Illinois Water Resources Center
Annual Technical Report
FY 2012

Introduction

The Illinois Water Resources Center serves the people of Illinois by providing science-based information and resources about the water research, legislation, and education developments in the state. Located at the University of Illinois at Urbana-Champaign, IWRC is near the center of a diverse state that includes both the third largest metropolitan area in the nation, Chicago, and some of the most active coalfields in the United States; its water resources are as disparate as Lake Michigan, the Mississippi River, and a vast network of ditches draining Illinois' rich agricultural fields. Consequently, the associated research and technology needs for these water resources are varied and rapidly changing, especially in 2012, which was marked by droughts leading to historically low water levels in Lake Michigan and the Mississippi River.

In 2012, IWRC researchers funded by 104B and 104G, the USGS, and the U.S. Army Corps of Engineers completed 5 projects and continued work on 2. Outreach and technology transfer activities have included our biennial conference, redesigning the website, launching a blog, establishing a social media presence, and supporting local agencies through Smallwatersupply.org and the Private Well Class.

Research Program Introduction

In 2012, IWRC leveraged over $796,000.00 for research projects in Illinois through funding from the U.S. EPA, the U.S. Geological Survey, the U.S. Army Corps of Engineers, and our own programmatic funds. The research supported by these funds has delved into the willingness of people to pay or volunteer to support low impact flood reduction techniques, determined new discharge ratings of the Chicago River, and the modeled the movement of Asian carp eggs in a bid to control these persistent invasive species. The majority of this research was conducted by young scientists just entering the field of water resources.

Influence of Algal/ Bacterial Interactions on Denitrification in Stream Biofilms

Basic Information

Title:	Influence of Algal/ Bacterial Interactions on Denitrification in Stream Biofilms
Project Number:	2011IL219B
Start Date:	3/1/2011
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Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	
Principal Investigators:	John Kelly, Kimberly Gray, Christopher Peterson, Miguel Rojas

Publication

1. Kalscheur, Katheryn; Miguel Rojas; Christopher Peterson; John Kelly; Kimberly Gray, 2012, Algal Exudates and Steam Organic Matter Influence the Structure and Function of Denitrifying Bacterial Communities, Microbiology Ecology, 64, 881-892.

Final Report

Project Title: Influence of Algal / Bacterial Interactions on Denitrification in Stream Biofilms

Principal Investigator: Dr John Kelly
Associate Professor
Department of Biology
Loyola University Chicago
1032 West Sheridan Rd.
Chicago, IL 60660
phone: 773.508.7097
email: jkelly7@luc.edu

Problem and Research Objective :

Over the last century, since the development of a commercially viable process for converting atmospheric nitrogen (N_2) to ammonium (the Haber–Bosch process), the input of nitrogen to the environment has increased dramatically. In fact, the amount of nitrogen fixed annually by industry now exceeds that fixed by combined biological processes in ecosystems worldwide (Vitousek et al. 1997). Much of the N that is fixed commercially is applied to agricultural soils, and much of this N ends up in freshwater or marine systems via leaching and runoff. Compared to pre-industrial times, annual N loading rates into freshwaters in the United States have increased 6 to 50-fold (Carpenter et al., 1998), with a concurrent 20-fold increase in N loading into the oceans from the world's largest river systems (Howarth et al. 1996).

Increased nitrogen inputs to surface waters can lead to decreased water quality. For example, high levels of nitrogen can result in eutrophication (Officer et al. 1984, Nixon et al. 1996) which can lead to excessive growth of algae. Algal blooms followed by algal decomposition can lead to a decrease in the amount of dissolved oxygen in the water, a condition known as hypoxia (Nixon et al. 1996), which can cause the death of fish and other aquatic organisms (Carpenter et al. 1998, Goolsby et al. 2001). A well publicized example of this phenomenon can be seen annually in the Gulf of Mexico. Nitrogen inputs to the gulf have tripled in the last 30 years and have been linked to the development of a huge seasonal hypoxic zone (Alexander et al. 2000, Goolsby et al. 2001). Fishermen refer to this hypoxic zone as a "dead zone" due to the lack of fish, crabs, and shrimp.

Nitrogen pollution of rivers and streams is an especially significant issue for the Midwestern United States and for the State of Illinois in particular due to the high levels of agriculture in this region. The soils in this region receive high inputs of N fertilizers, and much of this N finds its way into the Mississippi River and eventually to the Gulf of Mexico. Although Illinois represents approximately 6% of the drainage area of the Mississippi River, it is responsible for about 15% of the nitrates in the river (David and

Gentry 2000), so Illinois has a disproportionately large impact on downstream water quality.

Due to the significant environmental problems linked to N pollution, there is interest in biological processes that can remove nitrogen from aquatic ecosystems. Denitrification is a microbially catalyzed dissimilatory redox process through which nitrate (NO_3^-) is reduced to gaseous nitrogen (primarily N_2). Nitrate is a soluble, biologically available form of nitrogen, but N_2 is a gas that will diffuse to the atmosphere and has limited biological availability (it is only available to N fixing bacteria). Therefore, denitrification has the capacity to reduce N pollution of surface waters by removal of nitrogen from the system.

Denitrification is catalyzed primarily by denitrifying bacteria and it tends to be favored in anoxic habitats, so high rates of denitrification are often observed in aquatic sediments just below the sediment water interface. However, a number of studies have demonstrated that significant denitrification also occurs within periphytic biofilms (Nakajima 1979; Sorensen et al. 1988; Eriksson & Weisner 1997; Eriksson 2001; Toet et al. 2003; Sladeckova et al. 1983, Baldwin et al. 2006), which are mixed communities of algae and bacteria that can be found attached to solid surfaces within aquatic ecosystems (Wetzel 1983). Within these biofilms algae grow autotrophically producing fixed carbon and heterotrophic bacteria utilize the carbon produced by algae, supplied either via extracellular release or algal decomposition (Haack & McFetters 1982; Murray et al. 1986; Paerl & Pinckney 1996). Recent studies by Co-PI Gray have demonstrated that denitrification rates per cell can actually be much higher in periphytic biofilms as compared to sediments, thus suggesting that biofilms might be hot-spots for denitrification activity within aquatic ecosystems (Sirivedhin & Gray 2006). Biofilms therefore have the potential to make significant contributions to nitrogen removal from aquatic ecosystems. Unfortunately relatively little is known about the mechanisms driving denitrification in these biofilms.

The objective of our project is to explore the ecology of periphytic biofilms with a specific focus on denitrification. We will accomplish this goal through a combination of field and laboratory-scale experiments. This project will improve our understanding of the ecology of denitrification in streams and rivers, and it will provide insight into environmental factors that influence denitrification rates. This work could have significant implications for the management and remediation of nitrogen pollution and the design of systems for nitrogen removal, including wetland restoration and the construction of artificial treatment wetlands. Specifically, our project will provide details on environmental and ecological conditions that will maximize denitrification, details that could be incorporated into the design of treatment systems in order to maximize nitrogen removal.

Prior to the IWRC award our research team conducted a short term (28 day) field experiment in two streams in DuPage County Illinois that differed in degree of human impact. The low impact stream was in a restored prairie watershed and the high impact stream was directly downstream of a wastewater treatment plant that released its

effluent to the stream. Our results demonstrated that there was a tight coupling between the species composition of the algal and bacterial communities at the low-impact site, supporting the important role that algae can play in shaping bacterial communities within biofilms. However, at the high-impact sight the algal and bacterial communities were de-coupled, suggesting a significant role of anthropogenic inputs in shaping biofilm bacterial communities. The results of this study were recently published in the journal *FEMS Microbiology Ecology* (Peterson et al., 2011). These results generated several lines of inquiry into the ecology of periphytic biofilms:

Research Element 1: Explore the influence of algae on bacterial community composition within biofilms

Element 1 Part A: The short term field project described above suggested that algae could be drivers of bacterial community development in periphytic biofilms. We wanted to explore the potential influence of specific algal species on bacterial community development in controlled lab experiments. **We explored this question as part of the IWRC funded project, as detailed below.**

Element 1 Part B: We hypothesized that algae might influence bacterial community development in biofilms through the production and release of species specific organic compounds, which might select for different bacterial communities. **We tested this hypothesis as part of the IWRC funded project, as detailed below.**

Element 1 Part C: The short term field project described above suggested a tight coupling between algal and bacterial communities in one stream during the first 28 days of biofilm development. We wanted to determine if this coupling would be observed in a wider arrays of streams over a longer period of time. We conducted a larger scale investigation of periphytic biofilms using six streams over a period of 12 weeks. **The analysis of the bacterial communities in these samples was part of the IWRC funded project, as detailed below.**

Research Element 2: Explore the mechanisms by which anthropogenic inputs might influence bacterial species composition within biofilms

Element 2 Part A: We hypothesized that the organic compounds within wastewater effluent might be disrupting the coupling between algal and bacterial communities within biofilms. To explore this we used Pyrolysis GC/MS to compare the organics within rivers with varying degrees of

wastewater inputs. We found that the dissolved organic carbon in streams that received discharge from upstream wastewater treatment plants was distinct from the organic carbon in streams that did not receive discharge, and we were able to identify specific pyrolysis fragments that were indicative of upstream wastewater treatment plant discharges. This work was completed and recently published in the journal *Water Research* (Kalscheur, Penskar, et al., 2012). This research element was not part of the IWRC funded project, but this work did contribute to our understanding of these systems and lead to further research questions that were explored in the IWRC funded project, as detailed below.

Element 2 Part B: Our next step was to explore the impacts that organics from streams with and without wastewater inputs might have on bacterial communities within biofilms. **We explored this question as part of the IWRC funded project, as detailed below.**

Methodology for IWRC Funded Project

1. **Research Element 1 Part A: Influence of algal species identity on bacterial community development in biofilms**

   We established axenic cultures of four algal species that were numerically dominant at our field sites, *Achnanthidium minutissimum* (AM), *Achnanthidium lanceolatum* (AL), *Amphora pediculus* (AP) and *Nitzschia amphibia* (NA), and we collected a mixed bacterial community from streams at both of our field sites (the sites are described in Peterson et al., 2011). We then set up replicated lab-scale microcosms each consisting of a single algal species, and amended each microcosm with the mixed bacterial community. We monitored biofilm development in the microcosms over a period of 80 days. We used quantitative real-time PCR (qPCR) to quantify denitrifying bacteria within the biofilms. We used another leading-edge DNA-based approach, tag pyrosequencing, to assess the composition of the denitrifying bacterial communities within the biofilms. **This work was conducted by a graduate student at Loyola University Chicago, Miguel Rojas, who was supported by the IWRC award.**

2. **Research Element 1 Part B and Research Element 2 Part B: Effects of organic carbon source on the development of denitrifying bacterial communities in biofilms.**

   We were interested in exploring the effects of algal-derived organic carbon (Research Element 1 Part B) and organic carbon from stream sites that varied in

anthropogenic inputs (Research Element 2 Part B). To accomplish this we isolated organic exudates from axenic cultures of seven algal species that were common in our field sites. We also isolated organic carbon from the two streams in DuPage county that we had used for our short term study (see Peterson et al., 2011). We set up small-scale microcosms in which a mixed population of bacteria that was collected from the field was incubated with organic carbon from one of these sources. We measured rates of denitrification and development of denitrifying bacterial communitties using qPCR and tag pyrosequencing. **The analysis of the bacterial communities for this project was conducted by a graduate student at Loyola University Chicago, Miguel Rojas, who was supported by the IWRC award.**

3. **Research Element 1 Part C: Biofilm development in six streams of varied anthropogenic influence**

   Six streams in DuPage County Illinois that differed in degree of human impact, with some sites located directly downstream of wastewater treatment plant effluent discharge points and some streams running through protected areas, including prairies and forests, were selected for this field study. Ceramic tiles were incubated in each stream for a total of 12 weeks to provide a uniform substrate for the development of periphytic biofilms. Tiles were collected after 3, 5, 8 and 12 weeks and returned to the lab for biofilm analysis. On each sampling date, 500 ml of stream water was also collected to assess nutrient chemistry. **The analysis of the bacterial communities within these biofilms was conducted by a graduate student at Loyola University Chicago, Miguel Rojas, who was supported by the IWRC award.**

Principal Findings and Significance

1. **Research Element 1 Part A: Influence of algal species identity on bacterial community development in biofilms**

   The results of this project indicated that different algal species supported different abundances of denitrifying bacteria (Fig. 1). In addition, the species composition of the denitrifying bacterial communities was also influenced by the identity of the algal species (Fig. 2). This work was conducted by a graduate student at Loyola University Chicago, Miguel Rojas, who was supported by the IWRC award. Miguel is currently preparing a manuscript based on this project that will be submitted to a peer reviewed scientific journal. Miguel will serve as the first author on this manuscript and the support of IWRC will be acknowledged.

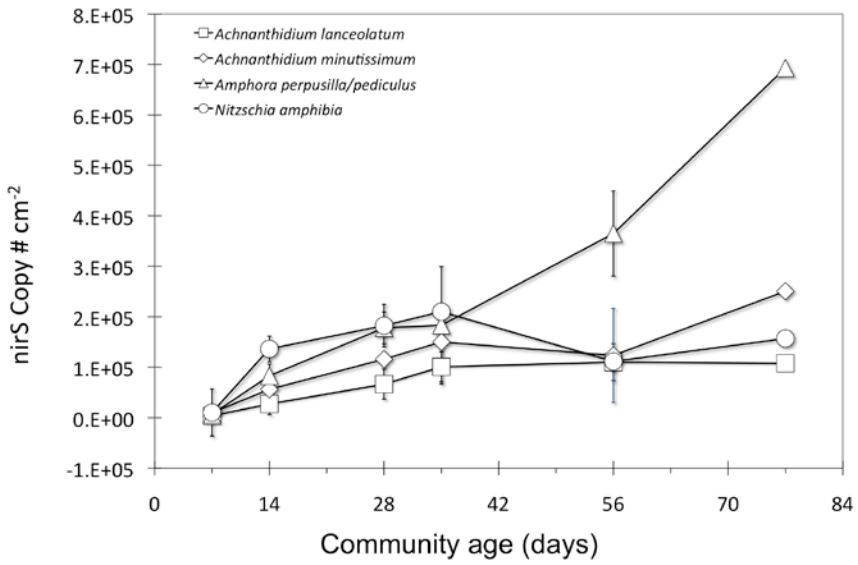

Fig 1. Effect of algal species identity on abundance of denitrifying bacteria as indicated by quantitative PCR (qPCR) quantification of *nirS* copy numbers. Each data point represents mean of four replicates and error bars represent standard error.

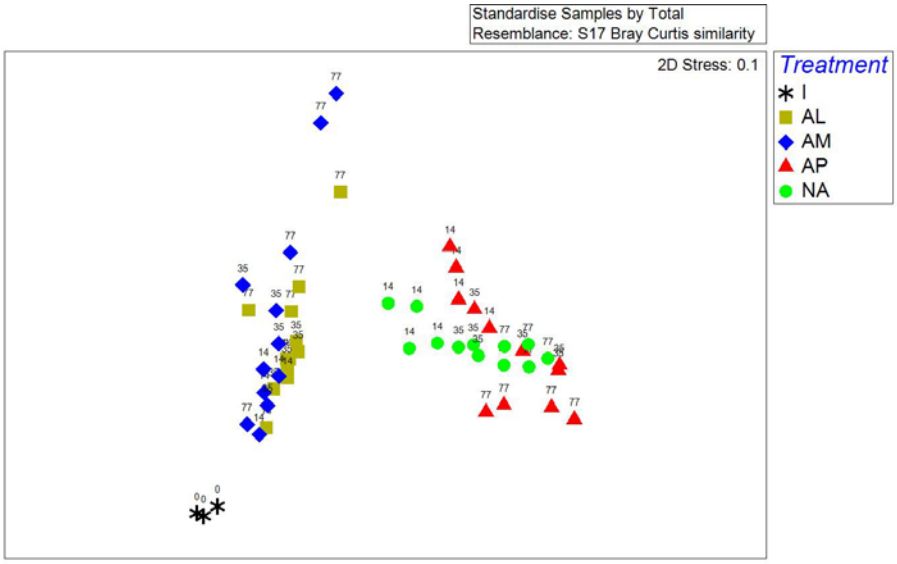

Fig 2. Effect of algal species identity on denitrifier community composition. Ordination is based on MDS analysis of *nosZ* gene sequences present within each biofilm. Gene sequences were determined via tag pyrosequencing of *nosZ* gene. Letters indicate algal species present in each microcosm (Achnanthidium lanceolatum (AL), Achnanthidium minutissimum (AM), Amphora perpusilla/pediculus (AP) and Nitzschia amphibia (AM). The initial bacterial inoculum is shown as "I" on the ordination. Numbers above each data point indicate duration of incubation (in days).

2. **Research Element 1 Part B and Research Element 2 Part B: Effects of organic carbon source on the development of denitrifying bacterial communities in biofilms.**

   Our analysis revealed differences in the organic composition of algal exudates and stream waters, which, in turn, selected for distinct communities of denitrifying bacteria. Organic carbon source also had a significant effect on denitrification rates of the communities. One especially interesting finding was that organic carbon from the stream with low human inpacts selected for a bacterial community that was similar to the community produced by incubation with algal-derived organic carbon. In contrast, organic carbon from the stream that received wastewater input selected for a distinct bacterial community that also showed the highest rates of denitrification. This result supported our earlier finding (as reported in Peterson et al., 2011) that anthropogenic inputs can alter the composition of bacterial communities within biofilms. Miguel Rojas, a graduate student at Loyola University Chicago who was supported by the IWRC award, contributed the bacterial community analysis to this project. **This work was recently published in the journal *Microbial Ecology* (Kalscheur, Rojas et al 2012) and the support of IWRC was acknowledged (see attachment).**

3. **Research Element 1 Part C: Biofilm development in six streams of varied anthropogenic influence**

   Our analysis revealed that the streams at our field sites varied significantly in a variety of physico-chemical characteristics, including depth, velocity, concentrations of N and P, and DOC concentration. Tiles from all sites developed biofilms over the course of the study, although the biofilm mass varied for the different sites. Half of the sites showed measurable rates of denitrification, and these tended to be the sites with higher levels of biofilm mass. The sites differed in algal species composition (Fig. 3). Miguel Rojas, a graduate student at Loyola University Chicago who was supported by the IWRC award, was responsible for analysis of the bacterial communities in these biofilms. Miguel used terminal restriction fragment lentgth polymorphism (T-RFLP) analysis to profile the bacterial communities within the biofilms (Fig. 4). This analysis did not show a clear separation of bacterial communities based on site. Miguel also profiled the denitrifying bacterial communities within the biofilms using tag pyrosequencing. This analysis focused on the denitrifying bacteria in particular based by targeting *nosZ*, a functional gene involved in the denitrification pathway. These results demonstrated a strong seperation of denitrifying communities based on site (Fig. 5). Our analysis of the relationships between algal and bacterial communities at these sites is ongoing, and we are in the process of preparing a manuscript based on this project that will be submitted to a peer reviewed scientific journal. Miguel will serve as a co-author on this manuscript, and the support of IWRC will be acknowledged.

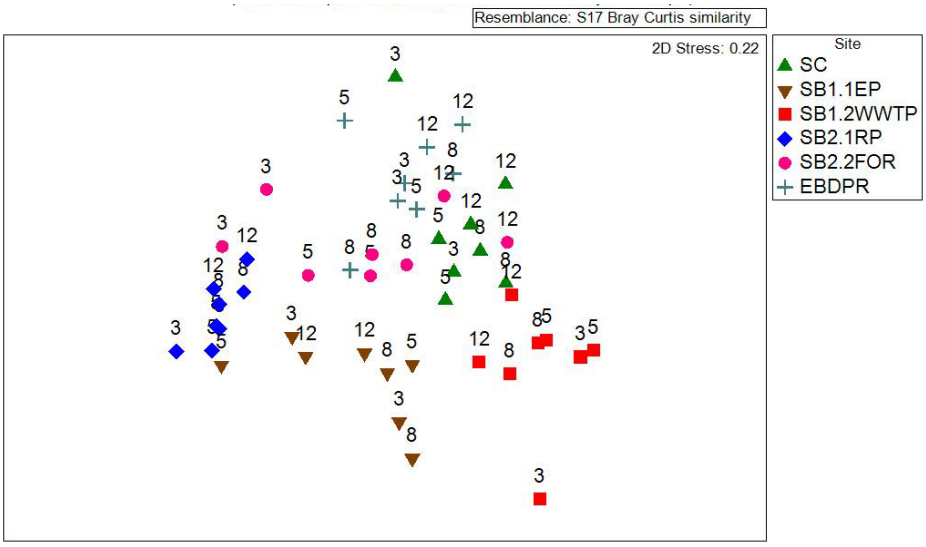

Fig 3. Algal community composition in biofilms from six field stream sites. Ordination is based on MDS analysis of algal species abundance data determined by microscopic identification of algal cells. Different colors and shapes indicate different stream sites, and numbers above data points indicate the number of weeks that tiles were incubated in the streams (i.e. time of biofilm development).

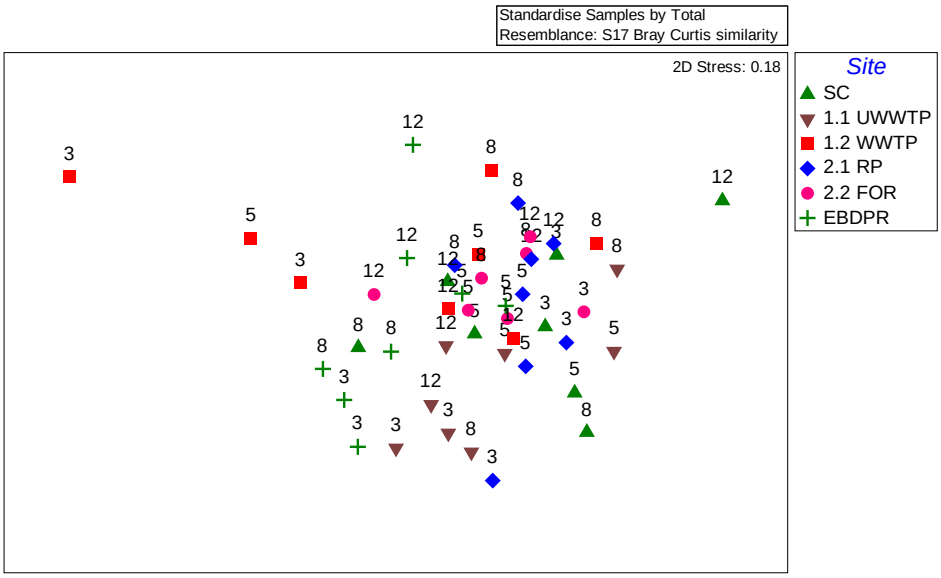

Fig 4. Total bacterial community composition in biofilms from six field stream sites. Ordination is based on MDS analysis of T-RFLP analysis of 16S rRNA genes. Different colors and shapes indicate different stream sites, and numbers above data points indicate the number of weeks that tiles were incubated in the streams (i.e. time of biofilm development).

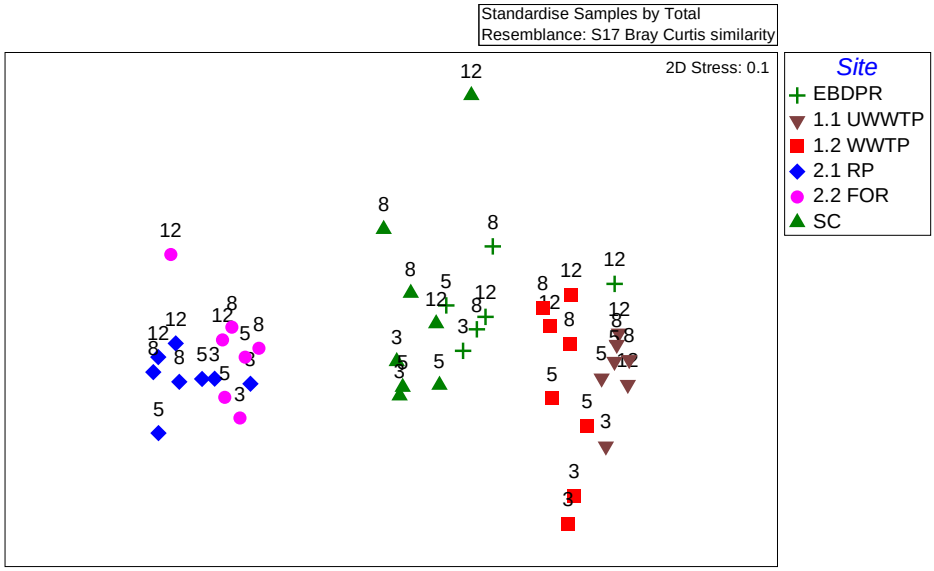

Fig 5. Denitrifying bacterial community composition in biofilms from six field stream sites. Ordination is based on MDS analysis of pyrosequencing analysis of *nosZ* genes. Different colors and shapes indicate different stream sites, and numbers above data points indicate the number of weeks that tiles were incubated in the streams (i.e. time of biofilm development).

Notable Achievements

- We demonstrated that the input of wastewater treament plant effluent to streams can disrupt the ecology of biofilms and influence an envrionmentally significant process, denitrification
- We demonstrated that pyrolysis GS/MS technology can be used to discriminate the organic carbon in streams with varying degrees of wastewater inputs, and that wastewater inputs can alter the organic composition of streams
- We have gained new insight into the realtionships between algal species and bacterial species within biofilm communities

Students Supported with Funding

Miguel Rojas, a graduate student in the MS program in Biology at Loyola University Chicago was supported by the IWRC award. Miguel entered the program in Fall of 2010 and is planning to graduate with his MS degree in Spring 2011.

Publications and Presentations

We have published one paper that included results obtained with IWRC support. This paper (which is attached) acknowledged IWRC support.

- Kalscheur, K.N., M. Rojas, C.G. Peterson, J.J. Kelly, and K.A. Gray. 2012. Algal exudates and stream organic matter influence the structure and function of denitrifying bacterial communities. Microbial Ecol. 64: 881-892.

We have two other publications in preparation that were also supported by the IWRC award. These papers will acknowledge IWRC support.

- Peterson, C.G., A.D. Daley, S.M. Pechauer, K.N. Kalscheur, M. Rojas, K.A. Gray, and J.J. Kelly. Development of algal and bacterial communities in periphytic biofilms in streams with varied anthropogenic inputs. *In preparation.*
- Rojas, M., K.N. Kalscheur, C.G. Peterson, K.A. Gray, and J.J. Kelly. Algal taxa influence the development of denitrifying bacterial communities within biofilms. *In preparation.*

Miguel Rojas, a graduate student at Loyola University Chicago who was supported by the IWRC award, presented a poster based on his work at the 2012 General Meeting of the American Society for Microbiology.

- Rojas, M., K.N. Kalscheur, K.A. Gray, C.G. Peterson, and J.J. Kelly. Algal species identity influences the development of denitrifying bacterial communities in periphytic biofilms. General Meeting of the American Society for Microbiology, June 16 - 19, 2012, San Francisco, CA.

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MICROBIOLOGY OF AQUATIC SYSTEMS

Algal Exudates and Stream Organic Matter Influence the Structure and Function of Denitrifying Bacterial Communities

Kathryn N. Kalscheur · Miguel Rojas · Christopher G. Peterson · John J. Kelly · Kimberly A. Gray

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Abstract Within aquatic ecosystems, periphytic biofilms can be hot spots of denitrification, and previous work has suggested that algal taxa within periphyton can influence the species composition and activity of resident denitrifying bacteria. This study tested the hypothesis that algal species composition within biofilms influences the structure and function of associated denitrifying bacterial communities through the composition of organic exudates. A mixed population of bacteria was incubated with organic carbon isolated from one of seven algal species or from one of two streams that differed in anthropogenic inputs. Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) revealed differences in the organic composition of algal exudates and stream waters, which, in turn, selected for distinct bacterial communities. Organic carbon source had a significant effect on potential denitrification rates (DNP) of the communities, with organics isolated from a stream with high anthropogenic inputs resulting in a bacterial community with the highest DNP. There was no correlation between DNP and numbers of denitrifiers (based on *nirS* copy numbers), but there was a strong relationship between the species composition of denitrifier communities (as indicated by tag pyrosequencing of *nosZ* genes) and DNP. Specifically, the relative abundance of *Pseudomonas stutzeri*-like *nosZ* sequences across treatments correlated significantly with DNP, and bacterial communities incubated with organic carbon from the stream with high anthropogenic inputs had the highest relative abundance of *P. stutzeri*-like *nosZ* sequences. These results demonstrate a significant relationship between bacterial community composition and function and provide evidence of the potential impacts of anthropogenic inputs on the structure and function of stream microbial communities.

K. N. Kalscheur · K. A. Gray (✉)
Department of Civil and Environmental Engineering,
Northwestern University,
Evanston, IL, USA
e-mail: k-gray@northwestern.edu

M. Rojas · J. J. Kelly (✉)
Department of Biology, Loyola University Chicago,
Chicago, IL, USA
e-mail: jkelly7@luc.edu

C. G. Peterson
Department of Environmental Science,
Loyola University Chicago,
Chicago, IL, USA

Introduction

Denitrification, the reduction of nitrate to dinitrogen, is an ecologically significant process that removes nitrogen from aquatic ecosystems and can ameliorate the effects of nitrogen pollution. However, the potential of surface waters to support denitrification has steadily diminished with the loss of habitats and aquatic buffer zones, such as forested and coastal wetlands, the losses of which continue to substantially outdistance gains [18]. With the amount of nitrogen fixed from the atmosphere for human use vastly exceeding biophysical thresholds, much of this new reactive nitrogen has accumulated in the environment, which has led to detrimental environmental changes [37] including the seasonal hypoxic zone in the Gulf of Mexico [36]. Recent results indicate that since 1980 little progress has been made in reducing nitrate concentrations in the Mississippi River and that flow-normalized nitrate inputs to the Mississippi are increasing in some areas [47]. Therefore, there is significant interest in understanding the factors that control rates of denitrification in aquatic ecosystems.

Within aquatic ecosystems, periphytic biofilms can serve as hot spots for a myriad of biogeochemical processes including denitrification [8, 46, 48, 50]. The metabolic processes of bacteria within biofilms can be influenced significantly by interactions with resident microalgae, as bacteria often rely on algal-derived organic carbon to fuel their metabolism [6, 14, 24]. The strength of algal/bacterial interactions and, by inference, their importance in mediating biogeochemical processes within biofilms can be influenced by exogenous factors such as nutrient concentrations and supply rates [33, 35, 42, 54]. Less is known about the degree to which variation in algal species composition influences the structure and metabolic performance of bacterial consortia in freshwater biofilms. Results of Ishida et al. [27] suggested that rates of denitrification in biofilms varied depending on the degree of dominance of certain algal genera within those biofilms. This led us to investigate more closely the relative influence of algal species assemblage on the composition and performance of consortia of denitrifying bacteria within benthic biofilms.

We hypothesize that algal communities influence the composition and function of denitrifying bacterial communities within periphyton through interspecific variation in the composition of organic exudates produced by the algae. The rationale for this hypothesis is based on previous data illustrating that bacteria within periphyton use algal-derived exudates as a carbon source to fuel their metabolism [6, 24], that different algal taxa can produce chemically distinct exudates [3, 25, 52], and that different algal species can support taxonomically distinct communities of satellite bacteria [22, 30, 40]. In addition, the ability to denitrify is widely dispersed among the prokaryotes, being found in about 50 bacterial genera [43]. There are differences among these genera in their oxygen thresholds, carbon requirements, and kinetic parameters [49], suggesting that the species composition of denitrifying bacterial communities may have significant functional implications. Several studies have demonstrated a relationship between the composition of denitrifying communities and the process of denitrification in terrestrial ecosystems [16, 26] and stream sediments [5].

To test our hypothesis, we designed an experiment that used a mixed population of bacteria obtained from local streams and incubated this mixed community with organic carbon isolated either from one of seven different algal species or from the waters of two streams that varied in degree of anthropogenic inputs. To determine if the organic carbon derived from these different sources influenced the composition and function of denitrifying bacteria, we quantified denitrifying bacteria based on quantitative real-time polymerase chain reaction (qPCR), assessed the composition of denitrifying bacterial communities using tag pyrosequencing, and measured potential denitrification rates using the acetylene inhibition method. Denitrifying bacteria were profiled based on functional genes involved in the denitrification pathway (*nirS* and *nosZ*), because the phylogenetic diversity of denitrifiers makes 16S rRNA gene-based approaches inadequate to target this functional guild [12]. In this experiment, we also used Py-GC/MS to fingerprint the salient chemical characteristics of each organic source and then used these data to determine if we can identify distinct chemical structural features that are associated with the selection of particular bacterial community composition and function [28, 35, 46].

Methods

Algal Exudates and Stream Water Organic Carbon

Triplicate axenic algal batch cultures were established for five diatoms [*Achnanthidium minutissimum* (Kütz.) Czarnecki (AM), *Achnanthidium lanceolatum* Bréb. ex Kütz. (AL), *Amphora pediculus* (Kütz.) Grun. (AP), *Gomphonema parvulum* (Kütz.) Kütz. (GP), *Nitzschia amphibian* Grun. (NA)] and two green algae [*Scenedesmus armatus* (R. Chodat) R. Chodat (SA) and *Stigeoclonium tenue* (C. Agardh) Kütz. (ST)] from algal inocula obtained from The Culture Collection of Algae at the University of Texas at Austin. These algal species were selected based upon previous studies and on our field and artificial stream observations of their potential influence on denitrification [27]. Cultures were grown in 250 ml Erlenmeyer flasks filled with 150 ml of Chu's Medium No. 10 and capped with a sponge and stainless steel cap. Growth was monitored by cell counts, and when stationary phase growth was observed, the extracellular organics were separated from the cells by filtering (pre-rinsed 0.45 mm Tuffryn® membrane filters).

The effects of organic carbon from the axenic algal cultures were also compared to the dissolved organic carbon isolated from two stream waters with contrasting anthropogenic influences. Water samples were collected on August 4, 2009 from two streams in DuPage County, IL, one within a Restored Prairie (RP) site that did not receive significant anthropogenic inputs and one located downstream of a wastewater treatment plant effluent release (DER). These sites are described in detail in [28, 35]. Nearly 100 % of the stream flow at the DER site on this date can be attributed to the Wheaton Sanitary District Plant's effluent discharge as calculated from field measurements and plant operating data. Stream waters were collected in acid-washed amber glass jugs, filtered (pre-rinsed 0.45 μm Tuffryn® membrane filters) and stored at 4 °C.

The dissolved organic carbon concentration in the filtered cultures and stream waters was measured by high

temperature catalytic oxidation (APHA Standard Method 5310 B; Dohrmann Apollo 9000). Each organic carbon sample was then split, with a portion reserved for organics analysis via Py-GC/MS and a portion used as the carbon source in the bacterial incubations.

Bacterial Inoculum

A mixed bacterial consortia was collected from periphyton attached to rocks in three streams with varying anthropogenic influences, including the RP and DER sites mentioned above and a site on the E. Branch DuPage River that had upstream input of wastewater treatment plant effluent. Periphyton was removed from rocks by scraping with a stiff-bristle toothbrush into stream water. The collected periphyton from all three sites was homogenized and filtered through a 5 μm Teflon filter (Micron Separations Inc.) with the filtrate containing the bacterial consortia preserved at -85 °C in a 15 % (v/v) glycerol solution. Initial experiments indicated insufficient bacterial mass for the measurement of denitrification. Therefore, prior to the start of the incubations with different carbon substrates, the bacteria were pre-fed M9 Minimal Media [39] with glucose (G) as the carbon source until exponential growth was observed. The bacteria were then washed ten times in M9 Minimal Media without a carbon source.

Incubations

Subsamples of the bacterial inoculum (IN) were incubated for 14 days with equal amounts (1 mg) of the different carbon substrates from seven algae and two stream waters. A G incubation was also included as a control. Each incubation was done in duplicate. Carbon substrates included G, carbon isolated from one of the monoalgal cultures (AL, AM, AP, GP, NA, SA, or ST) or carbon isolated from one of the two field sites (DER or RP). Incubations were conducted in 250 ml gas-tight jars (I-CHEM septa jars) containing 190 ml M9 minimal media (without glucose) and 40 mg l^{-1} NO_3-N as KNO_3 to provide enriched conditions for denitrification. At the start of the incubation, the jars were sealed and flushed with N_2 for 3 min. During the incubation, jars were agitated gently at 150 rpm, under dark conditions at room temperature (23± 1 °C). The dissolved organic carbon concentration in each of the jars was measured four times during the 14-day incubation period by removing 3 ml of solution by syringe through the jar's septa and measuring dissolved organic carbon (DOC) by high-temperature catalytic oxidation (APHA Standard Method 5310 B; Dohrmann Apollo 9000).

Measurement of Denitrification Potential

Denitrification potential (DNP) of incubated bacterial communities was determined by a modified version of the standard acetylene inhibition method for ecological research [2, 21, 46]. To ensure enriched conditions for the DNP analyses, 15 ml containing 40 mg l^{-1} NO_3-N as KNO_3 and 100 mg l^{-1} carbon as G were added to the jars by syringe along with 225 mg l^{-1} chloramphenicol to inhibit microbial growth. The DNP incubation started immediately after the jars were flushed with N_2 for 3 min and acetylene was added (10 % v/v) to inhibit transformation of N_2O to N_2. During the DNP incubation, the jars were agitated gently at 150 rpm under dark conditions at room temperature (23±1 °C). Headspace samples were measured using a gas chromatograph (Hewlett Packard 5890) equipped with ^{63}Ni electron capture detector at an operating temperature of 320 °C. A stainless steel Porapak Q (80/100 mesh) column was used to separate the gases at 60 °C with high purity N_2 as a carrier gas (18–20 ml min^{-1}). Denitrification rates were calculated from linear regression of N_2O accumulation in the headspace, after the concentrations were corrected for solubility using the Bunsen coefficient [51]. Effect of carbon source on DNP rates was assessed by analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test using Systat version 13 (Systat Software, San Jose, CA). DNP rates were square root transformed prior to ANOVA to account for non-homogeneity of variances.

Sample Collection and Processing

At the end of the incubation period jars were stored overnight at 4 °C. From each jar 200 ml of microbial suspension was removed and centrifuged at 12,000×*g* for 10 min. Supernatant was removed by decanting and the pellet was resuspended in 1.5 ml of supernatant and transferred to a 2 ml screw cap microcentrifuge tube. Each suspension was centrifuged at 12,000×*g* for 5 min, supernatant was removed by pipeting and the remaining pellet was stored at -20 °C. DNA was isolated from each pellet using the UltraClean microbial DNA kit (MoBio Laboratories, Carlsbad, CA). Successful DNA isolation was confirmed by agarose gel electrophoresis.

Organic Characterization by Py-GC/MS

For the stream waters, sample preparation involved first distilling approximately 4 L of the filtered water sample in a series of evaporations carried out using rotary vacuum evaporation (Buchi rotavapor Model R114 with B480 Waterbath) operating at 25 °C and under a vacuum pressure of approximately 27.5 in. Hg. To reduce salt interferences, which can exert matrix effects during pyrolysis, the concentrated liquid samples were dialyzed with a 2000 molecular weight cutoff membrane (Thermo Scientific Slide-A-Lyzer G2 Dialysis Cassette).

The concentrated liquid samples and filtered algal exudates were lyophilized, homogenized, and loaded into

pyrolysis tubes (unsealed quartz tube, Scientific Instrument Services, Inc.) to approximately 0.8–1.0 mg of carbon and plugged with deactivated glass wool. External standards of polystyrene and poly-L-tyrosine were run before each analysis series and within a series to verify proper instrument functioning. After preparation, the sample or standard was placed inside the platinum filament coil of the pyrolysis probe (Chemical Data Systems Pyroprobe, 2000) which was then inserted into the interface (Chemical Data Systems 1500 valved GC interface). Conditions of the pyrolysis unit used to ensure reproducibility of the analysis include: (1) pyrolysis interface temperature of 250 °C, (2) final pyrolysis temperature of 625±5 °C, (3) total pyrolysis time of 1 min, and (4) ramping rate of 20 °C ms^{-1}. After flash pyrolysis, the volatile pyrolyzates were directly swept onto a GC column to be separated (60-m, 0.25-mm internal diameter, cross-bond, carbowax column; Restek: Stabilwax®). The gas chromatograph (Fisons 8030) was operated in a splitless injection mode with a column head pressure of 25 psi. Oven temperature was held at 45 °C for 15 min, then ramped up to 240 °C at 2 °C min^{-1}, and finally, held at 240 °C for 10 min. The separated fragments were identified by MS (Fisons MD 800) that operated at 70 eV and scanned from 20 to 400 amu at 1 scan s^{-1}. Positive electron ionization (EI+) mode was used as an ion source. The source temperature was set at 200 °C and the GC/MS interface temperature was 250 °C. A GC/MS data acquisition software, Xcalibur version 1.2, was used to collect the mass to charge (*m*/*z*) scan and produce a pyrochromatogram. The National Institute of Standard Technology (NIST) Library (match at >800/1000) was then used to identify the fragments in the pyrochromatogram. Principal component analysis (PCA) was employed to assess between species differences in organic signatures. PCA of the organic profiles was performed using prcomp function in R (R Development Core Team, 2008).

Quantification of Denitrifying Bacteria

qPCR was used to determine copy number of *nirS* genes as an indicator of the number of bacteria within the communities with the potential for denitrification. qPCR reactions followed the protocol described by Mincer et al. [31]. Briefly, primers nirS1F and nirS6R [11] were used to amplify an 890 base pairs (bp) fragment of the *nirS* gene. The standard used for quantification was genomic DNA isolated from *Pseudomonas stutzeri* ATCC 11607, which was assumed to have a genome size of 4 Mbp and 1 *nirS* copy per genome [23]. The *P. stutzeri* dilution series included tenfold dilutions ranging from 1.2×10^5 to 12 copies of *nirS*. qPCR reactions were run using an MJ Research DNA Engine Opticon 1 thermal cycler equipped with Opticon software version 3.1 (Bio-Rad, Hercules, CA). The conditions for all qPCR reactions were as follows: 12.5 μl QuantiTect SYBR Green PCR Master Mix (Qiagen, Valencia, CA), 0.5 μM final concentration of each primer, 1 μL template, and water were added to a final 25 μL volume. All primers were synthesized by Eurofins MWG Operon (Huntsville, AL). All reactions were performed in low-profile 0.2 ml white strip tubes with optical ultraclear strip caps (Bio-Rad). Three analytical replicates were run for each sample. The specificity of qPCR reactions was confirmed by melting curve analysis and agarose gel electrophoresis. Thermal cycling was conducted as follows: initial denaturation at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 1 min, primer annealing at 57 °C for 1 min, extension at 72 °C for 1 min, hold at 78 °C for 1 sec, and plate read. Finally, a melting curve was run from 50 to 95 °C with a read every 1 °C and a hold of 1 s between reads. Effect of carbon source on *nirS* copy numbers was assessed by ANOVA and Tukey's honestly significant difference (HSD) test using Systat version 13 (Systat Software). Copy numbers were log transformed prior to ANOVA to account for nonhomogeneity of variances. Relationship between *nirS* copy numbers and DNP was assessed by determining Pearson product–moment correlation coefficients and probabilities using Systat version 13 (Systat Software).

Composition of Denitrifying Consortia

Tag pyrosequencing of *nosZ* genes was used to profile the bacteria within these communities with the potential for denitrification. DNA from each sample was sent to the Research and Testing Laboratory (Lubbock, TX). PCR amplification was performed using primers nosZF and nosZR [38]. Sequencing reactions utilized a Roche 454 FLX instrument (Roche, Indianapolis, IN) with titanium reagents. Sequences were processed using MOTHUR v.1.20.1 [41]. Briefly, any sequences containing ambiguities or homopolymers longer than eight bases were removed. Remaining sequences were individually trimmed to retain only high-quality sequence reads, and sequences were aligned using MAFFT (http://mafft.cbrc.jp/alignment/software/). Aligned sequences were trimmed to a uniform length of 136 bp, and chimeric sequences were removed using UCHIME [19] run within MOTHUR. After these pretreatment steps were completed, the data set included a total of 160,811 sequences for an average of 7657 sequences $sample^{-1}$. Sequences were clustered into operational taxonomic units (OTUs) based on 97 % sequence identity using the average neighbor algorithm. This clustering identified a total of 3963 OTUs. However, the communities were dominated by a relatively small number of OTUs, with the 20 most numerically dominant OTUs accounting for 83 % of the sequences. Therefore, subsequent analyses were based on this set of the 20 most numerically dominant OTUs. The community compositions of the individual samples were compared by

nonmetric multidimensional scaling (MDS) using the Primer V.5 software package (Primer-E, Plymouth, UK). For a full description of the MDS procedure, see Clarke and Warwick [17]. Briefly, the relative abundance of each of the top 20 OTUs within each of the samples was imported into Primer, and a similarity matrix was calculated using the Bray–Curtis coefficient [13]. The MDS procedure was then used to ordinate the similarity data following 100 random restarts. SIMPER analysis in Primer was used to compare the community composition of the samples that were DNP-positive, which showed production of N_2O in the DNP assay, and the samples that were DNP-negative, which did not show production of N_2O in the DNP assay. Selected OTUs were identified by comparing representative sequences to *nosZ* sequences available within Genbank via BLAST [1]. Primer was also used to quantify the diversity of the DNP-positive and DNP-negative samples based on the inverse Simpson index [44]. Finally, the relationship between the relative abundance of OTU1 and DNP rates was assessed by determining Pearson product–moment correlation coefficients and probabilities using Systat version 13 (Systat Software).

Results

Characterization of Organic Carbon

Initial comparisons of the Py-GC/MS fingerprints revealed that the organic mixtures of the algal exudates (*Achnanthidium lanceolatum* [AL] is provided as an example; Fig. 1) were very simple in contrast to the more complex organic fingerprint found in the stream waters (Fig. 2). For the algal exudates, the total number of peaks in the pyrochromatograms ranged from 35 to 53, while there were over 200 peaks in each of the stream water pyrochromatograms. Only seven peaks were identified in the exudates from *Amphora pediculus* [AP], since its low organic carbon concentration did not allow for detection of moderate to small peaks. Acetonitrile was the dominant peak (by area and height) in all of the algal exudate organic signatures, indicating a strong nitrogen containing aliphatic nature, which others have identified as a pyrolysis product of proteins [9, 32]. Other much less dominant common fragments include dipropylene glycol, phenol, styrene, propanols, and propenenitriles (Fig. 1). Differences among the algal exudates are found among the less dominant peaks, and we were unable to correlate these features with algal type or the denitrification potentials of the incubations.

In contrast, the stream waters showed a strong aromatic chemical signature with dominant pyrolysis fragments such as 1,2-dimethyl benzene, phenol and 4-methyl phenol (Fig. 2). Some clear differences between the organic signatures of the two stream waters are also evident (Fig. 2). The organic signature of RP water exhibited stronger peaks of 2-cyclopenten-1-one and 2-cyclopenten-1-one, 2-methyl-, which have been shown to be derived from soil polycarboxylic acids [10, 53], while the organic signature of DER water displayed the characteristics of wastewater effluent with dominant pyrolysis fragments such pyridine, pyrrole, and acetamide, products of protein, and aminosugar parent structures [7, 20, 28, 32, 34, 53]. To further compare the Py-GC/MS results, a semiquantitative technique [20] was used to assess differences in organic carbon signatures among all the samples analyzed. After the chemical peaks were identified in each sample, they were classified among four categories (aromatic, aliphatic, nitrogen-containing aromatic, and nitrogen-

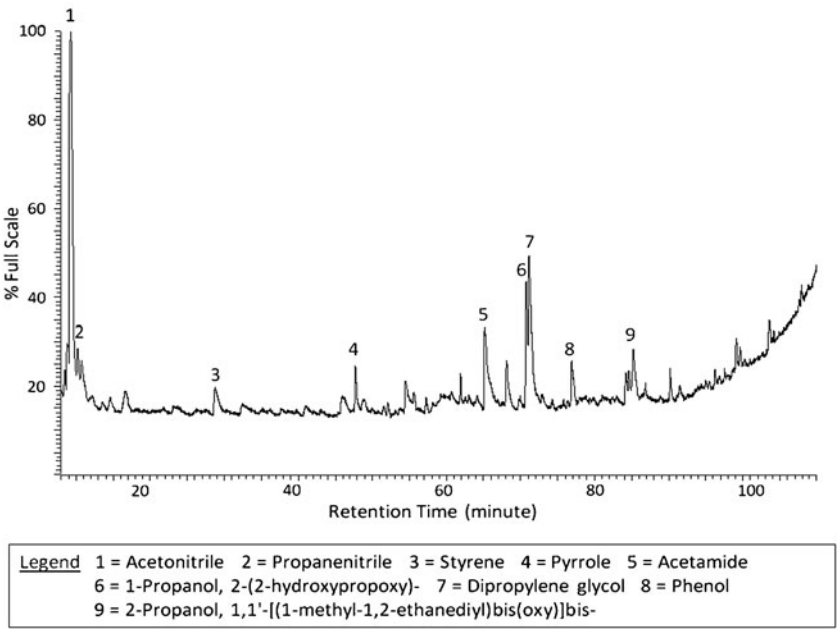

Fig. 1 Pyrochromatogram of *Achnanthidium lanceolatum* (AL) exudates

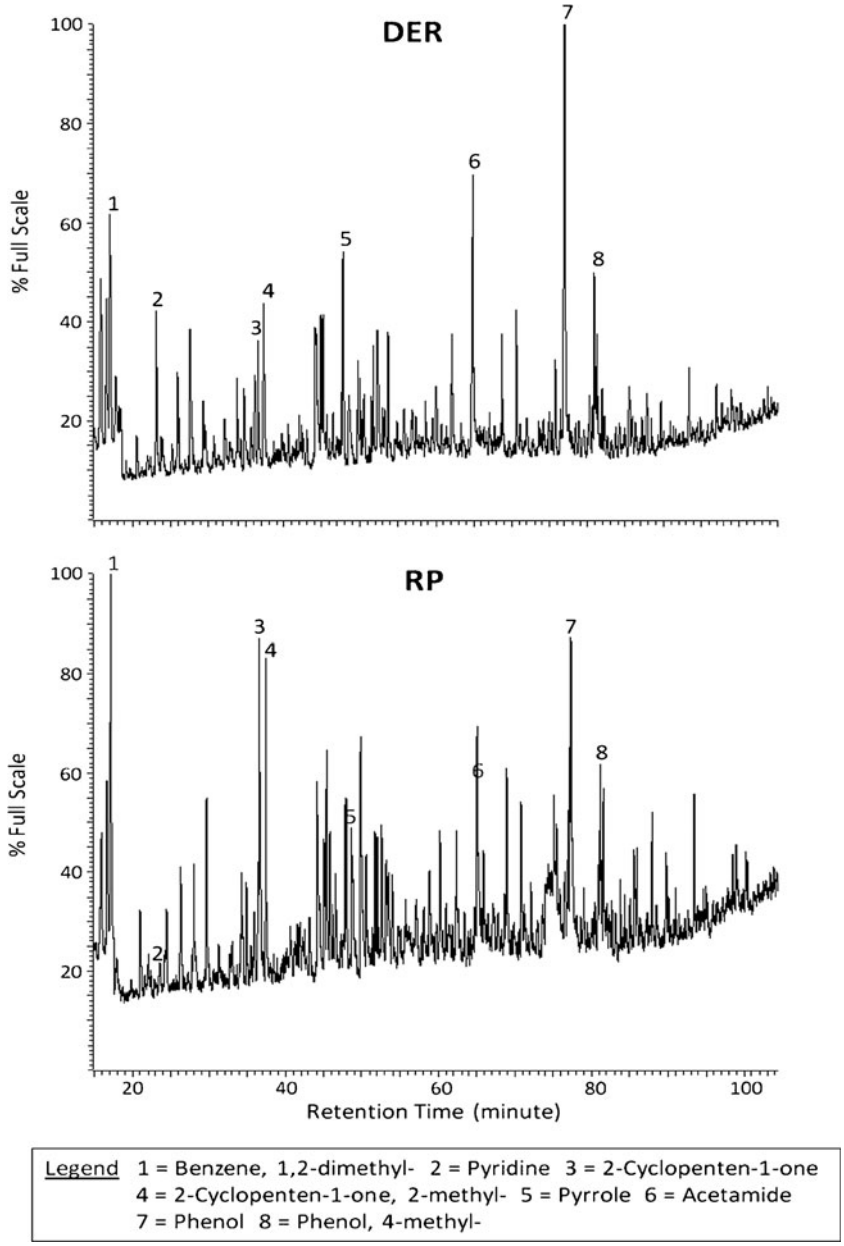

Fig. 2 Pyrochromatograms of organics from two field streams, DER and RP

containing aliphatic) with the percentage in each category based on the percentage of the total identified peak area. This comparison shows that the two stream waters had a much stronger aromatic signature than the algal exudates (Fig. 3, stream waters 60–67 % aromatic, algal exudates 13–29 % aromatic, Student's *t*-test, $t=10.2$, $p<0.007$). The AP sample was only 5 % aromatic, since its low organic carbon concentration did not allow for detection of moderate to small peaks and was therefore not included in the above statistical comparison of the stream waters and algal exudate aromatic signatures.

To further reduce the large amount of highly correlated data to a small number of independent variables while preserving most of the variance in the data, PCA was employed to assess among-species differences in organic signatures, which were comprised of the 57 unique pyrolysis fragments identified in the algal exudate pyrochromatograms. The peak areas were normalized by the maximum peak area of the identified fragments, which for all algal exudate samples was acetonitrile. PC1 accounted for 33.9 % of the total variance of the data, while PC2 accounted for 21.1 % (Fig. 4). The distance between all the samples on this score plot reveals that, while there are common dominant chemical fragments in the organic signatures, the underlying organic structure of all the algal exudates differs. The AP sample is located at the center of the score plot since its low organic carbon concentration did not allow for detection of moderate to small peaks, which would distinguish its

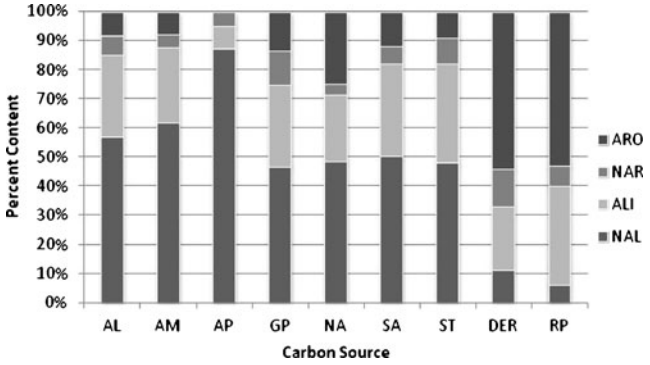

Fig. 3 Semi-quantitative analysis of organic signatures (ARO = aromatic , NAR = nitrogen-containing aromatic, ALI = aliphatic and NAL = nitrogen-containing aliphatic)

organic signature. However, the two green algae species (*S. armatus* [SA], *S. tenue* [ST]) did group near each other (PC1>3.7 and PC2>-0.2) and the two *Achnanthidium* species (AM, AL) also grouped near each other (PC1<-4.9 and PC2>0.9). Due to the highly differing nature of the algal exudate and stream water organic signatures, multivariate analysis comparing all these samples (not shown) was only able to highlight the dissimilarity of these two data sets, but was not useful for further comparison.

Bacterial Community Activity and Composition

Carbon source had a significant effect on denitrification potential rates ($p<0.001$), with measurable DNP in both replicates when bacteria were fed carbon isolated from the two stream waters (DER and RP) and in one of the two replicates when bacteria were fed G or organic carbon isolated from the diatoms AL and NA and the green alga ST (Fig. 5a). Bacterial communities fed carbon isolated from the DER site showed the highest DNP rate (Fig. 5a). There was also a significant effect ($p<0.001$) of carbon source on denitrifier abundance

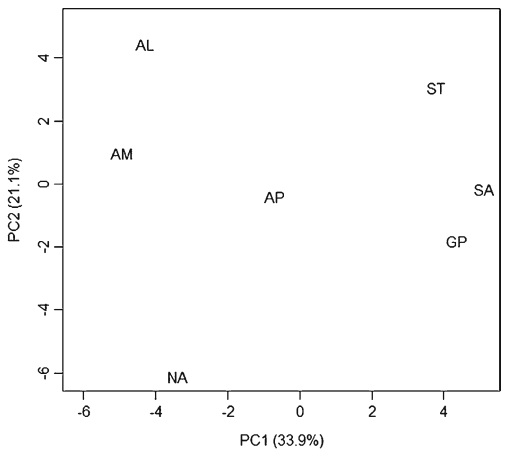

Fig. 4 Principal components analysis of algal exudate organic signatures

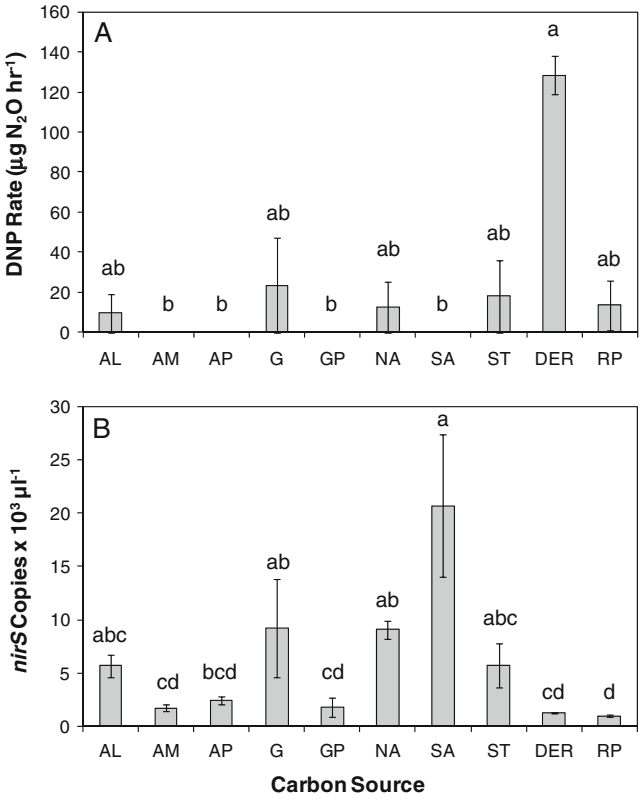

Fig. 5 Effect of carbon source on denitrification potential (**a**) and copy numbers of *nirS* genes (**b**). Each data point represents mean value with standard error bars ($n=2$). ANOVA indicated significant treatment effects on both DNP rate and *nirS* copy numbers ($p<0.001$). Different letters above bars indicate significant differences between treatments ($p<0.05$) based on Tukey's HSD test

based on copy numbers of *nirS* genes, with bacterial communities fed organics isolated from *S. armatus* showing the highest copy numbers (Fig. 5b). However, there was no significant correlation ($p=0.504$) between *nirS* copy numbers and denitrification potential (Fig. 6a).

MDS analysis of denitrifier community composition based on tag pyrosequencing of *nosZ* genes demonstrated that incubation of the bacterial consortia with the various carbon sources resulted in significant shifts in denitrifier community composition as compared to the initial inoculum (IN; Fig. 7). MDS indicated that all of the DNP-positive samples (those that showed production of N_2O in the DNP assay) were similar in composition and were generally distinct from the samples that were DNP-negative (those that did not show production of N_2O in the DNP assay; Fig. 7). For example, all of the incubations with organics from the natural streams (DER and RP) were DNP-positive and were located above and to the left of the inoculum on the MDS ordination. In contrast, all of the incubations with exudates from GP, AM, and SA were DNP-negative and were located to the right of the inoculum on the MDS ordination (Fig. 7). Results from some of the other treatments were less

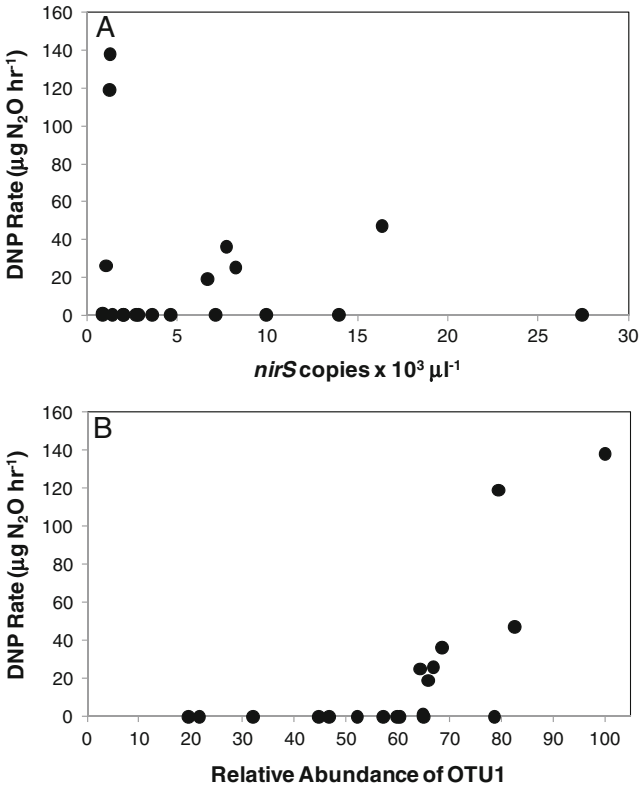

Fig. 6 Relationship between DNP rate and copy numbers of *nirS* genes (**a**) and relative abundance of OTU1 (**b**). Correlation analysis showed no significant correlation between DNP and *nirS* copy number (p=0.504) but a significant correlation between DNP and relative abundance of OTU1 (p=0.001)

consistent. For example, the replicate incubations with G were similar to each other in denitrifier species composition and were similar to the other DNP-positive samples in composition (based on their position on the MDS ordination), but only one of the replicate incubations was DNP-positive. Interestingly, the two G replicates had a large difference in denitrifier

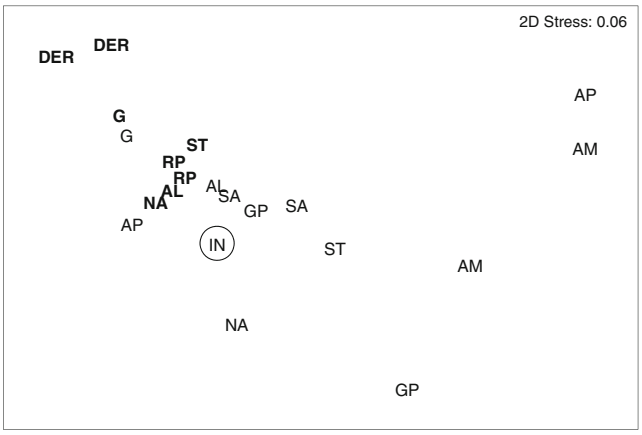

Fig. 7 MDS analysis of denitrifier community composition based on tag pyrosequencing of *nosZ* gene. Letters indicate carbon sources. Bacterial inoculum (IN) is circled. Samples in bold resulted in denitrification (i.e. production of N_2O) during DNP assay

abundance (based on *nirS* copy numbers) with the DNP-positive replicate having 1637 copies μl^{-1} and the DNP-negative replicate having 713 copies μl^{-1}. The AL and NA incubations also showed inconsistent results, with only one of the two replicates for each being DNP-positive. However, for both AL and NA exudates, there was a difference in denitrifier community composition between the replicates indicated by the MDS ordination, with the DNP-positive replicates being most similar to the other DNP-positive communities and the DNP-negative replicates being more similar to the other DNP-negative communities (Fig. 7). Finally, denitrifier communities incubated with organics from the DER site, which showed the highest rates of DNP (Fig. 5a), were clearly distinct in composition from communities from the other incubations (Fig. 7). In contrast, denitrifier communities incubated with organics from the RP site were similar in composition (Fig. 7) and DNP rate (Fig. 5a) to communities incubated with organics isolated from the algal species.

A comparison of the diversity of the denitrifier communities in the DNP-positive and DNP-negative samples based on the inverse Simpson index calculated from the tag pyrosequencing data indicated a significant difference in diversity (p<0.01), with the DNP-positive samples having significantly lower denitrifier diversity than the DNP-negative samples (Fig. 8a). SIMPER analysis of the tag pyrosequencing data indicated that the two most numerically dominant denitrifier OTUs, OTU1 and OTU2, accounted for 63 % of the variation between the DNP-positive and DNP-negative samples. ANOVA revealed that the relative abundance of OTU1 and the relative abundance of OTU2 were significantly different (p<0.05) between the DNP-positive and DNP-negative samples (Fig. 8b). Specifically, the relative abundance of OTU1 was significantly higher and the relative abundance of OTU2 was significantly lower in the DNP-positive samples as compared to the DNP-negative samples (Fig. 8b). Bacterial communities incubated with organics from the DER site, which showed the highest rates of DNP (Fig. 5a), also had the highest concentration of sequences from OTU1 (89 %) as compared to those incubated with G (80 %) or organics from the RP site (66 %) or the inoculum (57 %). These results suggested a possible relationship between the abundance of OTU1 and DNP rates, which was supported by a significant correlation (p=0.001) between these two variables (Fig. 6b). BLAST analysis indicated that sequences within OTU1 were most similar to *nosZ* sequences from *P. stutzeri* (100 % identity) and sequences within OTU2 were most similar to *nosZ* sequences from *Paracoccus denitrificans* (85 % identity).

Discussion

The results of this research demonstrate that DOC extracted from different algal species and stream waters varied in

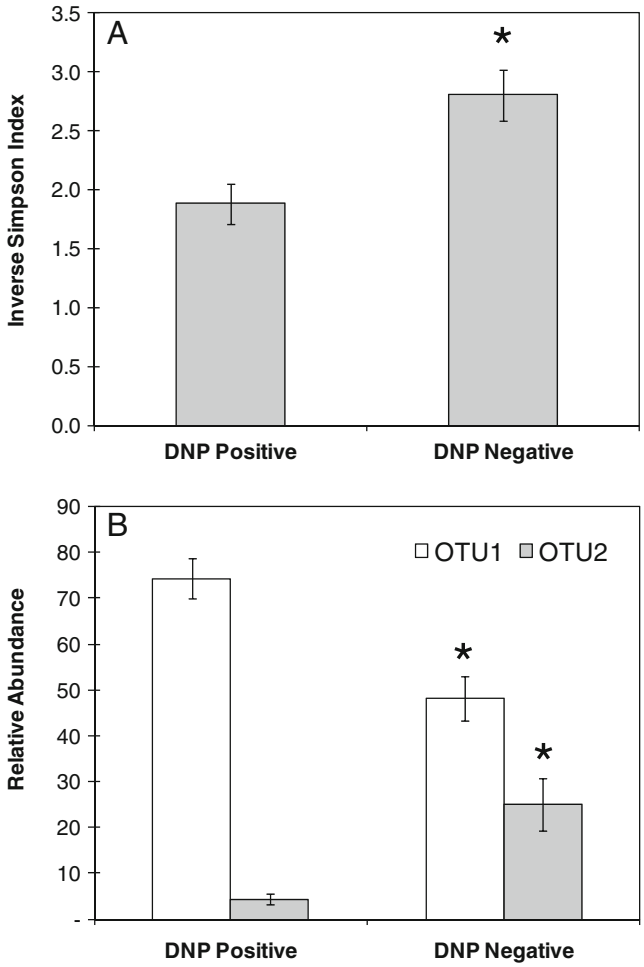

Fig. 8 (**a**) Diversity of denitrifier communities in DNP positive and DNP negative samples. ANOVA indicated significant difference between DNP positive and DNP negative samples ($p<0.01$). (**b**) Relative abundance of the two most numerically dominant OTUs in DNP positive and DNP negative samples. Asterisks indicate significant differences between DNP positive and DNP negative samples ($p<0.05$)

chemical quality and directly influenced the numbers, species composition and activity of denitrifying bacteria. Previous work by our group and others demonstrated that different algal taxa select for taxonomically distinct communities of associated bacteria [22, 30, 40] and that the chemical quality of organic carbon has a measurable effect on the denitrification potential of bacterial communities [35, 46]. However, this research is the first to directly connect DOC quality to both species composition and the activity of denitrifying bacteria, a group of microorganisms that are highly relevant to the nitrogen removal capacity of aquatic ecosystems. Specifically, the present study showed that incubation of bacteria with organic carbon from a site (DER) highly influenced by anthropogenic sources, specifically wastewater treatment effluent, showed the highest rates of DNP. In contrast, incubation of bacteria with organic carbon from a stream with relatively low anthropogenic influence (RP) resulted in DNP rates that were an order of magnitude lower than for DER and were similar to rates for incubations with algal-derived organics. This finding is consistent with our previous results [46]. However, the current study also demonstrated that DNP rates across all incubations were not correlated with the numbers of denitrifying bacteria but rather with the species composition of the denitrifying consortia. Specifically, denitrifier communities that showed measureable denitrification were less diverse and showed significantly higher relative abundances of *P. stutzeri*-like sequences and significantly lower relative abundances of *P. denitrificans*-like sequences. Furthermore, there was a significant correlation between DNP rate and the relative abundance of *P. stutzeri*-like sequences. The relationship between relative abundance of *P. stutzeri* sequences (OTU1) and DNP rate (Fig. 6b) suggested a threshold effect, as communities with less than 60 % relative abundance of *P. stutzeri* sequences showed no denitrification. This apparent threshold effect may reflect the sensitivity of the assay; that is, some communities with less than 60 % relative abundance of *P. stutzeri* sequences may have denitrified at a rate that was below the limit of detection of the DNP assay.

The lack of correlation between denirifier abundance and DNP rates was rather surprising, as we expected that overall denitrifier density would influence denitrification rates directly as others have reported [5]. However, these results highlight the potentially significant influence that microbial community composition can have on community activity. The distinct responses of *P. stutzeri* and *P. denitrificans* in the experimental incubations may be related to difference in the physiological properties of these two organisms. Both *P. stutzeri* and *P. denitrificans* are Gram-negative bacteria from the proteobaterial phylum that are common in terrestrial and aquatic environments. Both are facultative anaerobes capable of both aerobic respiration and denitrification [55]. Each has been shown to require both low oxygen conditions and the presence of nitrogenous oxides to induce the genes involved in the denitrification pathway [4, 29]. These two species also contain the cytochrome cd1 version of nitrite reductase (encoded by the gene *nirS*), the enzyme that catalyzes the second step of the denitrification pathway (the reduction of nitrite to nitric oxide), and nitrous oxide reductase (encoded by the gene *nosZ*), the enzyme that catalyzes the final step in the denitrification pathway (conversion of N_2O to N_2) [55]. However, in a previous study that compared the denitrification properties of these two organisms under conditions similar to our DNP assay (i.e. high organic carbon, high nitrate, oxygen purged), *P. stutzeri* showed significantly higher rates of N_2 production than *P. denitrificans* [15], which may explain the results of our study in which only communities dominated by *P. stutzeri* showed denitrification in the DNP assay. Interestingly, *P.*

denitrificans has a K_m for nitrate that is ten times lower than that of *P. stutzeri* [55], which could give *P. denitrificans* an advantage under low nitrate conditions, but under the high nitrate conditions in our DNP assay this difference in substrate affinity was likely not a factor.

The results of our study did indicate differences in the chemical composition of algal organics and organics from the two stream sites. These results confirm earlier work in which we used Py-GC/MS to distinguish natural versus anthropogenic organic signatures in aquatic systems [28, 45, 46]. We were unable, however, to identify which chemical features as characterized by Py/GC/MS are associated with DNP. The organic fingerprints of the algal exudates that produced detectable DNP do not display common chemical patterns and show little similarity to DER and RP. This is likely due to methodological limitations associated with isolating algal exudates under laboratory conditions, where we were unable to produce sample volumes sufficient to cultivate bacterial communities and resolve the fine details of organic quality by Py-GC/MS.

One of the most interesting findings of our study is the marked difference in the response of bacterial communities fed organics from stream water dominated by wastewater inputs (DER) compared to that of communities provided organics from a stream with relatively small anthropogenic influence (RP). The organics in water from both of these streams resulted in relatively low numbers of denitrifying bacteria, yet the organics from the DER site produced DNP rates an order of magnitude higher than any of the other carbon sources. Our data indicate that this high rate of denitrification was likely based on the fact that organics from DER selected for a denitrifier community that was distinct in composition, was less diverse and was dominated by *P. stutzeri* as compared with communities developed under any other carbon source. In contrast, organics from the RP site supported DNP rates and denitrifier community composition that were similar to those supported by organics from several of the algal species. These results are consistent with the conclusions of Peterson et al. [35] that bacterial denitrifiers and algae in biofilms developed in RP were coupled and in-biofilm processes were likely strongly influenced by autogenic factors, while, in DER, algal and bacterial assemblages were decoupled, and biofilm processes were more influenced by the organic carbon and nutrient pools in stream water. This highlights the potentially significant impacts of anthropogenic inputs on stream microbial communities. The role of anthopogenic impacts on internal structure and processes with periphytic biofilms warrants further research, as these communities serve as critically important sites of ecosystem services.

Care should be taken not to broadly generalize when interpreting our results for several reasons. First, while denitrifier communities dominated by *P. stutzeri* showed the highest denitrification rates in the DNP assay, it would be inappropriate to conclude from these results that bacterial communities dominated by *P. stutzeri* will be the most effective denitrifying communities under all circumstances. For example, the lower nitrate K_m for *P. denitrificans* suggests that it may perform better under nitrate limiting conditions. We can conclude from our results that bacterial communities dominated by *P. stutzeri* were the most effective denitrifying communities under the conditions of our DNP assay, i.e., high carbon, high NO_3^-, and anoxic. Secondly, while this study focused on the influence of organic carbon, it is possible that other components of the DER and RP stream waters, such as inorganic nutrients or the presence of other unidentified organic or inorganic wastewater contaminants, may also have influenced the development of the bacterial communities. While further work is needed to determine the specific components of the DER stream water that induced the denitrifying bacterial response we observed, our results suggest a potentially important role of species composition, both algal and bacterial, in the variation in rates of biogeochemical processes within periphytic biofilms.

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Determining the Fate and Toxicity of Polycyclic Aromatic Hydrocarbons Associated with Coal-Tar and Other Carbonaceous Material Particles in Urban Lakes

Basic Information

Title:	Determining the Fate and Toxicity of Polycyclic Aromatic Hydrocarbons Associated with Coal-Tar and Other Carbonaceous Material Particles in Urban Lakes
Project Number:	2011IL239G
Start Date:	8/1/2011
End Date:	7/31/2014
Funding Source:	104G
Congressional District:	15 IL
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Sediments, Surface Water
Descriptors:	None
Principal Investigators:	Charles J. Werth, Michael Jacob Plewa

Publications

There are no publications.

IWRC ANNUAL REPORT

Project Title:
Determining the Fate and Toxicity of Polycyclic Aromatic Hydrocarbons Associated with Coal-Tar and Other Carbonaceous Material Particles in Urban Lakes

Project PIs and Contact Information:
Charles J. Werth, Professor (PI)
Department of Civil & Environ. Eng., University of Illinois at Urbana-Champaign, 205 North Mathews Ave., Urbana, IL 61801, werth@illinois.edu, 217-333-3822

Michael J. Plewa, Professor of Genetics and University Scholar (co-PI)
Department of Crop Sciences, University of Illinois at Urbana-Champaign, 1101 West Peabody Dr., Urbana, IL 61801, mplewa@illinois.edu, 217-333-3614

Problem and Research Objectives:
Particle associated contaminants (PACs) have resulted in the impairment of thousands of streams, lakes, and reservoirs; PACs were responsible for fish-consumption advisories for 39 percent of total river mileage and 43 percent of total lake acreage in the United States in 2008. Results from recent water quality surveys indicate that metal, polychlorinated biphenyl, and DDT concentrations in freshwater sediments have generally decreased since their peak in the mid 1970's, consistent with their use and regulatory histories. However, total concentrations of polycyclic aromatic hydrocarbons (SPAHs) have increased, and generally with increasing urbanization. PAHs are toxic to aquatic life, and many are probable or suspected carcinogens. This is of special concern because many urban surface waters are used for human recreation (e.g., fishing, swimming) and/or drinking water.

Sources of particle-associated PAHs in urban lake sediments are located both within and outside the watershed. They include point (e.g., industrial emissions) and nonpoint (e.g., automobiles) combustion sources, asphalt from roads and parking lots, vulcanized rubber products such as automobile tires, and coal-tar and asphalt based sealcoats on parking lots and driveway pavement and roofs. Results from a number of our recent studies indicate that coal-tar pavement sealcoat is fluvially transported into urban streams and lakes with runoff, and can be the dominant source of PAHs in urban streams and lakes.

The overall goal of this study is to determine the fate and toxicity of PAHs associated with coal-tar particles in urban lake sediments. The specific objectives of this study are listed below.

1) Determine the sorption equilibrium and desorption kinetics of PAHs in coal-tar and other carbonaceous material particles that comprise urban lake sediments. We hypothesize that sorption capacities are low and release rates are high for PAHs in coal-tar and other less condensed carbonaceous materials (CMs) compared to highly condensed CMs like black carbon char and soot.

2) Determine PAH losses and redistribution associated with coal-tar particles in urban lake sediments. We hypothesize that lower molecular weight PAHs are released from coal-tar particles soon after burial (weeks to months) and taken up by more strongly sorbing black carbon, and that higher molecular weight PAHs are only lost to black carbon over much longer time scales (i.e., years) as phenolic and heterocyclic compounds that comprise coal-tar degrade. As a result, we hypothesize that PAHs are largely conserved in lake sediments and are not significantly released to the water column, and that sediment pore-water concentrations of PAHs decrease with aging.
3) Determine the toxicity of PAHs associated with coal-tar and other carbonaceous material particles in urban lake sediments. We hypothesize that toxicity of pore water in sediments decreases with time as PAHs and other organic pollutants redistribute from less strongly sorbing CMs like coal-tar to more strongly sorbing black carbon, and as less recalcitrant pollutants are biologically degraded over time. Such information is important because these lakes are sources of recreation and/or drinking water for large populations, and understanding coal-tar contributions to toxicity is an important step in protecting the environment and public health.

Methodology:

The proposed work combines bench scale laboratory experiments, field experiments, and laboratory analysis of field samples. It is divided into four tasks that cover 1) lake core retrieval, analysis, treatment, and in situ placement, 2) sorption isotherm and desorption kinetic profile measurement, 3) PAH and CM analysis of in situ cores, and 4) toxicity analysis of in situ cores.

All cores will be taken from Whitnall Lake near Milwaukee, Wisconsin, in the next two months. A set of cores will be used for sorption isotherm and desorption kinetic profile measurements in task 2, and another set will be amended with coal tar and other carbonaceous materials spiked with deuterated PAHs, and then placed back into Whitnall Lake during the sampling period which will occur over the next two months.

Sorption isotherm measurements will be performed to determine the affinity of PAHs for different carbonaceous materials, including asphalt, coal tar, soot, and charcoal. Sorption kinetic profiles measurements will be performed to determine the time scales that PAHs transfer between carbonaceous materials in lake sediments.

Spiked cores placed back in Whitnall Lake will be retrieved after 6 months, 1 year, and 2 years, and then analyzed to determine deuterated and non-deuterated PAH concentrations, and concentrations of PAHs on individual CM particles. Measurement of deuterated PAH concentrations will allow us to determine if PAHs associated with each type of CM were lost to the watershed, transferred to another CM, or degraded over time.

The toxicity of extracts from the original cores, and spiked cores retrieved at different time intervals, will be determined.

Principal Finding and Significance:

Due to a delay in hiring graduate students, project activities started in the summer of 2012. In the fall of 2012 and winter of 2013, we developed the methods necessary to amend the carbonaceous material particles with deuterated PAHs, and to separate out the different carbonaceous material particles from the sediments after core retrieval. We are now set to begin work at Whitnall Lake in the next two months.

Notable Achievements:

We have developed the methods necessary to amend the carbonaceous material particles with deuterated PAHs, and to separate out the different carbonaceous material particles from the sediments after core retrieval.

Students supported with funding:

In the summer of 2012 two graduate students were hired. Ms. Tory Boyd was hired to perform the all work except toxicity testing. Ms. Boyd obtained her MS degree at Illinois, and the work in this proposal represents the bulk of her PhD thesis. The other graduate student is Ms. Azra, who is performing the toxicity testing.

Publications and presentations:

Due to a delay in hiring graduate students, project activities are only starting in the summer of 2012. As a result, there are no publications or presentations to report.

Measuring Public Preferences over Stormwater Management Outcomes in Illinois: Willingness to Pay and Willingness to Help

Basic Information

Title:	Measuring Public Preferences over Stormwater Management Outcomes in Illinois: Willingness to Pay and Willingness to Help
Project Number:	2012IL248B
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End Date:	2/29/2013
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Descriptors:	None
Principal Investigators:	Amy Ando

Publications

There are no publications.

MEASURING PUBLIC PREFERENCES OVER STORMWATER MANAGEMENT OUTCOMES IN ILLINOIS: WILLINGNESS TO PAY AND WILLINGNESS TO HELP

SUMMARY REPORT

I. Principal Investigators

Amy W. Ando
Professor, Department of Agricultural and Consumer Economics
University of Illinois at Urbana-Champaign
1301 W. Gregory Dr., Urbana, IL 61801
217 (333)-5130
amyando@illinois.edu

Catalina Londoño
PhD Candidate, Department of Agricultural and Consumer Economics
University of Illinois at Urbana-Champaign
1301 W. Gregory Dr., Urbana, IL 61801
217 (848)-2986
londono1@illinois.edu

II. Problem and research objective

Problem: Stormwater runoff is the cause of many environmental problems in Illinois and elsewhere such as flooding, soil erosion and water pollution. Urbanization increases runoff problems; permeable surfaces are replaced by impervious surfaces that limit water infiltration, which starves streams of groundwater that supports base flows during dry periods. The flows of urban stormwater have been dealt with using large-scale engineering solutions that convey the water rapidly to streams, rivers, detention ponds. These technologies increase pollutant loads in streams and tend to make stream flows excessively fast and heavy during storms, scouring stream beds, and further degrading aquatic habitat in urban water bodies (NRC 2009; Brabec 2009).

New strategies for mitigating stormwater runoff have been developed that may manage stormwater with a broader suite of environmental benefits. These green infrastructure technologies (often called Low Impact Development (LID) tools or Best Management Practices (BMPs)) such as landscape infiltration systems, pervious pavements, and green roofs (USEPA 2000) mimic nature in order to capture, temporarily store, treat, and/or infiltrate stormwater. The results can be improved water quality, increased groundwater recharge, and/or healthier aquatic

habitat (NRC 2009). The U.S. EPA is considering new regulations (USEPA n.d) that would effectively require widespread implementation of BMP development approaches, but the total benefits of that change are hard to estimate given gaps in the literature on the benefits of stormwater management.

In the absence of strict federal stormwater regulations, the City of Chicago through its Water Agenda has taken several steps to address stormwater management issues. The Chicago City Council passed its "Chicago Stormwater Management Ordinance" designed to promote programs that minimize the negative stormwater impacts of new development and redevelopment in the city (City of Chicago 2011). The Ordinance applies to certain types of new developments and redevelopment and requires specific practices to ensure that stormwater is responsibly managed in accordance with the goals of the Water Agenda. Thus, the City has begun promoting green building design and BMPs, has taken steps to prevent polluted stormwater from roadways from discharging directly into Lake Michigan and the Chicago and Calumet Rivers, and is also working to comply with National Pollutant Discharge Elimination System (NPDES) Phase II requirements. There is a strong movement to encourage green infrastructure in order to comply with NPDES and regulation to incorporate LID requirements in new and re-developments where feasible. However, authorities struggle with the lack of benefit estimates to use in cost-benefit analyses of any such regulations.

Although there is general consensus on the environmental benefits and cost-effectiveness of BMPs and despite governmental efforts to promote them, they have not been widely implemented. Reasons for the lack of adoption include political issues, lack of professional education and training, regulatory conflicts and inflexibility, lack of funding for research and development and personal beliefs, knowledge and preferences (Roy et al. 2008).

Empirical evidence of people's preferences over stormwater management control can help to shape the way policy makers design incentives when they intend to promote the use of new environmental technologies and thus overcome the barriers that seem to hinder the widespread implementation of apparently cost-effective stormwater management tools. The particular results from this research could be used as a tool for the development of policies and sustainability efforts in the Chicago area and potentially in other urban areas in Illinois.

This project produces information about how much people value different kinds of environmental improvements that can be accomplished with LID stormwater management approaches. The results of this project can also help policy makers understand people's attitudes about using decentralized stormwater solutions instead of conventional concrete infrastructure, and people's willingness to volunteer to help maintain such decentralized solutions. That knowledge could help city, state, and federal policy makers to make choices about stormwater policies, since such policies vary in the extent to which they accomplish different environmental

objectives (Birol et al. 2006). Finally, this research contributes to academic research on choice experiment methodology by exploring payment through time as well as money.

Objectives: The objectives of the project are to improve public policy in Illinois regarding stormwater management and to advance scientific understanding of consumer behavior and the value of improving environmental attributes affected by stormwater control. Specific objectives are:

Objective 1: Estimate the values that citizens place on multiple attributes of stormwater management outcomes.
Objective 2: Assess whether people are willing to take on stewardship of stormwater control facilities where they live.
Objective 3: Inform stormwater policy making in Chicago and other urban areas of Illinois.
Objective 4: Identify the role that questions about volunteer time can play in choice experiment surveys to estimate values of a wide range of public goods.

II. Methodology

Choice experiment survey: Choice experiment (CE) survey methodology (Louviere and Hensher 1982) has been used widely for decades in marketing and economics to estimate consumers' willingness to pay for goods or services. This approach asks respondents to choose between different hypothetical goods or scenarios defined in terms of their attributes. We use a CE survey asking respondents to indicate preferences between different hypothetical stormwater-control scenarios which vary in key attributes: pollution levels in area water bodies; aquatic habitat in area streams/rivers; frequency of floods; monetary cost to the household; and time the household would have to spend maintaining and/or installing BMPs. The questions are given in varied orders to control for ordering and learning effects.

We use CE for several reasons. First, the outcomes of a stormwater-management strategy – flooding, water quality, cost, and aquatic habitat quality – can actually vary in different directions from one another depending on the nature of a city's strategy. For example, flooding can be reduced with large sewer infrastructure, but that is likely to worsen water quality. Thus, it makes sense to describe a scenario of stormwater management outcomes in which the attributes vary separately from each other. Second, given this feature of stormwater runoff management, CE methods provide valuable information to policy makers by determining which type of features of environmental quality people care about the most and consequently should be prioritized.

Survey instrument: An online choice experiment survey was answered by 500 residents of Chicago. Following recommendations for stated choice surveys (Mattews et al., 2006), the survey instrument provided respondents with background information about stormwater management problems and controls and then presented respondents with a number of binary-choice questions, each of which asked them to choose between a pair of hypothetical stormwater-control scenarios and the status quo, meaning that respondents could also choose to have no new stormwater management projects in their area. This opt-out option leaves flooding and environmental quality the same, and entails no cost. The choice questions are followed by a demographic questionnaire and questions about respondents' experiences with stormwater issues.

The final list of attributes and levels was refined according to input from water management experts and focus groups. The focus groups were moderated by hired experienced professionals, with the participation of 6-7 people per group and a total duration of 90 minutes each. The participants replied to advertisements posted in Craigslist and were rewarded with a $25 gift card. Each participant answered a complete questionnaire and the moderator asked about aspects such their perceptions of the general purpose of the survey, level of difficulty, language, amount of questions, attribute levels, own flooding experience and general suggestions.

We administered the survey online through the company Qualtrics, which provided both the software and the respondents' panel. Web-based surveys guarantee a given number of responses with panels of respondents who are paid to participate in surveys on a regular basis. The flexibility and relative low cost of web-based surveys make them a good alternative for research, especially with declining response rates in mail surveys (Groves, 2006; de Leeuw and de Heer, 2002). The company is able to ensure that the sample is representative of people from a wide range of income levels.

Experimental design: The experiment consists of three treatment groups, to which respondents were randomly assigned in equal numbers. To evaluate the effect of having a time attribute to capture the value people have for features of a scenario, Treatment #1 (Time and Money) was presented with both time- and money-cost attributes, Treatment #2 (Money-only) with only a money-cost attribute, and Treatment#3 (Time-only) with only a time-cost attribute.

We compare the total value estimates between groups to quantify the effect that a second non-monetary "payment" attribute has on those estimates. We use econometric methodology (mixed multinomial logit) to account for preference heterogeneity in the sample and to evaluate how the new attribute behaves in different sub-groups of the sample.

Statistical analysis: Choice experiments are based on random utility maximization theory (McFadden 1973) where the different attributes contribute to utility together with a random component to capture the unobserved differences. The indirect utility function capturing the

utility of each scenario to each respondent is specified to have a linear functional form. The coefficients on attributes capture the marginal utilities of those attributes to respondents. The coefficient on money captures the marginal utility of money. The coefficient on time is the sum of the scarcity value of time and the marginal utility that a respondent obtains from volunteer activity.

Neoclassical economic theory predicts that time is valued at its opportunity cost (i.e. the person's wage rate). We use data from respondents who report a positive wage to test whether individuals do value their time at their current wage rate. We also test whether marginal willingness to pay for stormwater projects varies with respondents' income levels.

III. Principal findings and significance

Preliminary results show that people in Chicago place a positive value on improving elements of environmental quality, both water quality and aquatic health. The survey shows that aquatic health and hydrological function are important to individuals in our sample. Flooding reduction is not significant in the treatment in which people are presented with both time and money payments, even after controlling for individual flooding experience. Only 33% of the respondents recall at least one flooding event in the past year and, out of those who did, fewer than 6% experienced more than four flood events. These results suggest that people do not see flooding as a major hazard or inconvenience and are not willing to pay both money and time to avoid flooding. Thus, promoting the environmental benefits of stormwater management projects appears to be an important factor in inducing public acceptance of different policies, perhaps more than emphasizing reduction in the prevalence of flooding. If individuals understand that fees they pay go toward maintaining or improving the environmental quality of that body of water, they might be more willing to accept and participate in the fee program in exchange for those benefits.

We calculated people's average marginal willingness to pay and willingness to help to obtain various attributes (flood reduction, environmental improvement). These numbers can be interpreted as the amount of dollars per month people would be willing to give or the number of hours people would spend each month in helping with decentralized stormwater management projects in their neighborhood for one percent reduction of flooding, or a discrete improvement in quality. These values can be used to calculate the total benefits of a stormwater management plan.

Results suggest that people do not value their time at their wage rate. If time were truly valued according to hourly wages, people reveal themselves willing to pay much more through in-kind contributions of time than through direct payments of money. In addition, current results

reveal the surprising result that high income people are willing to pay less money for stormwater improvements. This finding could have two sources. First, income and political affiliation could be correlated such that income is standing as a proxy for anti-tax ideology. Second, people of different income categories may have different direct experiences with the benefits of stormwater management. High income people might be located in areas where there is less significant flooding. Also, wealthy people are better able to afford substitute locations for local surface waters for activities such as fishing and swimming, so their willingness to pay to improve water quality in the waters in and around Chicago might be lower.

IV. Notable achievements

- Traditional economic valuation research only permits people to express value for environmental amenities in terms of money; we develop a novel valuation method that permits individuals to express value in terms of time spent as well as money paid.
- We find that people see contributing time helping as a valid mode of payment, and the wage value of the time they are willing to devote to helping is greater than the amount of money they would be willing pay directly for the same program.
- Our estimates of the values of water quality and aquatic habitat improvements can inform cost-benefit estimates of urban stormwater programs and regulations.
- The findings imply that programs that allow residents to pay both through fees and through helping may be better able to capture a full range of value from residents of diverse financial means.

V. Student supported with funding

Catalina Londoño
Ph.D. Agricultural and Applied Economics
University of Illinois at Urbana-Champaign
Expected degree date August 5, 2013

VI. Publications and presentations

- Very preliminary results were presented by Amy Ando at the W3133 Multistate Research Group Annual Meeting in Coeur d'Alene, Idaho in February 2013.
- Catalina Londoño defended her doctoral dissertation on May 8, 2013, which includes the results of this study.
- The final results of the project have not been presented or published yet. However, they will be presented at the Annual Meeting of the American Applied Economics Association in Washington DC in August 2013. The research will be part of the doctoral dissertation of

Catalina Londoño which will be deposited by August 2013 and at least two peer reviewed papers in academic journals.

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The Impacts of Cellulosic Biofuel Feedstock Production on Midwest US Hydrologic Cycle

Basic Information

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Principal Investigators:	Carl Bernacchi, Carl Bernacchi

Publications

1. VanLoocke A, Twine TE, Zeri M, Bernacchi CJ. 2012, A regional comparison of water-use-efficiency for miscanthus, switchgrass and maize. Agricultural and Forest Meteorology, 164, 82-95.
2. VanLoocke A, (2013) The impact of land-use and global change on water-related agro-ecosystem services in the Midwest US. Ph.D. Thesis, Atmospheric Sciences, College of Liberal Arts and Sciences, University of Illinois, Urbana, Illinois,

- **Project title and PI's name and contact information**

PI: Carl J. Bernacchi, Assistant Professor, Department of Plant Biology, University of Illinois. Email: bernacch@illinois.edu Phone: 217-333-8048.
Co-PI: Andy VanLoocke, Postdoctoral Researcher, USDA-ARS. Email: andyvanl@illinois.edu Phone: 217-300-0880

- **Problem and research objective**

Water is a crucial component in agricultural production (Wallace et al., 2000; Chaves et al., 2003; Oliver et al., 2009), the demand for which is predicted to increase in the future (Steduto et al., 2007). There are many uncertainties associated with future water availability, many of which are driven by predicted climatic changes (e.g., Wuebbles and Hayhoe, 2004). However, proposed changes to land use could potentially lead to large-scale alterations in the hydrologic cycle of Illinois and the Midwestern US. One potential land use change that is receiving relatively little attention is the onset of increased renewable energy production. The US 2007 Energy Independence and Security Act requires that production of ethanol from corn grain is capped near current production levels and that the majority of renewable fuels will be produced by cellulosic feedstocks by 2022 (Sissine, 2007). However, cellulosic feedstock production should not result in unsustainable agronomic practices or have negative impacts on ecosystem services, particularly those related to the hydrologic cycle. Little is currently known about the perturbations to the environment that will occur if production of cellulosic feedstocks were to be executed on a large scale (Rowe et al., 2009). This research will provide detailed insight on various bioenergy feedstock production scenarios and the effects of these land use changes on key components of the hydrologic cycle and on water quality.

The majority of the land area in Illinois was once host to tall grass prairies, thus the soils, climate, and topography are well suited for the production of tall perennial grass species. Two candidate species identified as ideal candidates for cellulosic feedstock production for this region are *Miscanthus* × *giganteus* (miscanthus) and *Panicum virgatum* (switchgrass; Heaton et al., 2008). Based on small-scale experiments, it is shown that miscanthus and switchgrass take up more carbon (Stampfl et al., 2007; Davis et al., 2010; Zeri et al., 2011), require less nutrient application (Heaton et al., 2004, 2010), but use more water (Hickman et al., 2010; McIsaac et al., 2010; VanLoocke et al., 2010) than current vegetation. Through small-scale impacts on the timing and rate of evapotranspiration of miscanthus and switchgrass compared to existing land cover, changing the land use to accommodate a perennial grass can have large-scale consequences on many components of the regional hydrologic cycle. The impact of an increase in water use associated with cellulosic biofuel production has been discussed previously (NRC 2008; 2011; McIsaac et al., 2010; Hickman et al., 2010), however, quantification at the State and Regional scale is lacking. This was the motivation for our first objective. Objective 1: Estimation of the total water use, biomass productivity and water use efficiency (WUE) of miscanthus and switchgrass relative to maize.

The goal of this objective was to compare total water use, productivity, and three WUE metrics for two leading cellulosic feedstocks against maize, the currently dominant bioenergy feedstock. The term water use efficiency (WUE) relates the amount of water used for a given amount of biomass production or carbon gain. An increase in the WUE of an agro-ecosystem can be considered an ecosystem service and is an important factor when selecting a cellulosic feedstock (Somerville et al., 2010). A number of different metrics can be used to calculate WUE; for this project we used the following three. First, WUE was calculated based on the total water used in evapotranspiration (ET) to achieve a given harvested biomass, defined as harvest WUE

(HWUE). Second, WUE was calculated as the total water used for the total annual net ecosystem productivity (NEP), which we defined as ecosystem WUE (EWUE). Finally, WUE was calculated based on the total water used for the total net biome productivity (NBP), where NBP is NEP minus the carbon removed at harvest, which we defined as biome WUE (BWUE). This objective allowed for a thorough analysis of the various WUE metrics throughout the Midwest, allowing for identification where ecosystem services for one species (e.g., switchgrass) might be more beneficial than for another species (e.g., miscanthus).

Land use changes to accommodate production of miscanthus and switchgrass are likely to influence more than the quantity of water flowing through various components of the hydrologic cycle. Perennial grasses require less fertilizer than maize (e.g. Heaton et al., 2010). Therefore, the potential exists to decrease the leaching of nitrogen into the water, improving water quality and providing an ecosystem service (Costello et al., 2009; McIsaac et al., 2010; Ng et al., 2010). Alternatively, if the hydrologic cycle is perturbed by an increase in rates of ET, less water flowing though streams and rivers could potentially be more concentrated in pollutants, thereby degrading water quality. In either case, large-scale changes in evapotranspiration and nutrient application compared to existing land cover has the potential to alter the flux of nitrate through the Mississippi River Basin to the Gulf of Mexico. Current research focusing on water quality changes associated with these feedstocks is limited to point measurements of nitrate movement within the soil profile (McIsaac et al., 2010) and watershed scale measurements that do not include validated mechanistic growth and physiology modules for miscanthus and switchgrass (Costello et al., 2009; Ng et al., 2010). This was the motivation for our second objective. Objective 2: Predict the impact of cellulosic biofuel production on streamflow and nutrient transport in streams, rivers, and discharge from the Mississippi River Basin.

This objective coupled a version of Agro-IBIS developed in objective one with the Terrestrial Hydrology Model with Biogeochemistry (THMB). We drove the model using different scenarios based on establishing a variety of fraction covers of miscanthus and switchgrass in place of the current mosaic of vegetation. This model coupling allowed the excess water in each grid cell to be routed through known streams and rivers throughout the basin and allowed us to quantify changes in streamflow in the region. Through various simulated nutrient applications, we also determined the nitrate leaching from different vegetation types,

The outcome of this research helped us to understand the consequences of land use change to accommodate cellulosic feedstocks grown for renewable energy. Clear benefits in terms of offsetting fossil fuel usage and improving economic development are already widely known, but our research has important implications in driving the best management practices and to help inform producers, policy makers and private and commercial stake holders. Climate also has an unavoidable impact on daily life, from agricultural productivity, to biodiversity, and human health. Thus, understanding the consequences of land use change on climate can help to minimize uncertainties associated with various production scenarios. This research coupled advanced, highly mechanistic ecosystem modeling with state-of-the-art measurements to provide the most defensible and accurate simulations possible before a cellulosic feedstock industry emerges. While the purpose of the research was hypothesis-based and intended for scientific audiences, the model simulations and outputs will be integrated into a web-accessible graphical user interface for use by non-technical users, including the general public.

- **Methodology**

Our research addressed the regional environmental impacts and ecosystem services of cellulosic feedstocks on the Midwest regional hydrologic cycle. Objective 1 was addressed using the Agro-

IBIS (Integrated Biosphere Simulator–Agricultural version; Kucharik and Brye, 2003) ecosystem model, described in detail below, at local and regional scales. Agro-IBIS served as the central model for this project and different vegetation was explicitly represented to accomplish the first objective. For the second objective, the fluxes of water out of the soil column (drainage) as well as nutrients (nitrogen leaching) simulated by Agro-IBIS was routed by the Terrestrial Hydrology Model with Biogeochemistry (THMB; Donner and Kucharik, 2008) through a realistic network of streams through the region. Our research modified Agro-IBIS to simulate the growth and biophysical processes (e.g. water use, and carbon uptake) of miscanthus and switchgrass. We used state-of-the-art sensors to validate the model using the most complete measurements on the carbon, nitrogen and hydrological cycles associated with standard agronomic species (maize and soybean) compared with miscanthus and switchgrass. We coupled Agro-IBIS with THMB in order to route water and nitrogen fluxes in each grid cell through the region to the Gulf of Mexico.

Since Agro-IBIS is a semi-mechanistic model, we used published values for certain physiologically based parameters for each species. However, given the nascent nature of this research, there is a tremendous amount of parameterization that is required where current data does not exist. In this case, we collected the data necessary to parameterize the model using a variety of equipment designed specifically for characterizing key physiological traits associated with vegetation. This includes portable gas exchange systems for measuring key photosynthesis and respiration variables, leaf area meters to determine canopy characteristics, and a range of laboratory based equipment analyzing nitrogen and carbon ratios in the plant tissues. Proper parameterization is critical to maximize the predictive ability of a model, but data for proper validation is equally important. Incorporated into this research project was the opportunity to use equipment that provides ecosystem-scale measurements of carbon, water and energy fluxes using a technique referred to as eddy covariance. This method provides temporal resolution ranging from half-hourly fluxes of water vapor and carbon dioxide for measuring key biogeochemical fluxes. Additional data includes monthly and annual mean streamflow [$m^3 s^{-1}$] and nitrate export [$kg\ yr^{-1}$], daily maximum and annual surface temperature [°C] and precipitation [mm]. We conducted a rigorous validation of the model by comparing the predicted fluxes of key biogeochemical variables against the gold standard in measuring these same fluxes as the data is collected.

The temporal and spatial domain for the second stage of simulations using THMB was the same as the stage one simulations in Agro-IBIS. THMB simulations were run at 5' × 5' spatial resolution to resolve the variation in elevation that drives streamflow. Monthly mean values of drainage and nitrate leaching were integrated into the THMB structure, where the water and nutrient was routed through the region to the Gulf of Mexico. For the control simulation, current land use and management practices in the region were represented based on the approach in Donner and Kucharik (2008). Simulations were then conducted using various fractions of land devoted to miscanthus and/or switchgrass relative to current crops to determine consequences of their production on stream flow and water quality. Additional simulations were conducted with varying levels of nutrient application based on predicted management recommendations for each cellulosic feedstock.

- **Principal findings and significance**

The principal findings of this research added considerable insight into water-related ecosystem services associated with large scale production of the key cellulosic bioenergy

feedstocks, miscanthus and switchgrass, and can be summarized in three categories: 1) Water Use 2) Water Use Efficiency and 3) Water Quality. The findings with respect to Water Use and Water Use efficiency were produced through the efforts to address objective 1 described above. Through the detailed parameterization and validation of the updated version of Agro-IBIS, we were successfully able to produce the best estimates to date of water use by miscanthus and switchgrass, and the relative gain in carbon for that water use (i.e. Water use efficiency). The results produced from the objective 1 analysis indicated that miscanthus will use more water across the Midwestern US relative to maize (the current dominant feedstock) and switchgrass. However when three different metrics of carbon gain were used to obtain water use efficiency, we showed that miscanthus uses water more efficiently than maize and switchgrass for most of the Midwestern US. We also showed that for belowground carbon production, switchgrass was more water use efficient than maize, despite being less efficient in terms of grain or aboveground production. By putting the water use of these cellulosic feedstocks in the context of their relative carbon gain, this research showed that, if properly implemented, miscanthus could produce more bioenergy and sequester more carbon, getting a much larger return for the water used. This finding is of critical significance given the predicted increased demand on water going into the future.

The principal findings with respect to objective 2 was that the decrease in streamflow associated with the increased rates of water use for miscanthus and switchgrass where minor relative to the large improvements in water quality associated with greatly reduced rates of nitrate leaching. This was driven by the perennial nature of miscanthus and switchgrass, combined with internal nutrient recycling which allows for a more efficient use of nutrients. This reduced the demand for fertilizer and the opportunity for nitrate to leach into streams. Another important finding from objective 2 was that the most aggressive biofuel production scenarios showed the potential for a reduction in nitrate leaching to be great enough to meet the EPA targets for reducing the size of the Gulf of Mexico Hypoxic Zone.

- **Notable achievements**

A key achievement of this research was the development of a modeling framework capable of simulating the hydrologic impacts of cellulosic bioenergy production at the scale of the Mississippi River Basin. It was vital to achieve this large scale framework to make quantitative estimates of the direct impacts of policy and management decisions on water quality and quantity in the Mississippi River Basin. Given the direct relevance of cellulosic biofuels and the Gulf Hypoxic Zone to policy, this research has provided a greatly improved, and highly defensible, method for guiding future policy. Because we were able to combine the agro-ecosystem model directly to the hydrologic transport model, we achieved a tool to assess key water-related issues for bioenergy production in the Midwestern US and the Mississippi River basin. Finally, as new decisions are made, and the cellulosic production system evolves, we will be able to make quantitative estimates as to what the impacts are likely to be, in a way that can inform future policy and management decisions.

- **Students supported with funding**

Andy VanLoocke, Department of Atmospheric Sciences, University of Illinois. Ph.D. awarded in October of 2012. He is current a postdoctoral research associate with the USDA-

ARS and will begin a position as assistant professor in the Department of Agronomy at Iowa State University in January 2014.

- **Publications and presentations**

VanLoocke A, Twine TE, Zeri M, Bernacchi CJ. (2012) A regional comparison of water-use-efficiency for miscanthus, switchgrass and maize. Agricultural and Forest Meteorology, 164, 82-95.

VanLoocke A, (2013) The impact of land-use and global change on water-related agro-ecosystem services in the Midwest US. Ph.D. Thesis, University of Illinois. https://www.ideals.illinois.edu/handle/2142/42334

This research was included in 9 presentations/posters; however none of these were published as conference proceedings.

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USGS Award no. G12AP20115 Modeling of Mixed Open Channel and Pipe Flow by Metroflow

Basic Information

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Descriptors:	
Principal Investigators:	Brian Miller, Marcelo Horacio Garcia

Publications

There are no publications.

Modeling of Mixed Open Channel and Pipe Flow by MetroFlow

Principal Investigator
Marcelo H. Garcia

University of Illinois at Urbana-Champaign
Ven Te Chow Hydrosystems Laboratory
Department of Civil and Environmental Engineering
205 N Matthews Ave
Urbana, IL 61801

Email: mhgarcia@illinois.edu

Tel: 217-244-4484

May 2013

Problem and Research Objective

Other the past 9 years, the University of Illinois has developed a suite of models that simulate various hydrologic and hydraulic processes with special emphasis on complex urban systems. In total, three key models have been developed that simulate the various layers in an urban environment (e.g. hydrology, sewers, interceptors, and deep tunnels):

- Illinois Urban Hydrologic Model: probabilistic based model to determine subcatchment outflow hydrographs
- Illinois Conveyance Analysis Program: quasi-unsteady conveyance analysis program based on hydraulic performance graphs
- Illinois Transient Model: one-dimensional hydraulic transient code based on the Godunov scheme

These three models, which are under continuous improvement, have been integrated together, along with the EPA SWMM 5 model, into a single cohesive modeling framework entitled MetroFlow. The MetroFlow modeling package provides a single unified graphical user interface enabling the end-user to have a seamless modeling experience by automating the inter-model communications, data storage, and analysis and reporting of simulations. This streamlined approach enhances the ability of researchers, scientists, and engineers to analyze, simulate, and forecast phenomena in the world of water.

Currently, the MetroFlow framework is being used to analyze the complex flow within the pipe/tunnel networks in and around the city of Chicago with special emphasis on combined sewer overflows and hydraulic transients. These tools have proven useful in modeling various physical phenomena (e.g., flow reversal and geysering) and determined numerous potential problematic regions throughout the network depending on the various hydrologic and hydraulic forcings.

While the MetroFlow software provides a robust framework, to improve and help promote wider adoption of MetroFlow among the scientific and engineering communities, we proposed expanding the MetroFlow to utilize common data formats, e.g., USGS National Water Information System (NWIS) and US Army Corps of Engineers Data Storage System (HEC-DSS), which are *de facto* standards for time series data in water resources. Allowing MetroFlow to easily import and export these two formats will greatly enhance the model usability and desire of researchers to use these new scientific tools.

Methodology

To implement the HEC-DSS import/export capability, a software library was developed to interface to HEC-DSS libraries and the MetroFlow user interface. The library allows for export, import, query, sorting, and filtering of data imported or exported from HEC-DSS libraries. To support this software library, the MetroFlow timeseries management engine was streamlined and efficiency improved.

In addition to model development, an accompanying user's manual was created to assist new users to quickly become proficient with the package. To help demonstrate and clarify difficult sections in the manual, screencast (i.e., movie-based tutorials) were developed and integrated into the user's manual to streamline information and allow unified/logical access to the training material.

Principal Findings and Significance

By implementing HEC-DSS import and export, improving the timeseries management engine, and creating user manuals with integrated videos, the usability of the MetroFlow software package has enhanced and lends itself to wider adoption by the general scientific and engineering communities.

Notable Achievements

This research resulted in the following achievements:

- Development of software library for interacting with HEC-DSS data files from the C, C++, and C# programming languages
- Enhanced efficiency for timeseries management for MetroFlow
- Enhanced user experience and documentation for the general scientific and engineering communities

Students Supported with Funding

The following students and staff in the Department of Civil & Environmental Engineering, University of Illinois at Urbana-Champaign were supported with the research grant:

- Genevieve Nemeth, undergraduate 2013
- Blake Landry, postdoctoral research associate
- Nils Oberg, staff

Publications and Presentations

The research grant allowed the following media to be created: MetroFlow User Manual and Videos for users.

USGS Award no. G13AP00004 Discharge Rating of Chicago River Controlling Works

Basic Information

Title:	USGS Award no. G13AP00004 Discharge Rating of Chicago River Controlling Works
Project Number:	2013IL266S
Start Date:	12/1/2012
End Date:	11/30/2013
Funding Source:	Supplemental
Congressional District:	
Research Category:	Engineering
Focus Category:	Hydrology, Surface Water, Management and Planning
Descriptors:	
Principal Investigators:	Brian Miller, Marcelo Horacio Garcia

Publications

There are no publications.

Discharge Rating of Chicago River Controlling Works

Principal Investigator
Marcelo H. Garcia

University of Illinois at Urbana-Champaign
Ven Te Chow Hydrosystems Laboratory
Department of Civil and Environmental Engineering
205 N Matthews Ave
Urbana, IL 61801

Email: mhgarcia@illinois.edu

Tel: 217-244-4484

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Problem and Research Objective

As a part of the Chicago Area Waterway System (CAWS), the Chicago River has an important role in flood-control efforts for the Chicago metropolitan area. Water levels in the Chicago River can be lowered during intense rainfall events in order to minimize backwater effects on tributary streams, thereby minimizing upstream flooding. Water-levels in the Chicago River can be controlled by opening and closing a series of locks and sluice gates at the Chicago River Controlling Works (CRCW), located on Lake Michigan at Chicago Harbor; opening the lock at on the Chicago Ship and Sanitary Canal in Lockport, Illinois; and opening the lock on the North Shore Channel in Wilmette, Illinois. Of particular interest is a characterization of the total volume of water that back flows from the Chicago River into Lake Michigan via the CRCW. This location is relatively close to the drinking water intakes for the City of Chicago and poses a potential contamination risk from combined sewer overflow (CSO) events that occur along the CAWS during large storm events.

Previously, the CAWS has been investigated via a three-dimensional numerical model (Sinha et al., 2012) in order to characterize flow conditions and contaminant transport during wet-weather events. At this time, however, the conveyance capacity of the CRCW lock and sluice gates are not well known. There has only been a single equation available for estimation of the flow discharge through a single sluice gate and it is not representative of the total discharge when multiple sluice gates are open as well as the lock gates. Therefore, development of a method to more accurately estimate flow discharge through the lock and sluice gate structures at the mouth of Chicago River has become important for flood risk management and the Lake Michigan diversion.

The main objective of the current study is to develop discharge rating curves and hydraulic performance graphs (Yen and González-Castro, 2000) for the lock and southern/northern sluice gate batteries at the Chicago River Controlling Works (CRCW).

To obtain discharge ratings of the lock and sluice gate structures at CRCW (Chicago River Controlling Works), several flood cases are investigated by using 3-Dimensional Computational Fluid Dynamics (3D-CFD) simulations. For this purpose, a physical domain that includes the hydraulic structures and a portion of the Chicago River and Lake Michigan is generated and used as the computational domain with various boundary conditions applied for different test runs. The result of the individual modeling runs are used to construct a discharge rating curve for the structures. Additionally, another numerical simulation was implemented to determine the

influence of trash screens on energy losses through the sluice gates (Baines and Peterson, 1951).

The overall procedures for this study are detailed as follows:

- Examine the effect of different initial/boundary conditions on flow through the CRCW.
- Examine the effect of different operating scenarios on flow through the CRCW (i.e., different combinations of sluice gate/lock-gate openings)
- Development a discharge rating curves of each hydraulic structure for the different operating scenarios.
- Perform analytical and numerical studies on the effects of trash screens on the discharge rating curves for the sluice gates.
- Application of the discharge rating curves to a real flood case and calculation of total flow discharge throughout the flood.

Moreover, to better understand the hydraulics and fluid dynamics at the CRCW, flow patterns occurring in the vicinity of the locks and sluice gates are investigated and visualized.

Methodology

In this study three finite-volume CFD codes are employed to investigate the flow through the hydraulic structures and to develop the discharge rating curves: 1) ANSYS FLUENT, 2) Flow-3D, and 3) SSIIM (Sediment Simulation In Intakes with Multiblock option).

Firstly, the non-hydrostatic finite-volume commercial CFD codes, ANSYS FLUENT and Flow-3D are employed to solve the three dimensional Reynold's-averaged Navier-Stokes (RANS) equations and to investigate the discharge ratings for the hydraulic structures at CRCW as well as the hydrodynamics in that area during the flood. Both codes apply the volume-of-fluid method to track the free-surface (Hirt and Nichols, 1981), with a turbulence closure. Because field measurement during the flood is not possible, there are not real data available for the comparison. Therefore, the two commercial CFD codes, which are well-known to be verified by many cases, are implemented to support the results of each other. In the simulations, the combinations of the following boundary conditions and scenarios are considered.

- Four operation steps of the hydraulic structures, 1) opening all southern gates, 2) opening all northern and southern gates, 3) opening the lock gates halfway while maintaining step 2, and 4) opening the lock gates fully with all sluice gates

opened. These steps are exactly matched to the real operating procedures for flood control at CRCW.

- Lake level ranges between -3 ft CCD and +4 ft CCD.
- Gates open when river stage reaches +3.0 ft CCD through +4.0 ft CCD provided that river stage > lake stage.
- The initial water surface level in the Lake Michigan is controlled not to change during the simulation. A no-slip boundary condition is applied at all water-solid surface interfaces.

In addition, the RANS model, SSIIM (Olsen, 2009) is employed to perform the calculation of the time-averaged flow through a sluice gate with a screen. The screen effect in the study is investigated by substituting the netted geometry of the screen with solidity ratio of the screen. The solidity ratio is used in a porosity approach which is regarded as a force from the roughness on the water in each cell suggested by Engelund (1953). The porosity modeling is able to give smooth interactions between areas with small and large roughness so that it represents the effect of open or closed part of screen on the flow field.

Principal Findings and Significance

In this study we investigate the discharge rating curve at the CRCW in order to optimize efficient operation of the lock during high-water flow event on the CAWS. A single flow discharge equation through a sluice gate has been developed from the rating curve and has been compared to an existing formula used for flow though the sluice gates. An individual rating curve of each hydraulic structure as well as hydraulic performance graph at CRCW is developed based on CFD modeling results.

Based on the analysis, it appears that flow discharge through the hydraulic structures is very dependent on the initial water surface level at Lake Michigan. This is an important finding because the water-surface level at Lake Michigan has being observed to be decreasing for the last several decades. This means that the discharge characteristics are likely to vary over time and a single equation is unlikely to be sufficient to estimate discharge though the CRCW over time. Moreover, determination of the velocity distribution and turbulence characteristics of the flow through the hydraulic structures will aid in updating the operation guidelines (if necessary) and will help in developing guidelines for maintaining the structures against a flood situation.

Notable Achievements

This research resulted in the following achievements:

- Development of discharge rating curve as well as hydraulic performance graph for efficient operation of the lock and sluice gates at Chicago River Controlling Works.
- Estimation of Screen effect on the flow field through sluice gates by using a porosity approach in numerical simulations.
- Visualization of flow field including turbulent flow structures around hydraulic structures.

Students Supported with Funding

The following staffs in the Department of Civil & Environmental Engineering, University of Illinois were supported with the research grant:

- Su Jin Kim, postdoctoral research associate

Publications and Presentations

Due to the on-going nature of the project, no official reports or presentations have been based on this work at this time.

References

Baines, W. D., E. G. Peterson, 1951, An investigation of flow through screens, Transactions of the American Society of Mechanical Engineers, 73, 467-480.

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Yen, B., J. González-Castro, 2000, Open-channel sapacity determination using hydraulic performance graph, Journal of Hydraulic Engineering, 126(2), 112-122.

Information Transfer Program Introduction

None.

Transferring Water Resources Information to the People of Illinois

Basic Information

Title:	Transferring Water Resources Information to the People of Illinois
Project Number:	2012IL253B
Start Date:	3/1/2012
End Date:	2/29/2013
Funding Source:	104B
Congressional District:	15
Research Category:	Not Applicable
Focus Category:	Education, None, None
Descriptors:	None
Principal Investigators:	Lisa Merrifield

Publications

There are no publications.

Illinois Water Resources Center Technology and Outreach Annual Progress Report
FY 2012

IWRC Webpage

The IWRC website has not been substantially updated since 2006. Consequently, much of 2012 was spend reorganizing the page for increased ease of use and maneuverability. This included sorting research publications by topic and creating separate tabs for funding opportunities and memberships in national organizations. Recognizing the role of IWRC in supplying water resources information to the people of Illinois, we also added a resources section to the webpage. This included information for private well owners, water resources professionals, K-12 age students, and university students. While much of this content is still under development, the resources for K-12 and university students includes curriculum for use in classrooms and information regarding water resources careers.

Blog

In January 2013, we launched the IllinoisWater blog (illinoiswater.blogspot.com). The blog provides us with an easily updated platform to share Illinois water news, including funding opportunities, legislative updates, and research highlights. The most popular material, however, has proved to be a series discussing water-related careers. Since January, we have interviewed a fish biologist, sustainable real estate developer, sanitation engineer, and a zoology professor about their jobs as part of our ongoing efforts to support the entrance of young professionals into water-related careers. The blog is targeted at a middle-school level to provide accessible follow-up material for any of our school outreach activities.

Social Media

In fall of 2012, IWRC debuted a social media presence on both Facebook (https://www.facebook.com/IllinoisWaterResourcesCenter) and Twitter (@IllinoisWater). While our followings are small, they are comparable to those of other NIWR members. To measure the influence of our social media presence, we created a Klout account, which rates our blog, Twitter feed, and Facebook presence on a scale of 1 to 100. IWRC's Klout score is 44, while that of The Nature Conservancy in Illinois is 45, and the Water Texas Resources Institute (a fellow NIWR member) is 49. For reference, the Klout score for the White House is 85.

Our social media connections have proved to be excellent information resources. Two of our subjects for the Water Jobs interviews published on our blog were found through Twitter interactions, while our greatest number of blog visits were generated by a cross-posting on Facebook. Additionally, Twitter has allowed us to share funding information

with Illinois researchers and to interact with other Illinois research and environmental organizations, thus increasing our outreach opportunities.

Illinois Water Conference

IWRC has hosted a biennial water conference since the 1990s to provide an opportunity for government employees, educators, corporate representatives, citizen groups, and non-government organization members to present and discuss the latest water resources research and policy directions for Illinois.

The Water Conference 2012 was held in Urbana, Illinois on September 24th and 25th at the Illini Union. Over 200 water professionals from academia, private industry, and government agencies, as well as over 50 undergraduate and graduate students, attended the conference. 12 students received scholarship funding to attend the meeting. Student opportunities included a careers panel as well as a poster competition. The Illinois Chapter of the American Water Resources Association provided judging for the 43 posters submitted, and awarded prizes to the best undergraduate and graduate posters presented.

Sessions addressed aquatic invasive species, water supply planning, 2011 flooding, new inland recreational water quality standards for bacteria, and Lake Michigan foodwebs. The Plenary session addressed the 2012 Drought and included State Climatologist Jim Angel, and hydrologist Vern Knapp.

The USGS Illinois Water Science Center hosted a workshop in Sediment Transport Modeling and Monitoring on Wednesday, September 26, which was attended by approximately 22 people. The workshop addressed the use of HEC-RAS modeling to determine sediment transport.

Governor's Conference on the Management of the Illinois River System

IWRC staff helped plan this biennale event, which will be held in Peoria, IL from October 1-3, 2013. In 2012 planning was still in the preliminary stages, but IWRC staff have committed to designing the abstract book, providing a place to upload conference presentations, and producing the electronic conference proceedings.

Drinking Water Education and Protection

Small communities and rural areas face real challenges obtaining and maintaining safe water supplies. The Illinois Water Resources Center is a partner with the Illinois State Water Survey on two websites funded by the US EPA, in partnership with the Rural Community Assistance Partnership (RCAP), which address education and outreach barriers to safe water.

The Private Well Class

PrivateWellClass.org centers on a 10-week email course (The Private Well Class) that teaches homeowners how to properly care for and maintain their water well.

This includes introductory information on geology, well contamination and water testing. The site is designed to serve the 45 million Americans who rely on a private well for their drinking water and includes a pre- and post-test quiz to test knowledge improvement. Understanding how to prevent groundwater contamination, both on the property and via cross-connection control, is addressed in the lessons as well as during a series of three live webinars. To date, more than 2100 individuals have signed up for The Private Well Class.

Small Water Supply

SmallWaterSupply.org is a one-of-a-kind resource website on many drinking water, wastewater, and utility management topics. It aggregates information from across the web so that users can save significant time finding the documents, training events, and news they need. The site is designed to serve water operators in small communities and tribes across the United States.

The team behind SmallWaterSupply.org has indexed more than 23,000 events and 11,000 documents to date. A biweekly newsletter reaches more than 1100 water industry professionals with the most timely and relevant information available.

USGS Summer Intern Program

Basic Information

Start Date:	3/1/2012
End Date:	2/28/2013
Sponsor:	
Mentors:	Marcelo Horacio Garcia
Students:	Tatiana Garcia

Internship Evaluation

Question	Score
Utilization of your knowledge and experience	Very Good
Technical interaction with USGS scientists	Very Good
Treatment by USGS as member of a team	Very Good
Exposure and access to scientific equipment	Very Good
Learning Experience	Very Good
Travel	About Right
Field Experience Provided	About Right
Overall Rating	A

Additional Remarks

The USGS Student Internship Program provided me with the opportunity to work on a highly critical and relevant project studying Asian carp at early life stages. Throughout my internship experience I received valuable mentoring from Elizabeth Murphy, Hydrologist - USGS Illinois Water Science Center, and Ryan Jackson, Hydrologist - USGS Illinois Water Science Center. Not only did they guide me through the research process but also taught me valuable skills as well.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	0	0	0	1	1
Masters	0	0	0	0	0
Ph.D.	2	2	1	0	5
Post-Doc.	0	0	0	2	2
Total	2	2	1	3	8

Notable Awards and Achievements

Dr. John B. Braden of the University of Illinois, Urbana-Champaign, and a former director of Illinois Water Resources Center, received the 2012 Warren A. Hall Medal from the Universities Council on Water Resources. The Warren A. Hall Medal recognizes the “distinctive scholarly accomplishments of an individual in the water resources field.” It is awarded annually to an educator who works in the advancement of water resources in teaching, research, or public service. The award will be presented at the 2013 Universities Council on Water Resources Conference in Lake Tahoe, CA.

During 2012, The Private Well Class saw more than 1200 homeowners in 46 states enroll in free online training to improve understanding of proper well care and ensure their water remains safe to drink.

Over the past three years, Small Water Supply has served over 255 communities in Illinois and 2669 nationwide through their online resources that assist small communities with operating and maintaining their public water and wastewater.

Graduate student research has examined the willingness of Chicago-area residents to volunteer or pay for stormwater reduction techniques. This research is particularly timely as Chicago prepares new strategies to address the historic flooding it experienced in the spring of 2013.

Research by IWRC-supported graduate students has also modeled the water use of bioenergy crops and found that a shift from traditional corn agriculture to use of native grasses would not only reduce nutrient pollution contributing to the Gulf of Mexico Hypoxia zone, but could also result in increased carbon storage and decreased water use.

Publications from Prior Years

1. 2010IL207B ("Linking microbial community structure to water quality function: investigating nitrogen cycling during early floodplain development") - Articles in Refereed Scientific Journals - Peralta, A.L., J.W. Matthews, D. N. Flanagan, and A.D. Kent. 2012. Environmental factors at dissimilar spatial scales influence plant and microbial communities in restored wetlands. Wetlands 32(6):1125-1134.
2. 2004IL52G ("Carbonaceous Material Fractions in Sediments and Their Effect on the Sorption and Persistence of Organic Pollutants in Small Urban Watersheds") - Articles in Refereed Scientific Journals - Yang, Y., P. Van Metre, B. Mahler, J. Wilson, B. Ligouis, M. Razzaque, D. Schaeffer, C.J. Werth, 2010. The Influence of Coal-Tar Sealcoat and Other Carbonaceous Materials on Polycyclic Aromatic Hydrocarbon Loading in an Urban Watershed, Environmental Science & Technology, 44, 1217-1223.
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4. 2009IL184G ("Attachment and Transport Mechanisms of Cryptosporidium parvum oocysts in karst aquifers: Role of Natural Organic Matter and Iron Oxide") - Articles in Refereed Scientific Journals - Liu, Y., M.S. Kuhlenschmidt, T. B. Kuhlenschmidt, T. H. Nguyen, 2010, Composition and conformation of Cryptosporidium parvum oocyst wall surface macromolecules and their effect on adhesion kinetics of oocysts on quartz surface. Biomacromolecules , 11 (8), 2109-2115.
5. 2009IL173B ("Attachement and Transport Mechanisms of Cryptosporidium parvum Oocysts in Subsurface Environments: A Multi-Scale Study") - Articles in Refereed Scientific Journals - Liu, Y., C. Zhang, M. Hilpert, M.S. Kuhlenschmidt, T. B. Kuhlenschmidt, T. H. Nguyen, 2012, Transport of Cryptosporidium parvum oocysts in a silicon micromodel. Environmental Science & Technology, 46 (3), 1471-1479.
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7. 2010IL202B ("An Agent-Based Model of Nitrogen and Carbon Trading at the Watershed Scale") - Articles in Refereed Scientific Journals - Ng, Tze Ling; J. Wayland Eheart, Ximing Cai, John B. Braden, and George F. Czapar, 2012, Agronomic and stream nitrate load responses to incentives for bioenergy crop cultivation, reductions of carbon emissions and fertilizer use. Journal of Water Resources Planning and Management, ASCE, accepted for publication.
8. 2010IL202B ("An Agent-Based Model of Nitrogen and Carbon Trading at the Watershed Scale") - Articles in Refereed Scientific Journals - Ng, Tze Ling; J. Wayland Eheart, Ximing Cai, and John B. Braden, 2011, An agent-based model of farmer decision-making and water quality impacts in a watershed under markets for carbon allowances and second-generation bioenergy crops. Water Resources Research, 47, W09519,