate HABs and cyanotoxins. Attendees included stakeholders from municipalities, state agencies, research institutions, non-profit groups, environmental consulting firms, and the general public from throughout Arkansas and the region. This was a great venue for regional water managers to come to share ideas and learn about the successes and challenges that other managers have encountered.

Seventeen students presented their research during the poster presentation session. Undergraduate students in the Ecosystems Services Research Experience for Undergraduates (EcoREU) program, funded by the National Science Foundation, presented the work completed during their 10-week summer project under a faculty advisor. Graduate students also presented their research, many of whom received funding through the 104B program.

Monthly Electronic Newsletters:

The AWRC distributed monthly electronic newsletters to several hundred people from local and state agencies, municipalities, academia, non-profit organizations, consulting firms, students, and many other stakeholders. Electronic newsletters continue to be a valuable means of distributing important information related to water resources. The open rate is about 37% on average, much higher than the national average for Mailchimp enewsletters.

The Center published news articles on current research being done throughout the State, especially projects funded through the USGS 104B program, recent activities of the Center, the USGS, and other organizations, funding opportunities, and other timely water-related news. The AWRC populates a section of the newsletter for "Upcoming Events" to highlight not only Center- related events and activities, but also those of other local or national organizations such as ADEQ, ANRC, Beaver Watershed Alliance, Illinois River Watershed Partnership, and the US EPA. AWRC also updates a "Jobs" section each month aimed to provide recent graduates or early career people some guidance and examples of current job openings related to water science and engineering.

Websites:

The AWRC website (arkansas-water-center.uark.edu) is the primary portal for stakeholders to access important and useful water resources information. During this past year, Center-staff have worked to improve the usability of the website and the availability of water resources information. The website serves as a platform to provide:

- Immediate electronic availability of almost all AWRC publications
- A warehouse of raw data provided as water-data reports associated with research and monitoring projects
- Information about submitting a water sample to the AWRC Water Quality Laboratory
- Information on upcoming conferences and funding opportunities, especially USGS 104B and 104G grants, and other events.

Maintenance of the AWRC website is a critical component of the AWRC's information transfer program.

The Center also developed a new website (watercurrents.uark.edu) devoted to publishing and archiving stories from the electronic newsletters. Housing news articles on a designated website enhances searchability and aesthetic quality of important news and information.

Reports and Fact Sheets:

AWRC published 7 technical reports and 2 water-data reports on the Center's website during this past project year (March 2016-February 2017). These technical reports included water research and monitoring reports from projects funded by state or local water organizations, as well as reports by scientists not related to the Center in an effort to make available important information in addition to or in lieu of peer-reviewed articles. Water-data reports are published on AWRCs website and provide easy access to years-worth of Center-related water quality monitoring data associated with the data collected for the technical reports. These data reports are available to the public and can be accessed as neatly-organized Microsoft Excel data files.

The Center also developed and published 6 fact sheets. These fact sheets provide information to stakeholders, especially those who submit water samples to the AWRC Water Quality Lab for analysis. The lab offers analytical "packages" that include parameters of interest for various intended uses. These uses include aquaculture, livestock watering, poultry watering, domestic, and irrigation. Fact sheets are associated with each of the analytical "packages" and describe how a water sample should be collected, and how people can interpret their lab results. A fact sheet on reporting limits, method detection limits, and censored values is also available.

Social Media:

The AWRC continues to expand its presence on social media. During this past year, staff utilized Facebook and twitter to disseminate information about the activities of the Center including funding opportunities, conference materials, and research findings. Facebook followers continue to grow as the Center currently has 506 likes and "boosting" posts to advertise monthly electronic newsletters has increased the "reach" by over 2,600%. The Center also ventured into the world of Instagram. Social media has been a great way to network and share ideas and stories among water stakeholders and organizations. The Center shares posts from other water or water-related organizations about current news or upcoming events.

Infographics:

The AWRC developed infographics to target students in various departments at the University of Arkansas in Fayetteville. For example, the Center created infographics specific to topics studied in the agriculture department, the engineering department, and even the home economics department. This activity was done in an effort to reach the student body at the university to enhance their understanding of water issues important to them, and their awareness of the Center and its activities.

Other News Outlets:

The AWRC continues to coordinate with communications staff at the University of Arkansas, University Relations Department, and the Division of Agriculture to increase the Center's reach and inform the greater public through additional news outlets. Specifically, AWRC worked with University Relations to run a story on a research being done by the Center on river impairment in a priority watershed in northwest Arkansas. This news article was distributed via email to over 25,000 faculty, staff and students at the University of Arkansas. The Center has also used Newswire as an outlet to disseminate information about student job opportunities, conferences, and other relevant information.

Publications, Presentations and Degrees:

When soliciting research proposals through the USGS 104B program, AWRC emphasizes several objectives, including the future publication of research results in peer-reviewed scientific literature. During this past year, 22 publications have been submitted or accepted into peer-reviewed scientific journals. These publications are listed within each project report or in the section for publications from previous project years.

AWRC also emphasizes the presentation of research results at local, national and international meetings and conferences, and the support of graduate research assistants. During this past year, 38 oral and poster presentations were given by student and faculty researchers at conferences around the country. Additionally,9 graduate students either successfully completed their graduate studies and have published their thesis or dissertation, or are expected to graduate in coming years.

Summary:

One of the primary missions of the AWRC is the transfer of information to water resources stakeholders. Through the use of an annual water conference, electronic newsletters, maintenance of the websites, publication of reports and fact sheets, engagement through social media, new efforts to inform the student body through targeted infographics, use of additional news outlets, and scientific publications and presentations, AWRC continues to reach a broad audience throughout Arkansas and even the Nation. The Center has helped to ensure that water resources managers have the information necessary to help guide important management decisions.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	10	0	0	0	10
Masters	4	0	0	0	4
Ph.D.	3	0	0	0	3
Post-Doc.	2	0	0	0	2
Total	19	0	0	0	19

Notable Awards and Achievements

Benjamin Runkle -2016AR383B - Graduate student Colby Reavis was awarded the best student poster award at the 2017 Arkansas Soil and Water Education Conference.

Benjamin Runkle - 2016AR383B - Graduate student Colby Reavis received a travel grant from the AsiaRice Foundation. In January, 2017, he visited Youngryel Ryu's research group at Seoul National University in South Korea. There, he learned how to use the Breathing Earth System Simulator (BESS) product, based on remote sensing products and ecophysiological relationships and built by Ryu's group (Ryu et al., 2011; Jiang and Ryu, 2016). The visit to Korea also involved a visit to a rice research site with an eddy covariance tower and discussions about how to better parameterize and clarify the role of rice phenology as an important factor in field ET. Together the site visit and rice phenology discussion highlighted the need to take advantage of cutting edge site-monitoring tools such as drone-based imagery and solar-induced fluorescence.

Sally Entrekin - 2016AR387B - Student received outstanding ecology poster: Huggins, B., Brass, A. Entrekin, S. and Gifford, M. Influence of Common Salt Concentrations on Detritivore Respiration. Arkansas Academy of Science, Conway, AR May 7-8, 2017.

Brian Haggard - 2016AR390B - AWRC got the Beaver Lake Watershed Guardian Award from the Beaver Watershed Alliance

Brian Haggard - 2016AR390B - Graduate student Megan Reavis received 1st Place in the Graduate Student Poster Competition – Geosciences Subdivision, Arkansas Academy of Science, Annual Meeting, Fayetteville, Arkansas; Lord (Reavis), M. and B. Haggard. 2016. Floodplain soils: a potential source of phosphorus to the Illinois River? Arkansas Academy of Science Annual Meeting, Fayetteville, Arkansas.

Brian Haggard - 2016AR390B - Undergraduate freshman engineering students received Best Paper Award – Process Design and Improvement, FEP Honors Research Symposium, Fayetteville, Arkansas; Mitchel, A. and A. Smith. 2016. Evaluating Labs and Methods for Testing Nitrate Concentrations in Surface Water, 8th Annual Freshman Engineering Program Honors Research Symposium, Fayetteville, Arkansas

Publications from Prior Years

1. 2016AR336 ("Development and Implementation of Nutrient Runoff Reduction Measures for Poultry Houses") - Conference Proceedings - Sharpley – invited presentation "Live production area BMPs" at the U.S. Poultry & Egg Association, 2016 Environmental Management Seminar. Destin, FL. September 22, 2016.
2. 2012AR336 ("Development and Implementation of Nutrient Runoff Reduction Measures for Poultry Houses") - Conference Proceedings - Sharpley – invited presentation "Live production area BMPs" at the U.S. Poultry & Egg Association, 2016 Environmental Management Seminar. Destin, FL. September 22, 2016.
3. 2012AR336 ("Development and Implementation of Nutrient Runoff Reduction Measures for Poultry Houses") - Articles in Refereed Scientific Journals - Herron, S.L., K.R. Brye, A.N. Sharpley, D.M. Miller, and M.B. Daniels. 2015. Nutrient composition of dust emitted from poultry broiler houses in Northwest Arkansas. J. Environ. Protect. 6(11):1257-1267. doi: 10.4236/jep.2015.611110. http://www.scirp.org/journal/PaperInformation.aspx?PaperID=61058.
4. 2016AR336 ("Development and Implementation of Nutrient Runoff Reduction Measures for Poultry Houses") - Articles in Refereed Scientific Journals - Herron, S.L., A.N. Sharpley, K.R. Brye, D.M. Miller, S. Watkins, D. McCreery, and M.B. Daniels. 2016. Determination of nutrient concentrations in simulated rainfall-runoff from poultry house dust deposited adjacent to exhaust fans. J. Environ. Protect. 7:27-40. doi: 10.4236/jep.2016.71003. http://www.scirp.org/Journal/PaperInformation.aspx?PaperID=62587.
5. 2016AR336 ("Development and Implementation of Nutrient Runoff Reduction Measures for Poultry Houses") - Articles in Refereed Scientific Journals - Herron, S.L., A.N. Sharpley, K.R. Brye, and D.M. Miller. 2016. Optimizing hydraulic and chemical properties of iron and aluminum byproducts for use in on-farm containment structures for phosphorus removal. J. Environ. Protect. 7:1835-1849. http://file.scirp.org/pdf/JEP_2016112322303649.pdf.
6. 2013AR342 ("Improving Surface Water Quality by Reducing SOD and Nutrients") - Conference Proceedings - Richardson, G. A., G. S. Osborn. 2014. Effects of Sediment Resuspension and Oxygenation on Oxygen Uptake Rate. Paper No. 14-1896896. Annual Meeting ASABE 2014, Montreal, CA.
7. 2016AR342 ("Improving Surface Water Quality by Reducing SOD and Nutrients") - Conference Proceedings - Osborn, G. S. 2014. Dissolved Air Flotation for Removal of Algae and Nutrients from Surface Water. 2014 Annual Meeting ASABE, Montreal, CA.
8. 2013AR342 ("Improving Surface Water Quality by Reducing SOD and Nutrients") - Conference Proceedings - Richardson, G. R. and G. S. Osborn. 2013. Reducing Sediment Oxygen Demand in Eutrophic Lakes. Presentation ASABE International Meeting. Kansas City, MO. Presentation 131606450.
9. 2013AR345 ("Economics of On-Farm Reservoirs across the Arkansas Delta Region: A conjunctive management approach to preserving groundwater and water quality") - Conference Proceedings - Kovacs, K. 2016. Sustaining agricultural economic returns and a shallow aquifer on a landscape using conjunctive water management. Selected Presentation, UCOWR/NIWR Annual Water Resources Conference, Pensacola, FL, 2016, June 21st-23rd
10. 2013AR345 ("Economics of On-Farm Reservoirs across the Arkansas Delta Region: A conjunctive management approach to preserving groundwater and water quality") - Articles in Refereed Scientific Journals - Kovacs, K., M. Mancini. 2017. "Conjunctive water management to sustain agricultural economic returns and a shallow aquifer at the landscape level." Journal of Soil and Water Conservation, forthcoming.
11. 2013AR345 ("Economics of On-Farm Reservoirs across the Arkansas Delta Region: A conjunctive management approach to preserving groundwater and water quality") - Articles in Refereed Scientific Journals - Kovacs, K., G. West. 2016. "The influence of groundwater depletion from irrigated

agriculture on the tradeoffs between ecosystem services and economic returns” PLoS One, 11(12), e0168681.

12. 2014AR350 ("Is persistence of plasmids in antibiotic resistant E. coli isolated from stream water impacted by integrons and conjugation or mobilization genes?") - Articles in Refereed Scientific Journals - Suhartono, S., & Savin, M. (2016). Conjugative transmission of antibiotic-resistance from stream water Escherichia coli as related to number of sulfamethoxazole but not class 1 and 2 integrase genes. Mobile Genetic Elements, 6(6), e1256851.
13. 2014AR350 ("Is persistence of plasmids in antibiotic resistant E. coli isolated from stream water impacted by integrons and conjugation or mobilization genes?") - Articles in Refereed Scientific Journals - Suhartono, S., M. C. Savin, and E.E. Gbur. 2016. Genetic redundancy and persistence of plasmid-mediated trimethoprim/sulfamethoxazole resistant effluent and stream water Escherichia coli. Water Research. 103:197-204. http://dx.doi.org/10.1016/j.watres.2016.07.035.
14. 2014AR353 ("Microbial community under the changing pre-oxidation regime at Beaver Water District") - Articles in Refereed Scientific Journals - Walden, C., F. Carbonero, and W. Zhang. 2016. Preliminary Assessment of Bacterial Community Change Impacted by Chlorine Dioxide in a Water Treatment Plant. Journal of Environmental Engineering, 142(2), 04015077.
15. 2014AR354 ("Economics of multiple water-saving technologies across the Arkansas Delta Region") - Conference Proceedings - Knapp, T. 2016. Return on investment in on-farm storage reservoirs: A landscape perspective. Selected Presentation, SERA35: Delta States Farm Management Group Annual Meeting, Vicksburg, MS, 2016, May 25th-27th.
16. 2014AR354 ("Economics of multiple water-saving technologies across the Arkansas Delta Region") - Articles in Refereed Scientific Journals - Kovacs, K. A. Durand-Morat. 2017. “The influence of on- and off-farm surface water investment on groundwater extraction from an agricultural landscape.” Journal of Agricultural and Applied Economics, doi:10.1017/aae.2016.39
17. 2015AR365 ("REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta") - Conference Proceedings - Suvočarev K, Reba M, Runkle BRK (2016) for presentation at the 32nd Conference on Agricultural and Forest Meteorology, 22nd Symposium on Boundary Layers and Turbulence, and Third Conference on Biogeosciences, Salt Lake City, Utah, 20-24 June 2016.
18. 2015AR365 ("REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta") - Conference Proceedings - Runkle BRK, Paddy Rice Research Group, Global Research Alliance talk, July 14, 2016, at Stuttgart meeting, and field site presentation. Talk titled: “Alternate Wetting and Drying as an Effective Management Practice to Reduce Methane in Arkansas Rice Production”
19. 2015AR365 ("REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta") - Conference Proceedings - Runkle BRK, Presentation at Ameriflux PI meeting, Golden Colorado, September, 2016: Neighboring fields, neighboring towers: Testing climate-smart irrigation strategies to reduce methane emissions from rice fields
20. 2015AR365 ("REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta") - Conference Proceedings - Reba ML, Fong B, Runkle BRK, Suvocarev K, Adviento-Borbe A: Winter fluxes from Eastern Arkansas Rice-Waterfowl Habitats, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA
21. 2015AR365 ("REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta") - Conference Proceedings - Suvocarev K, Reba M, Runkle, BRK: Surface renewal: micrometeorological measurements avoiding the sonic anemometer, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA
22. 2015AR365 ("REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta") - Conference Proceedings - Runkle BRK, Suvocarev K, Reba ML, Novick KA, White P, Anapalli S, Locke MA, Rigby J, Bhattacharjee J, Variation in agricultural CO2 fluxes during the growing season, collected from more than ten eddy covariance towers in the Mississippi Delta Region, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA
23. 2015AR365 ("REWARD: Rice Evapotranspiration and Water use in the Arkansas Delta") - Conference Proceedings - Roby M, Reavis C, Reba M, Suvocarev K, Runkle BRK, Testing the

reduction of methane emissions from alternate wetting and drying in rice fields: two years of eddy covariance measurements from Arkansas, Presented at American Geophysical Union Fall Meeting, Dec., 2016, San Francisco, CA

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