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List of Abbreviations

CRM	Certified reference material
CV-AFS	Cold vapor atomic fluorescence spectrometry/spectrometer
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
Hg	Mercury
Hg^{II}	Mercuric mercury
Hg^0	Elemental mercury
HgTU/IC	Mercury thiourea complex ion chromatography system for mercury speciation analysis
HCl	Hydrochloric acid
ICP-MS	Inductively coupled plasma-mass spectrometry/spectrometer
IRB	Iron-reducing bacteria
ISTC	Illinois Sustainable Technology Center
LCHg	Low-charge mercury species detected by HgTU/IC
MMHg	Monomethylmercury
NRES	Department of Natural Resources and Environmental Sciences, UIUC
PETE	Polyethylene terephthalate
QFF	Quartz fiber filters
SPE	Solid phase extraction
SRB	Sulfate-reducing bacteria
TU	Thiourea
UIUC	University of Illinois at Urbana-Champaign
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

Abstract

Several operational woodchip bioreactors were installed at the outlets of agricultural drainage systems located in east central Illinois. The potential for monomethylmercury (MMHg) production and export in these bioreactors was investigated from summer 2008 to summer 2010. The basic approach was to compare the chemistry of simultaneously-collected bioreactor inlet and outlet water samples in order to assess the extent of nitrate depletion, consumption of sulfate, and production of MMHg, plus other low-charge mercury species (LCHg). In making such a comparison, we implicitly assume that the reactor is near steady state, which is a reasonable approximation given hydraulic residence times on the order of hours. All mercury (Hg) speciation measurements were made using a first-generation mercury thiourea complex ion chromatography system for Hg speciation analysis, which reliably separates MMHg and Hg^{II} (mercuric mercury), but combines MMHg and a newly-discovered, unidentified Hg species of low charge (LCHg). Due to this analytical artifact, the results reported here constitute an upper bound on true Hg methylation.

In no season was MMHg ever detected in inlet samples at concentrations at much above the detection limit of ~0.1 ng/L. However, levels of MMHg+LCHg over 2 ng/L were observed in the outlets during warm seasons when nitrate had become depleted within the bioreactor. Sulfate depletion was also observed in most samples with elevated [MMHg+LCHg]. The combination of sulfate depletion and MMHg production is consistent with nitrate inhibition of iron and sulfate reduction and with MMHg concentrations observed in other highly anaerobic environments, e.g., lake hypolimnia and wetland porewaters. The maximum [MMHg+LCHg] observed in any given bioreactor followed an inverse function of the bioreactor loading density, i.e., the ratio of the area drained to the area of the bioreactor pit. The function has a form similar to that observed for bioreactor denitrification efficacy and suggests that optimal bioreactor designs that permit substantial denitrification while minimizing Hg methylation are feasible.

Finally, extremely high MMHg+LCHg levels were observed when stagnant water conditions occurred within the bioreactors. Thus, it is recommended that bioreactors not be built with bottom depresssional areas where stagnant water can reside, in order to avoid developing anoxic conditions where methylation occurs. For the same reasons, bioreactors should not be used simultaneously with controlled drainage (water table management) if restricting the drainage results in keeping the bioreactors flooded for long periods of time.

1. Introduction

1.1. Mercury Pollution

Mercury pollution is a cause of abundant concern from an environmental health perspective. This concern is not focused primarily on mercury (Hg) in its inorganic forms of mercuric mercury (Hg^{II}) and elemental mercury (Hg^0) that are emitted to the atmosphere (Demissie and Keefer, 1998), but on excess monomethylmercury (MMHg) which is produced by the microbially-mediated methylation of Hg^{II}. This occurs after Hg deposition into aquatic ecosystems. The very efficient biomagnification of MMHg in aquatic food webs gives rise to the principal exposure pathway for humans: consumption of fish containing high levels of MMHg. Although sites with legacy contamination from local sources have been documented and geological sources of mercury cannot be neglected, there is much more concern about widespread contamination of ecosystems by atmospheric Hg deposition traceable to coal combustion for electric power generation and metal smelting, and to the incineration of Hg-containing wastes (Sigler and Lee, 2006). The scope of this contamination is evident in the recent USGS finding that more than 75% of stream systems sampled across the U.S. contained levels of Hg in fish that are of concern for wildlife health (Scudder et al., 2009). Although the issue is less pronounced in Illinois than in other states, there is a statewide advisory on the consumption of predatory fish by sensitive human populations, namely pregnant women and children. In addition, specific fish consumption advisories have been issued for 34 lakes and rivers (IDPH, 2014).

While it is clear that regulating atmospheric emissions offers the most direct means of mitigating Hg pollution, it is much less widely appreciated that other anthropogenic environmental alterations can indirectly reduce or potentially worsen its impacts. Hg bioaccumulation depends on the natural bacterially mediated methylation process that produces the organometallic compound monomethylmercury (CH_3Hg^+ or MMHg) from inorganic mercury (Hg^{II}) in deposition (Reaction 1):

$$Hg^{II} + CH_3 - \text{cobalamin} \xrightarrow{IRB,SRB} CH_3Hg^+ + \text{cobalamin} \qquad (1)$$

where methylcobalamin is the methyl donor for the enzymatic process (Choi and Bartha, 1993). Mercury methylation is crucial because MMHg biomagnifies in food webs much more efficiently than Hg^{II} (Mason et al., 1996), accounting for the fact that >90% of the total Hg in fish muscle typically occurs in this form. Thus, the human health impact of Hg pollution is largely determined by the extent of bacterial Hg methylation in the environment. As a consequence, differences in environmental factors that drive methylation can cause significant variations in Hg levels in fish across ecosystems, even for those that receive similar Hg inputs from atmospheric deposition. Similarly, other human activities and non-Hg forms of pollution, e.g., acid deposition, can affect the extent of Hg methylation in particular ecosystems.

We anticipate that such indirect influences on Hg methylation are important primarily based on research over the past 30 years showing that iron- and sulfate-reducing bacteria, IRB and SRB, respectively, are the principal bacterial populations responsible for this process in the environment (Gilmour et al., 1992; Fleming et al., 2006). Not only do these microbes have specific genes for Hg methylation, but one of the most reliable predictors of high MMHg levels in any specific aquatic ecosystem is its hydrologic connection to anaerobic zones where IRB and SRB reside (St. Louis et al., 1994). Oxygen and nitrate become depleted in these environments, leaving IRB and SRB as the predominant decomposers of organic matter. In stratified lakes, such conditions can develop during the summer in the hypolimnia. For rivers and streams, where anaerobic conditions generally do not develop in the water column, the biggest source of MMHg appears to be wetlands, as evidenced by the strong correlation of dissolved MMHg levels with the fraction of watershed area classified as wetland (Brigham et al., 2002). Wetlands are well-known loci of these anaerobic biogeochemical processes (Faulkner and Richardson, 1989), because they provide extensive contact with iron-rich soils, and because significant levels of sulfate are present in atmospheric deposition in much of the northern hemisphere. Thus, bacteria capable of methylation are ubiquitous in wetlands (St. Louis et al., 1994). Therefore, when formulating environmental management policies that encourage the development of constructed wetlands and managed anaerobic ecosystems for the important ecological services that they can provide, it is necessary to be cognizant that such development also has the potential for unintended negative consequences, such as amplifying the impacts of Hg pollution, primarily due to increased Hg methylation.

1.2. Nitrate Pollution and Managed Anaerobic Ecosystems

Nationally managed anaerobic ecosystems are being constructed for a variety of reasons, one of the most common being the mitigation of nitrate exports from agricultural watersheds. This form of pollution is especially meaningful in Illinois, where nitrate concentrations in rivers often exceed drinking water standards, e.g., in Lake Decatur (Demissie and Keefer, 1998), and whose nitrate exports to the Mississippi River contribute significantly to the formation of hypoxic “dead zones” in the Gulf of Mexico (Goolsby et al., 2001; Rabalais et al., 2001). Tile-drained watersheds where crops are fertilized with nitrogen are the dominant sources of riverine nitrate loads in the upper Mississippi River basin (David et al., 2010). Although nitrate pollution can be mitigated to an extent by managing rates of fertilizer application, high levels of nitrate export from Illinois seem inevitable due to the widespread use of subsurface agricultural (tile) drainage. Tile drainage is used extensively in much of the Midwest because it can enhance agricultural productivity (David et al., 2009), but tile drains also allow agrochemicals and nitrate to move rapidly from fields into surface waters. For this reason, “end of pipe” means of nitrate-pollution mitigation is being seriously considered by policy makers and government agencies responsible for managing the environmental impacts of agriculture.

The main types of managed anaerobic ecosystems used to mitigate “end of pipe” nitrate pollution are (i) constructed wetlands, (ii) fields with controlled water tables, and (iii) denitrifying bioreactors (Woli et al., 2010). All of these systems are designed to create anaerobic conditions that are conducive to denitrification, i.e., the transformation of nitrate to dinitrogen gas (N_2). Such conditions typically develop within water-saturated soils or stagnant waters after aerobic microbial decomposers consume most of the available oxygen. The requirement that oxygen be depleted prior to nitrate consumption results from the fact that oxygen respiration provides slightly more energy to microbes than nitrate per unit mass of organic matter decomposed. This fact, and the general pattern of competition between microbes with different anaerobic metabolism mechanisms, is conventionally explained by considering the “thermodynamic ladder” (Bethke et al., 2011). The process of organic matter decomposition yields energy by transferring the electrons released from breaking the C-C and C-H bonds in organic matter (Reaction 2):

$$\tfrac{1}{6}C_6H_{12}O_6 + H_2O \xrightarrow{-4e^-} CO_2 + 4H^+ \qquad (2)$$

to an available oxidant, i.e., the first species shown in Reactions 3 a-g:

$$\begin{array}{lll}
a)\ \textit{Aerobic respiration} & O_2 + 4H^+ & \xrightarrow{4e^-} 2H_2O \\
b)\ \textit{Denitrification} & NO_3^- + 6H^+ & \xrightarrow{5e^-} \tfrac{1}{2}N_2 + 3H_2O \\
c)\ \textit{Mn reduction} & MnO_2(s) + 4H^+ & \xrightarrow{2e^-} Mn^{2+} + 2H_2O \\
d)\ \textit{Fe reduction} & Fe(OH)_3(s) + 3H^+ & \xrightarrow{e^-} Fe^{2+} + 3H_2O \\
e)\ \textit{Sulfate reduction} & SO_4^{2-} + 10H^+ & \xrightarrow{8e^-} H_2S + 4H_2O \\
f)\ \textit{Methanogenesis} & CO_2 + 4H^+ & \xrightarrow{4e^-} CH_4 + H_2O \\
g)\ \textit{Homoacetogenesis} & 2CO_2 + 7H^+ & \xrightarrow{8e^-} CH_3COO^- + 2H_2O
\end{array} \qquad (3)$$

These oxidants are arranged in order of decreasing energy yield. Oxygen is the first to be consumed because it provides the greatest amount of energy for metabolism and growth for a given supply of organic matter. Oxidation of nitrate, better known as denitrification, yields only slightly less energy than oxygen respiration, making nitrate the next most favorable oxidant. Iron and sulfate respiration yield substantially less energy and can be metabolized only by IRB and SRB, which are responsible for Hg methylation. Where O_2 and NO_3^- are present, IRB and SRB are out-competed by bacteria that are less specialized.

Of the managed anaerobic ecosystem types being implemented, bioreactors have certain advantages that make them strong candidates to be widely adopted as a best management practice across the Midwest (Schipper et al., 2010). First, they use a proven technology and require little or no maintenance. Second, they require little or no modification of current farming methods and have no impact on the effectiveness of existing drainage systems. Third, the cost of installation is modest and does not require any (or much) land to be taken out of production. For these reasons, the efficacy of these bioreactors has been an active subject of research in central Illinois. It must be noted that the scale at which these bioreactors can be used is potentially very large. Nearly all farms in central Illinois are hydrologically-connected to streams and rivers via subsurface drainage systems, or tiles, and tile discharge has been established as the main avenue for nitrate export to rivers and streams (David et al., 2009). Thus, denitrifying bioreactors could be installed in very large numbers across the region if serious actions to mitigate nitrate pollution were to be taken.

1.3. Denitrifying Bioreactors

The denitrifying bioreactors that are the focus of this project are typically constructed at the ends of tile lines and managed so that tile drainage water flows through them just before being discharged into ditches and streams. Each reactor is comprised of a large pit lined with inexpensive polyethylene sheeting and filled with woodchips and one or more flow-control structures. Dissolved organic matter (DOM), derived from the depolymerization of woodchip cellulose, serves as the electron source for the reactor's heterotrophic microbes (Reaction 2). The abundant supply of organic matter allows the microbes to consume oxygen rapidly enough that the water within the reactor becomes sub-oxic to anoxic, thereby permitting the growth of denitrifying bacteria. When the flow of water slows enough and/or denitrification rates are high enough that the microbes can consume all of the nitrate in the inlet water, e.g., during summer (Robertson et al., 2007; Robertson and Merkley, 2009; Elgood et al., 2010), highly-reducing conditions favorable to iron and sulfate reduction can also develop (Vogan, 1993; Benner et al., 1997).

There are several possible ways in which bioreactors could have unintended negative impacts on environmental quality. Although carbon dioxide (CO_2) is always produced by decomposition of organic matter (Reaction 2), the potential emission rates of bioreactors are not large relative to other anthropogenic sources of CO_2. Of greater concern are the potential by-products of anaerobic microbial metabolism. It is important to avoid producing the more potent greenhouse gases, particularly nitrous oxide, which is a denitrification by-product and methane, which is the product of methanogenesis (Reaction 3). The end-product of sulfate reduction, hydrogen sulfide (Reaction 3; Elgood et al., 2010), is itself an undesirable potential constituent of discharge from the reactors. Scant information is available at this time on MMHg production in denitrifying woodchip bioreactors.

Of course, it is theoretically possible to create conditions that are conducive to IRB and SRB growth, yet free of Hg to methylate. In practice, very low levels of Hg are ubiquitous, but ultimately it will be important to identify the most important sources of Hg to bioreactors. As a strongly-sorbed metal, Hg^{II} from deposition should not be transported very far by water slowly percolating in soils. Preferential flow, however, could transport Hg^{II} from the surface to drainage tiles. Any Hg^{II} in tile drainage could be adsorbed on the wood chips and then later released after undergoing methylation, because MMHg adsorbs less strongly to most solid materials than Hg^{II}. In addition, the wood chips used in bioreactors are themselves a source of Hg. Tree leaves absorb substantial amounts of gaseous Hg^0 from the atmosphere, with some of it becoming incorporated into bole wood (Laacouri et al., 2013). Typical levels of Hg in wood vary from 0.1 to 10 ppb (100 to 10,000 ng/L) (Poissant et al., 2008). Also there is some Hg^{II} present in the soil into which bioreactor pits are dug (Dreher and Follmer, 2004). However, the plastic linings used should minimize the mobilization of Hg from this source.

Given the universal association between Hg methylation and sulfate reduction in aquatic systems and the ready source of bioavailable Hg in atmospheric deposition, it is virtually certain that MMHg is produced in bioreactors to some degree. This was confirmed in the only published work on Hg methylation in bioreactors to date. Shih et al. (2011) examined the production of MMHg in a woodchip bioreactor located in a stream bed and found that MMHg concentrations in the outlet increased with the onset of sulfate reduction, suggesting that such bioreactors do function as a source of MMHg production to the stream. However, when the nitrate concentration in the bioreactors was maintained above 0.5 ppm (500,000 ng/L), the production of MMHg was suppressed.

Note also that the initial observations from this study, obtained during the summer of 2008, have been available in unpublished form for some time (Hudson and Cooke, 2010). These observations provided evidence that very high levels of Hg methylation could indeed occur in bioreactors, at least during summer. In several bioreactor outlets, MMHg likely exceeded 1 ng/L, while the inlet water contained non-detectable levels. These levels can be compared to "typical" concentrations of MMHg in streams of about 0.1-0.3 ng-Hg/L (Scudder et al., 2009). Other recent work at the University of Illinois at Urbana-Champaign (UIUC) on bioreactors was reported by Bell (2013). There, evidence of mercury methylation was found in samples collected during the initial months of bioreactor operation when very low (nearly zero) nitrate concentrations developed.

1.4. Study Objectives

Different scenarios under which the export of MMHg from bioreactors would be of concern can be identified for small and large watersheds. At the smallest scale, one can imagine a single reactor draining a field of less than 100 acres (0.4 km^2) and discharging into a farm pond. A farmer with such a system may well be interested in maintaining a population of fish that are safe to eat and would want to be sure to operate the bioreactor in a way that minimizes MMHg export. In a larger watershed draining to a water supply reservoir, such as Lake Decatur, one can imagine a scenario in which thousands of tile systems are equipped with bioreactors in order to improve drinking water quality. In either case, the MMHg in water discharged from the bioreactors should not cause drinking water standards for total mercury (2,000 ng/L; USEPA, 2009) to be violated. However, water quality criteria for MMHg are much more stringent. The US Environmental Protection Agency (USEPA) has recommended that ambient MMHg levels be kept low enough prevent its accumulation in fish to more than 0.3 ppm (300,000 ng/L) (USEPA, 2010). Since bioaccumulation factors for MMHg range from 120,000 L/kg (trophic level 2) to 2,700,000 L/kg (trophic level 4), ambient dissolved MMHg should be kept below 2.5 ng/L to prevent excessive accumulation in trophic level 2 fish or 0.11 ng/L for fish of trophic level 4. Thus, a scenario in which many bioreactors discharge MMHg-laden water into a watershed and thus cause fish mercury levels to exceed the standard is a legitimate concern. The fish consumption advisories this scenario could trigger would be detrimental to the value of recreational fisheries.

Based on what is already known about bioreactors specifically, and managed anaerobic ecosystems in general, it is prudent to pre-emptively mitigate these possible sources of MMHg to surface waters. This is particularly important when measures to reduce agricultural nitrate export are implemented, particularly when they involve creating new zones where sulfate reduction can occur.

This study was designed to investigate the methylation of Hg in denitrifying bioreactors coupled to sub-surface agricultural drainage systems. The goal was to identify the conditions under which MMHg discharge occurs and investigate whether the reactors could be designed or operated in ways that minimize such MMHg export. This question was approached by measuring MMHg concentrations in samples of inputs to and discharges from operational woodchip bioreactors in central Illinois farm fields over a three-year period.

2. Study Sites

2.1. Overview

The bioreactors monitored in this study are located in east central Illinois between Champaign-Urbana and Decatur (Figure 1). The main bioreactors evaluated in the project are located at three sites: Decatur, Amenia, and Deland. All three sites are located within the watershed of Lake Decatur (Sangamon River), which is the water supply for the city of Decatur. Two other bioreactors, at Mt. Zion and Bloomington, were included but only sampled one time. This report also incorporates some environmental monitoring data from USGS stream gages (Lake Fork and West Okaw rivers) and National Weather Service (NWS) meteorological stations.

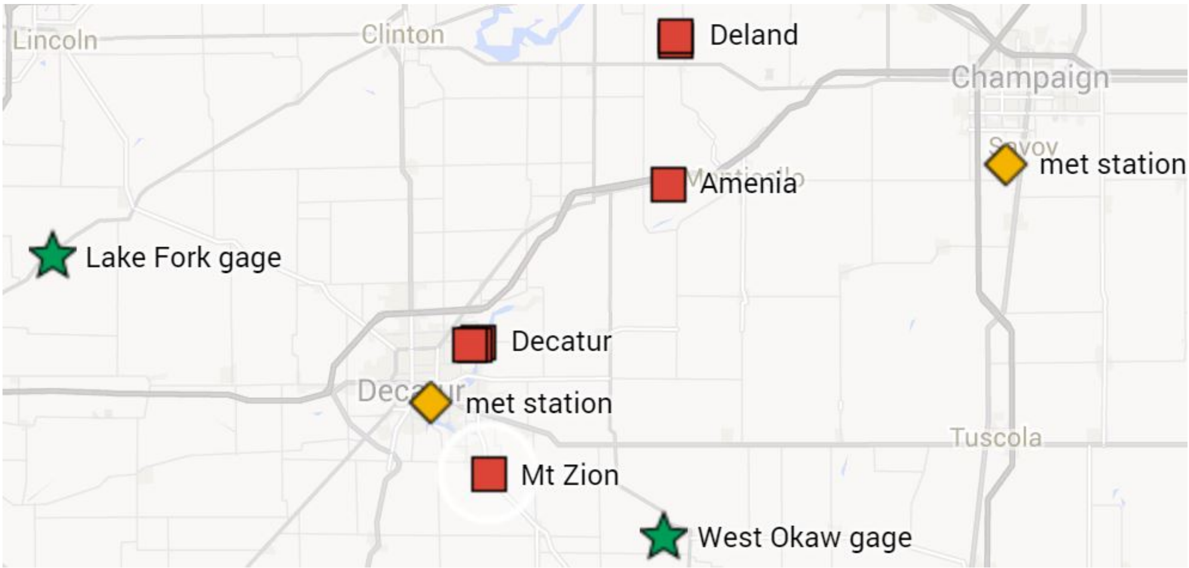
Lincoln
Clinton
Deland
Champaign
Savoy
met station
Amenia
Monticello
Lake Fork gage
Decatur
Decatur
met station
Tuscola
Mt Zion
West Okaw gage

Figure 1. Locations of bioreactors (red squares) and meteorological stations (yellow diamonds) within the study region. The Bloomington bioreactor (not shown) is directly north of Clinton. USGS gages (green stars) 5579500 (drainage area 214 mi^2) and 05591700 (drainage area 112 mi^2) were used to compute “regional runoff.” Image from Google Maps.

The bioreactors were constructed between the fall of 2006 and 2009 using one of two basic designs (Figure 2). All have Agri-Drain^{TM} control structures containing (i) stop boards to manage the water table depth and to direct flow through or around the bioreactor and (ii) a weir to permit flow measurement. Each bioreactor is fed by a tile system that drains a field ranging in size from 5 to 39 acres (0.02 to 0.16 km^2) (Table 1).

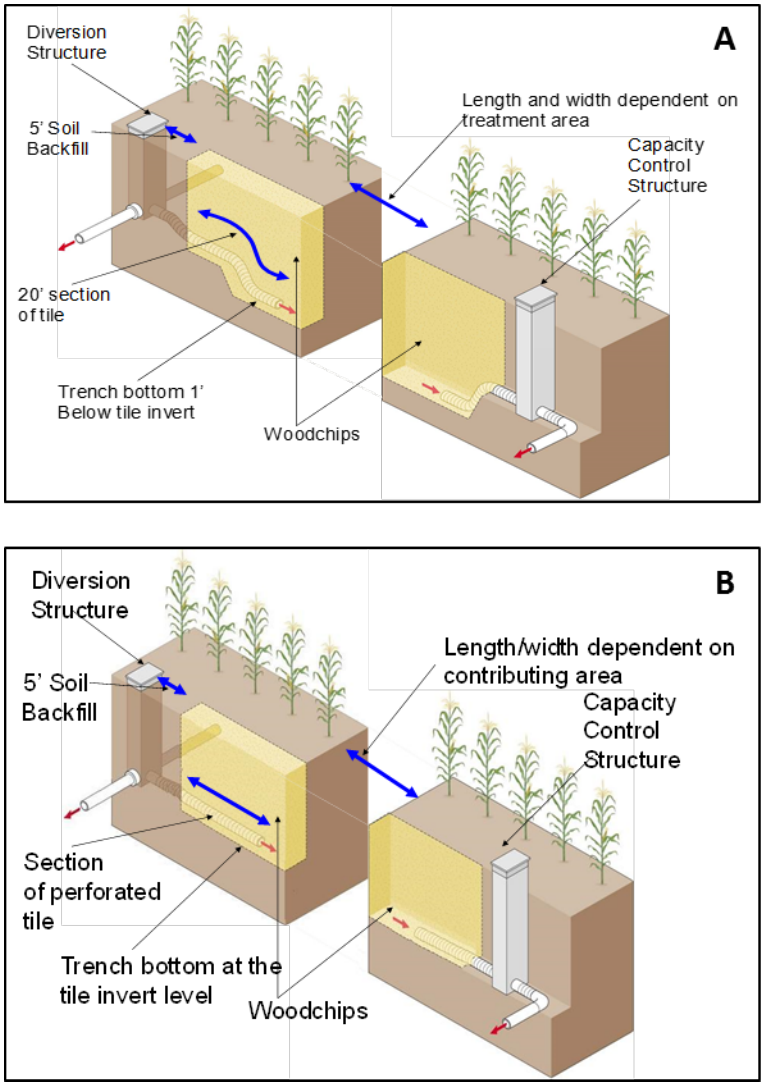
A
Diversion Structure
5' Soil Backfill
Length and width dependent on treatment area
Capacity Control Structure
20' section of tile
Trench bottom 1' Below tile invert
Woodchips
B
Diversion Structure
5' Soil Backfill
Length/width dependent on contributing area
Capacity Control Structure
Section of perforated tile
Trench bottom at the tile invert level
Woodchips

Figure 2. Schematic of second (A) and third (B) generation bioreactor designs. The key difference is the depth of the depression at the bottom of the reactor and the type of control structure.

Table 1. Characteristics of bioreactors sampled in this study.

Site	Area Drained (Ac)	Dimensions (L×W×D) (ft)	Lined Pit?	Wood Type	High Flow Bypass?	Date Constructed (Generation)	Crops
Decatur West[a]	5	20×20×5	No	Mixed	No	August 2006 (2nd)	Corn/alfalfa/ Wheat
Decatur East[a]	16	80×5×3	No	Mixed	No	August 2006 (2nd)	Corn/soybean
Decatur 1[a]	30	100×3×5	No	Mixed	No	August 2006 (2nd)	Corn/soybean
Amenia[b]	15	40×10×6	Yes	Hardwood	Yes	Fall 2007 (2nd)	Corn/soybean
Deland East[c]	34	40×10×7	Yes	Mixed	Yes	Fall 2007 (3rd)	Corn/soybean
Deland West[c]	28	93×10×6	Yes	Mixed	Yes	Fall 2006 (3rd)	Corn/soybean
Deland North[c]	39	56×10×6	Yes	Mixed	Yes	Fall 2009 (3rd)	Corn/soybean
Mt. Zion	70	40×10×4	Yes	Mixed	Yes	Fall 2009 (3rd)	Corn/soybean
Bloomington North	20	56×10×4	Yes	Mixed		Fall 2008 (3rd)	Corn/soybean

[a] See Section 2.2. [b] See Section 2.3. [c] See Section 2.4.

2.2. Decatur (Progress City)

Three bioreactors located on or adjacent to the campus of Richland College in Decatur, Illinois (Figure 3), were monitored in this study. These reactors were installed in the fall of 2006 as a part of the Illinois Land Improvement Contractors Association exhibit for the biennial Farm Progress show. The Decatur East and Decatur West bioreactors receive drainage from tile-drained fields planted in a conventional corn/soybean rotation and in corn/alfalfa/wheat, respectively (Table 1). A third bioreactor, Decatur 1, is located on private land just to the east of the Decatur East reactor.

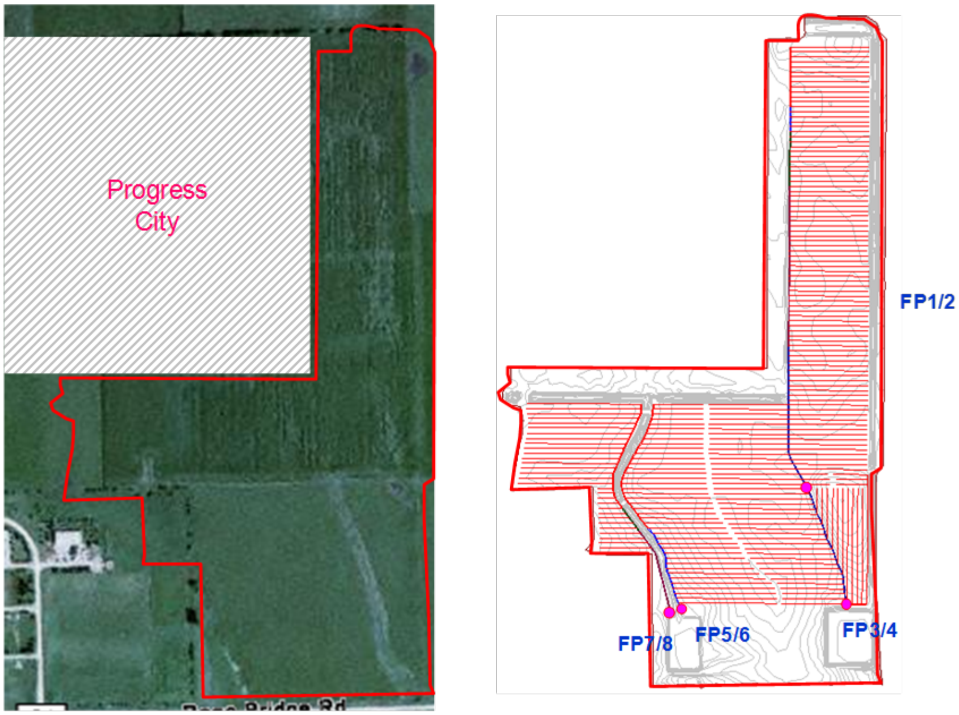

Figure 3. Aerial photo and topographic/tile map of the Decatur bioreactor sites. Decatur West (FP7/8), Decatur East (FP3/4) and Decatur 1 (FP1/2) reactors are shown in right diagram.

2.3. Amenia (Near Monticello)

The Amenia site, near the larger town of Monticello, is located on a private farm operated by an individual cooperator. This bioreactor was installed in the fall of 2007 as a component of a 15-acre (0.06 km^2) managed drainage system. This reactor was filled with paper-grade hardwood chips, rather than chips of mixed wood. A free-flowing drainage system is also located at this site (Figure 4).

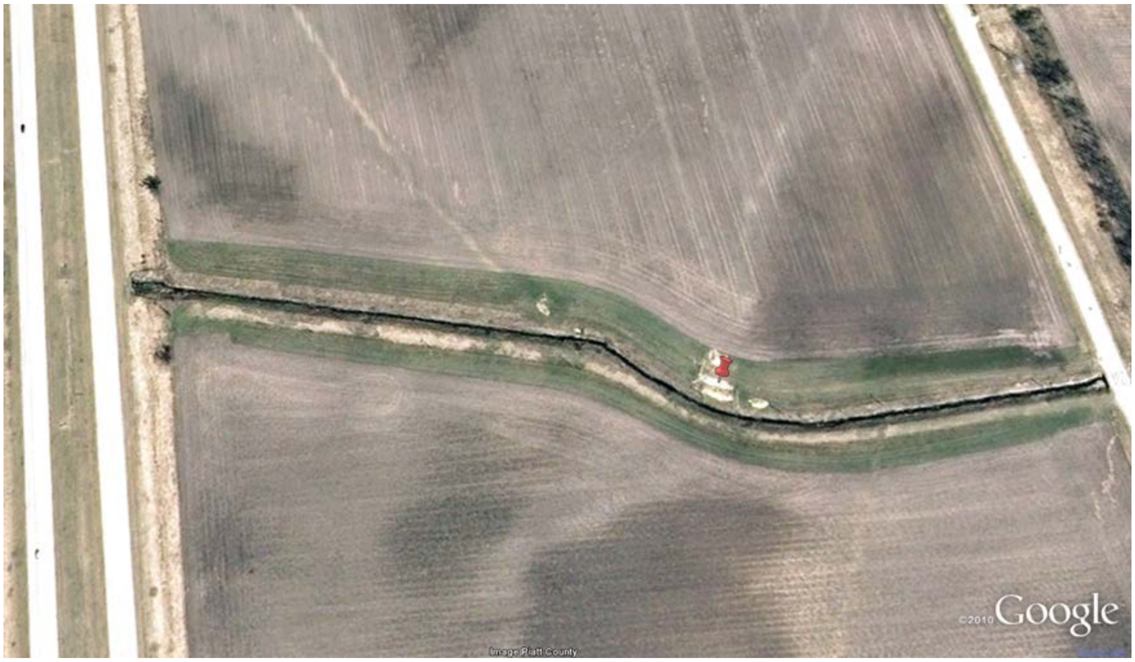

Figure 4. Aerial photo showing location of Amenia bioreactor (red pin). Light colored soil streaks in upper part of photo show tile lines running to ditch (left) and bioreactor (right). The highway along the left edge of the photo is Interstate 74. (Image from Google Maps).

2.4. Deland

The Deland site is located along a drainage ditch that divides a private farm field (Figure 5). Three tile systems have bioreactor/water table management systems and one is a free-draining system. New field tile was installed at the site in 2003. The first bioreactors installed at the site – Deland West and Deland East – are located on opposite sides of the drainage ditch in the southern third of the field. They were installed in fall 2006 and fall 2007, respectively. The third bioreactor – Deland North – drains the northwest corner of the field and was installed in 2009 (Table 1). All three bioreactors are located at the edge of the fields, close to a deep ditch with steep banks. The Deland East reactor outlet was submerged in the stream and water was frequently backed up in the reactor. An extensive study of the nitrogen budgets of the Deland East and West reactors was published recently (Woli et al., 2010).

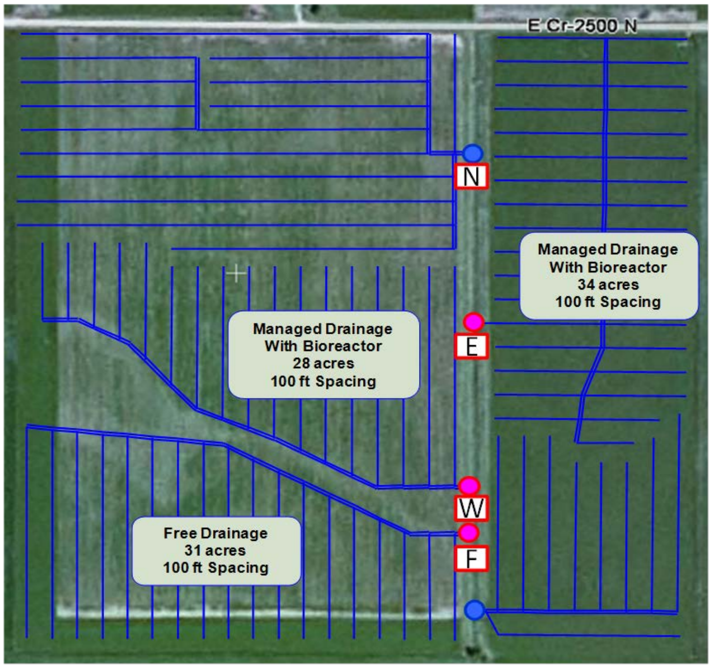
E Cr-2500 N
N
Managed Drainage
With Bioreactor
34 acres
100 ft Spacing
Managed Drainage
With Bioreactor
28 acres
100 ft Spacing
E
W
Free Drainage
31 acres
100 ft Spacing
F

Figure 5. Diagram of bioreactor locations and tile map for Deland site. North (N), East (E), west (W), and free-draining tile (F).

2.5. Other Bioreactors

Two additional bioreactors, located in Bloomington and in Mt. Zion (just south of Decatur), were sampled once in a 2010 survey (Figure 1). Limited information on their characteristics is included in Table 1.

2.6. Hydrology

Many of the reactors have pressure transducers connected to data loggers located at the control structures in order to record the depth of water behind the stop blocks in the reactor. From this depth, the rate of water flow into the bioreactors can be calculated using the rating equation for a V-notch weir (Cooke, 2009). These data were not continuous at all sites, however, so a regional-scale, area-normalized measure of flow was derived from USGS monitoring data. These daily values, referred to herein as “regional runoff,” are used as a qualitative indicator for the timing of major storm events and seasonal changes in flow experienced by the bioreactors. Bioreactor flow derived from highly localized precipitation could not be detected.

Streamflow in central Illinois is comprised of the sum of discharges from tiles and groundwater, with direct runoff occurring only occasionally. Thus, when small streams in the area show surges in flow, it can be reliably determined that tiles are discharging. The USGS gages on medium-sized streams nearest to the study sites are located on the Lake Fork near Cornland (5579500) and on the West Okaw near Lovington (05591700) (Figure 1). After normalizing observed daily flows at these two gages by their respective watershed areas, we computed the geometric means of the flows for each day during the study period (Figure 6A). As used here, the term “runoff” does not signify surface flowpaths, but rather area-normalization (USGS, 2015).

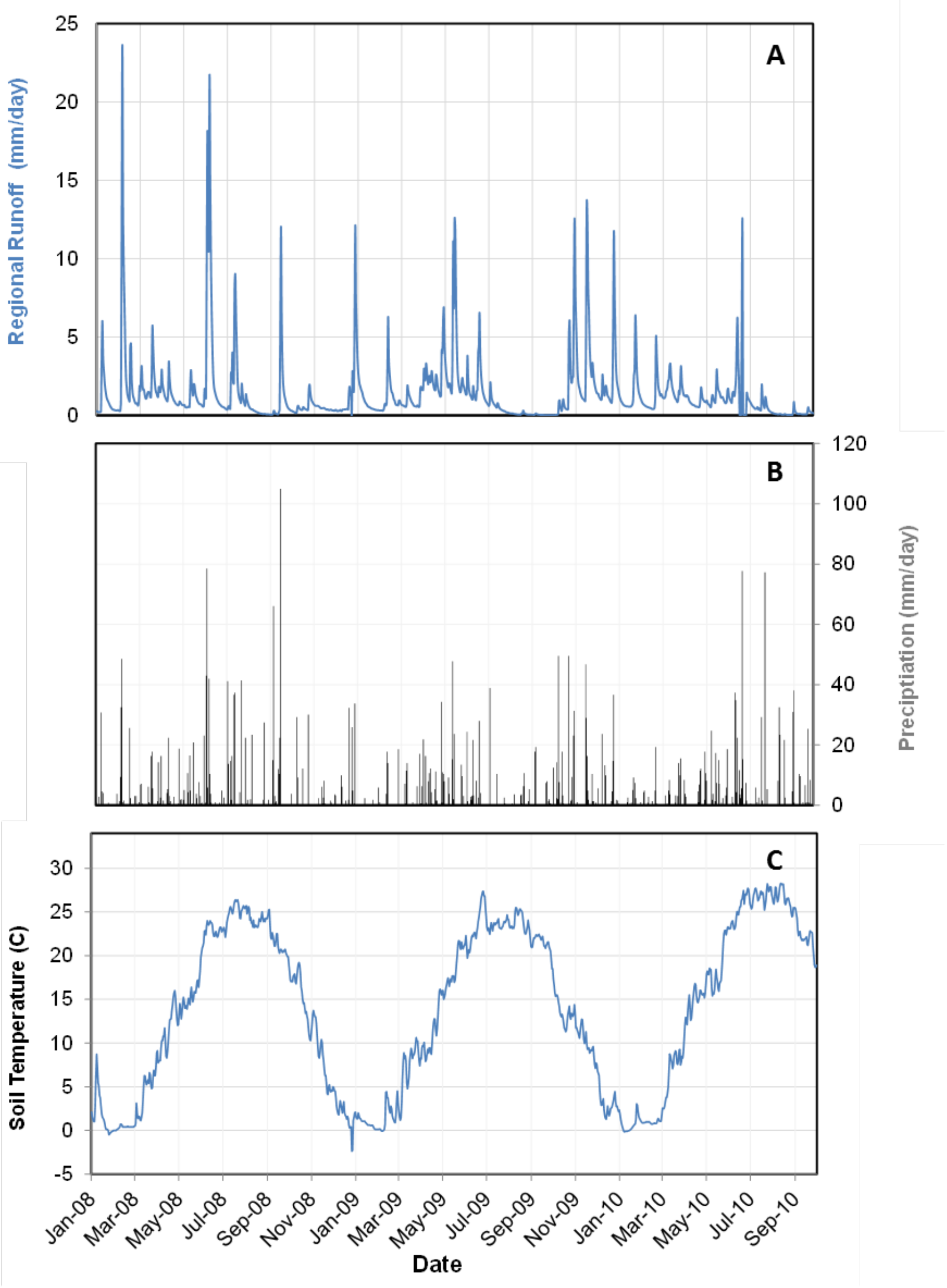

Figure 6. Physical environmental variables. A) Mean regional runoff (geometric mean of area-normalized flow at USGS gages 5579500 and 05591700). B) Daily precipitation at Decatur airport (NCDC). C) Daily average soil temperature at 8” depth at Champaign airport (WARM).

3. Methodology

3.1. Water Sampling

Water samples were collected from each bioreactor's inlet and outlet within a span of 5-10 minutes. Whenever possible, the bioreactor outlets were sampled by filling a bottle with water flowing out of the outlet pipes into the nearest drainage ditch. This was usually possible for Decatur East, Decatur West, and Amenia bioreactors, but not the reactors at Deland, Mt. Zion, or Bloomington. For the latter bioreactors, outlets were sampled by immersing the 500-mL bottles in the control structure wells. The inlets of all bioreactors were sampled in the same manner. The sample bottles were fastened to the end of an aluminum pole so that they could be submerged and filled at the bottom of the control structure.

Sample bottles consisted of new 500-mL polyethylene terephthalate (PETE) drinking water bottles that had been sealed inside double Ziploc® bags in the clean lab. Bottles were handled by field workers wearing clean nitrile gloves. Care was taken to avoid contamination at all stages of handling. However, at the time of this study we did not have a method for rigorously preventing dust from the control structures from entering the bottles. Filled bottles were re-sealed in the Ziploc® bags and transported back to the lab in a cooler, generally within 2-4 hours of collection. On the same day, samples were either prepared for analysis or frozen and stored at -20°C until the next step in processing was performed at a later date.

3.2. Sample Preparation

All water samples were prepared for analysis by vacuum-filtration through quartz fiber filters (QFF) that had been baked at 400°C to reduce the background Hg level. To permit minimal contact with container surfaces during filtration, a vacuum desiccator modified to hold a Savillex Teflon filter holder (Lewis and Brigham, 2004) was employed, which allowed filtrate to directly flow into the bottle used for sample storage. In all cases, these were acid-cleaned or 400°C-baked borosilicate glass bottles (I-Chem). Filtered samples were acidified with 0.4% v/v HCl (Fisher Trace Metal grade) and stored in a refrigerator.

To prepare samples for analysis, 20- or 40-mL aliquots of filtered/acidified samples were transferred to clean 60-mL I-Chem vials and weighed. Enough 1 M thiourea (TU) stock was added to each sample to reach 40 mM. After allowing overnight reaction, solid phase extraction (SPE) was performed with a thiol-functionalized, polydivinylbenzene resin to concentrate MMHg from the sample matrix (Vermillion and Hudson, 2007). The potency of TU-catalyzed SPE derives from its two-step exchange of MMHg from matrix ligands to those on a resin (H-SR). In the first step, the preserved sample was buffered to pH 3.5 and the dissolved organic matter (DOM)-bound MMHg was allowed to react with added thiourea (Reaction 4). In the second step, TU-bound MMHg exchanged to resin-bound sulfhydryls as the sample was pumped through the resin (Reaction 5).

$$MMHg - S - DOM + TU + H^{+} \longrightarrow MMHg - TU + H - S - DOM \tag{4}$$

$$MMHgTU + H - S - \mathrm{Resin} \longrightarrow H^{+} + TU + MMHg - S - \mathrm{Resin} \tag{5}$$

This step also separated the Hg from matrix components, namely dissolved organic matter that could potentially interfere with analysis. Once trapped on the resin, the Hg species from the original sample (Figure 7) could be eluted into just 4 mL of acidic thiourea solution (see mobile phase in Section 3.3) and frozen until analysis by HgTU/IC-CVAFS.

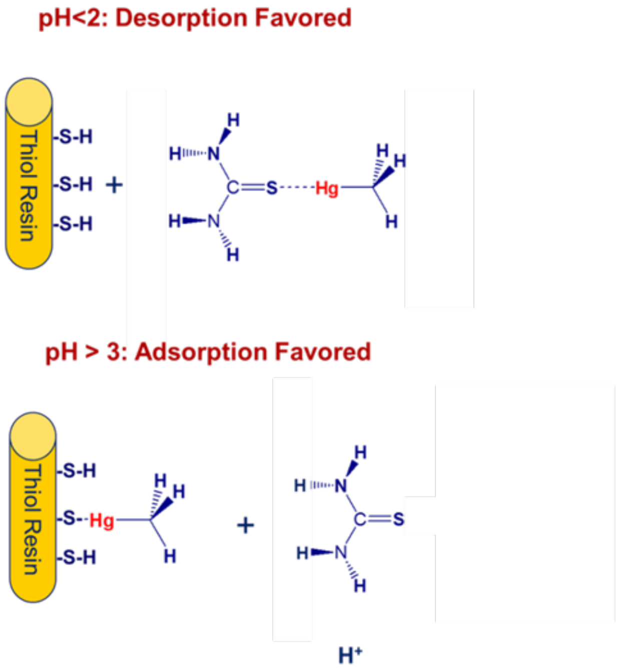
pH<2: Desorption Favored
Thiol Resin
-S-H
-S-H
-S-H
+
H
H
N
C
S
Hg
H
H
N
H
H
H
pH > 3: Adsorption Favored
Thiol Resin
-S-H
-S-Hg
-S-H
H
H
H
+
H
H
N
C
S
H
N
H
H+

Figure 7. Principal reactions of the pH-modulated thiourea-thiol switch. This reaction is used here to pre-concentrate MMHg from natural water samples and concentrate Hg species for injection into the analytical system mobile phase. At pH 3-4, the equilibrium favors the adsorption of MMHg onto thiol resins (or complexation by DOM-associated thiols in natural waters). At low pH, thiols are protonated while TU is not, causing the equilibrium to favor the binding of MMHg by TU in solution. Based on Shade and Hudson (2005).

3.3. Hg Speciation Analysis by HgTU/IC-CVAFS

Mercury speciation analysis was performed using the aqueous-phase ion chromatography method that has been under development in our lab over the past decade (Figure 8). The speciation system separates thiourea complexes of $MMHg^+$ and Hg^{2+} by virtue of their different ionic charges. In the mobile phase, the complexes retain the charge of the metal ions because thiourea (TU) is a strong electroneutral ligand (Shade and Hudson, 2005).

To quantitate Hg species using HgTU/IC-CVAFS, the pH of each prepared sample was adjusted to about 4. Next, the buffered sample was pumped through a small column containing thiol resin (TT in Figure 8) in order to concentrate the Hg species for injection. The trap consisted of a small column packed with a custom-synthesized thiol-functionalized resin (mercaptopropyl-functionalized poly-divinylbenzene). Finally, trapped Hg^{2+} and CH_3Hg^+ ions are eluted from the column into the analytical system by the acidic thiourea mobile phase.

Briefly, the system comprises three main parts: (1) an HPLC system for separation of charged $Hg(TU)_x^{2+}$ and CH_3HgTU^+ complexes across an ion chromatography column, (2) a flow-injection (FI) system for on-line Hg^0 cold vapor generation by UV post-column oxidation of MMHg and thiourea followed by reduction of Hg^{II} to Hg^0 and transfer to the argon carrier gas, and (3) a gas-phase detection system (CVAFS). The components and capabilities of the analytical system are described in detail in Shade and Hudson (2005).

All monitoring data reported herein were obtained using the HgTU/IC-CVAFS system operated using 1 M HCl in the mobile phase. Note that the system was operated very reliably until spring 2009 by Brian Vermillion (see acknowledgments section). From that time until the summer of 2010, data could not be generated reliably. This lack of data generation was due to problems with the post-column chemistry used to generate volatile Hg^0 from the separated Hg species (see results in Section 5). In 2010 and 2011, we were again able to generate usable data, although our detection limit was not as low as in 2009. In 2012, the system was revised and updated, as described in Section 3.4.

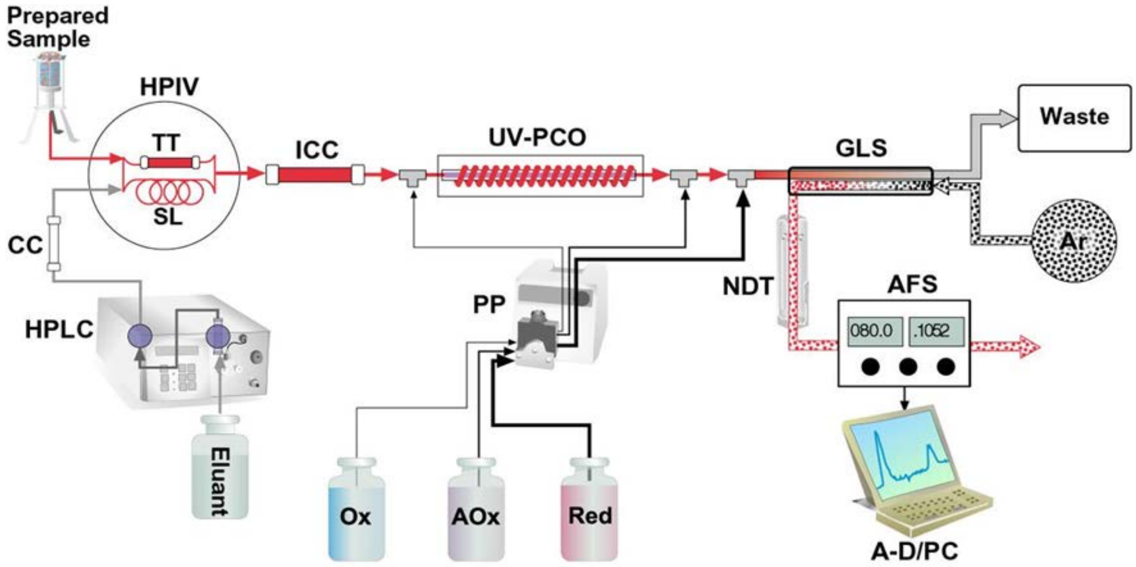

Figure 8. Schematic of the original Hg-thiourea complex ion chromatography system. The MMHg in prepared samples is concentrated onto the online thiol trap (TT) and eluted into the mobile phase at the high-pressure injection valve. The Hg species are separated on the ion chromatography column (ICC) and subjected to post-column oxidation and reduction before Hg^0 transfer to the gas phase in the gas-liquid separator (GLS). Reagents include: Eluant, an aqueous solution of HCl (1 M), acetic acid (1.75 M), and thiourea (0.15 M); Oxidant (Ox), H_2O_2; Antioxidant (AOx), sodium ascorbate; and reductant (Red), alkaline $SnCl_2$. Based on Shade and Hudson (2005).

3.4. Hg Speciation Analysis by HgTU/IC-ICPMS

In the late stages of this work, additional improvements to the analytical system were employed in order to examine the results obtained using CV-AFS. The post-column chemistry of this latest system was highly modified from the original (Figure 9). An Agilent 7500S ICP-MS was used as the Hg detector, giving us the capability to conduct isotope dilution studies and methylation/demethylation rate assays using tracers enriched in stable Hg isotopes.

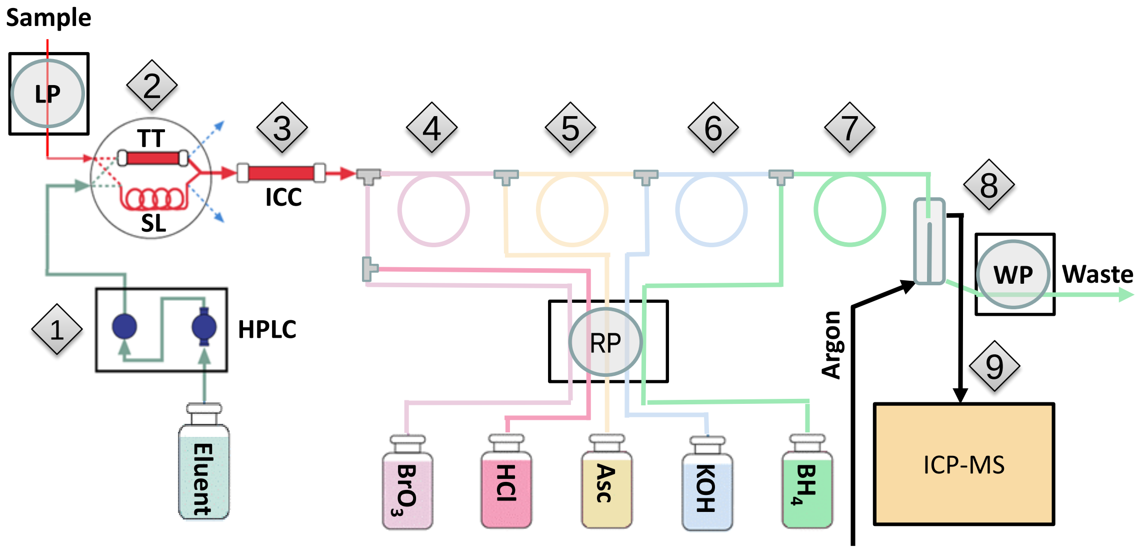

Figure 9. Schematic of the revised HgTU-IC system. (1) HPLC pump (2) sample injection valve, (3) IC column, (4) oxidation loop, (5) antioxidant loop, (6) acid neutralization loop, (7) reduction loop, (8) gas-liquid separator (GLS), and (9) Hg detector. Peristaltic pumps are labeled as follows: LP is for loading samples; RP for reagents; and WP for draining waste from GLS. The custom high pressure thiol resin preconcentrator is labeled TT. The sample injection loop is labeled SL. The first step post-column in the online reaction system is oxidation, using $KBrO_3$ at 40°C (BrO_3), where TU is oxidized and MMHg is converted to Hg^{II}. Next, the oxidation is quenched with sodium ascorbate and hydrophobic oxidation by-products are kept in solution with Triton X (Asc). Following the antioxidant injection, the pH of the sample stream is raised by introducing a base (KOH) and the heat released is absorbed in an ice bath. The final reaction step is the reduction of Hg^{II} to Hg^0 by alkaline borohydride (BH_4). The sample stream then passes through a gas/liquid separator where the Hg^0 is stripped into an argon stream and carried to the detector. Based on Olsen (2014).

While the system was still able to analyze both MMHg and Hg^{II} in aqueous samples, a significant modification had to be made to the original method in that these distinct forms of Hg were analyzed separately with different mobile phase compositions. The main difference was a much lower acid content – 0.1 M HCl – was used in the mobile phase when analyzing MMHg, while for Hg^{II}, the original 1.0 M HCl was used.

Typical chromatograms record ion counts for the five main Hg isotopes (198-202). Areas of appropriate peaks (MMHg) were integrated using HP Chromstation Chromatographic Data Analysis Software after Savitzky-Golay smoothing. For samples where isotopic tracers were not added, the total ion count (sum of all isotopes) was integrated. For samples containing tracers, peaks corresponding to isotopes 198-202 were integrated and isotope pattern deconvolution was employed to determine the contributions of each tracer in the sample (Rodríguez-González et al., 2005). Hintelmann and Ogrinc (2003) used a similar approach to speciated isotope dilution, differing only in the mathematics of solving the system of linear equations.

3.5. Analysis of Ancillary Chemical Parameters

Dissolved organic carbon (DOC) concentrations were analyzed by measuring total organic carbon in QFF-filtered water samples. Data reported here were obtained either from the Illinois Sustainable Technology Center (ISTC) or the Natural Resources and Environmental Studies (NRES) biogeochemistry lab (M. David; see acknowledgments section).

Anion concentrations (nitrate and sulfate) were measured using ion chromatography at either ISTC or the NRES biogeochemistry lab. Some of the nitrate data reported in Section 5.1 below were measured using flow injection with chromium reduction in the Department of Agricultural and Biological Engineering's wet lab.

4. Measuring Dissolved MMHg by Hg-Thiourea Ion Complex Chromatography

The results of this study are linked with the historical development and evolution of the novel method for Hg speciation analysis used herein: Hg-thiourea complex ion chromatography (HgTUIC). This analytical system very effectively discriminates between MMHg and Hg^{II} in aqueous sample preparations on the basis of the ionic charges of H_3CHg^+ and Hg^{2+}, and its ability to accurately measure MMHg has been demonstrated using certified reference materials (CRM). The first published result for a CRM was for sediment prepared using a slightly modified conventional method (Shade and Hudson, 2005). Later, analysis of a biological tissue prepared using a novel digestion method was shown to yield the same MMHg results as the conventional method (Shade, 2008). In this work, MMHg was measured in water extracted from the sample matrix using the novel solid-phase extraction procedure, described in Section 3. Although there is no CRM for MMHg in water, it was shown that this novel sample preparation procedure effectively extracted MMHg from natural water samples – even those containing very high levels of DOM – and did not create additional MMHg when Hg^{II} was added to the sample prior to analysis. Thus, by the time this study began in 2008, there was ample evidence that the new method met the same standards as the conventional method for effective recovery and lack of Hg^{II} methylation during analysis (Vermillion and Hudson, 2007; Horvat et al., 1993; USEPA, 2001).

The next step in validation of the method was to compare results with those of the conventional method when analyzing the same samples. Initial comparisons with a well-established lab showed that HgTUIC results were highly correlated with those of the standard method (Figure 10A) and effectively reproduced the well-known increase in MMHg in anaerobic compartments of aquatic ecosystems: the hypolimnion of a lake, in this case (Figure 10B).

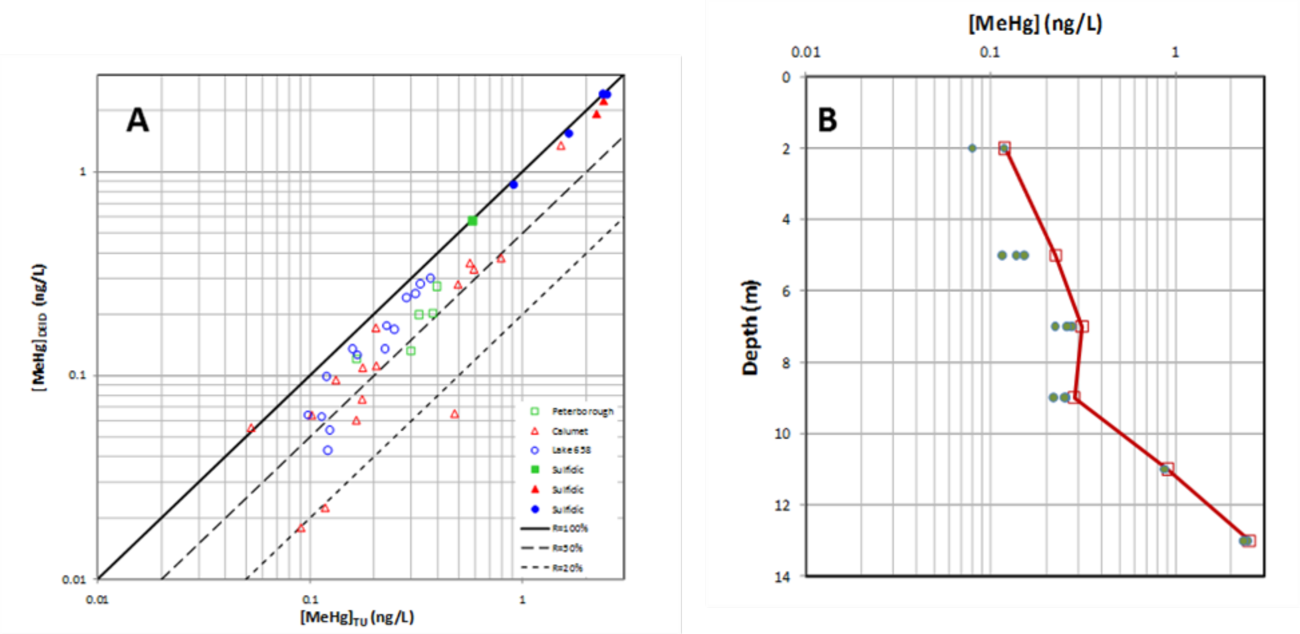

Figure 10. Comparison of MMHg analyzed by conventional and HgTU/IC methods. Samples obtained in 2006-2007; analyses performed in 2007-2008. Distillation/ethylation with isotope dilution ICP-MS ([MeHg]DEID) and HgTU/IC-CVAFS ([MeHg]TU this lab). (A) Survey of sites from NW Indiana, Ontario and the Experimental Lakes Area (ELA). (B) Profile of west basin of Lake 658 (ELA) obtained under stratified conditions in September 2008. [MeHg]DEID (red squares) and [“MeHg”]TU (green dots). Unpublished data from H. Hintelmann lab (Trent University) and B. Vermillion (this lab).

At low MMHg levels, a positive bias that averaged 50-100% of the conventionally-determined MMHg was observed, but there was no bias at high MMHg. Further very detailed examination of the causes of the difference led to a hypothesis that the conventional method was under-reporting MMHg due to incomplete distillation of MMHg strongly bound to dissolved organic matter (data not shown).

In March 2009, a problem with the post-column wet chemistry of the speciation system developed and prevented a means of resolving the cause of the inter-method differences in a timely manner. After this date, it was found that the system could no longer operate without very small particles of $Sn(OH)_2$(s) forming in the final Hg^{II} reduction step (Figure 8). These precipitates caused random spikes in the chromatogram that precluded the accurate quantitation of small amounts of MMHg in water. Apparently, conditions in the system had been just barely below the point where these particles formed, and some change in reagent quality or other variable pushed it above a threshold. Although minor revisions of the system’s wet chemistry made it possible to generate some results by fall 2010, the system was not as reliable or accurate as it had been before 2009.

In 2012, a new grant enabled the purchase of an ICP-MS (Agilent 7500S) and a thorough redesign of the wet chemistry of the system. This work culminated in the M.S. thesis of T. Olsen (2014). Olsen solved the wet chemistry problems in the system and replaced the AFS detector with the ICP-MS. Note well that cold vapor generation was still used rather than nebulizing liquid samples, as is usually done with ICP-MS. This version of the system is far superior in stability and accuracy, in addition to its capabilities to analyze Hg isotopes.

Returning to samples that exhibited differences between methods, the power of the isotopically-labeled internal standards in ICP-MS detection was employed to investigate the cause of this difference. The introduction of internal standards in natural samples quickly made it clear that the conditions in the study system did not allow for complete separation of MMHg and other unknown Hg species. Upon decreasing the HCl in the mobile phase from 1.0 M to about 0.1 M, it was found that MMHg was cleanly separated from one or more other Hg species (Figure 11). Because these other Hg species were retained less than MMHg on the ion column, they will be referred to as low-charge Hg species, or “LCHg.” Tests to date have shown that these compounds are not Hg^0, monoethylmercury, or any complex of Hg^{II} with thiols or bisulfide (data not shown). Most likely, these are a very stable chelated or nanoparticulate form of Hg^{II}, or possibly chelated MMHg.

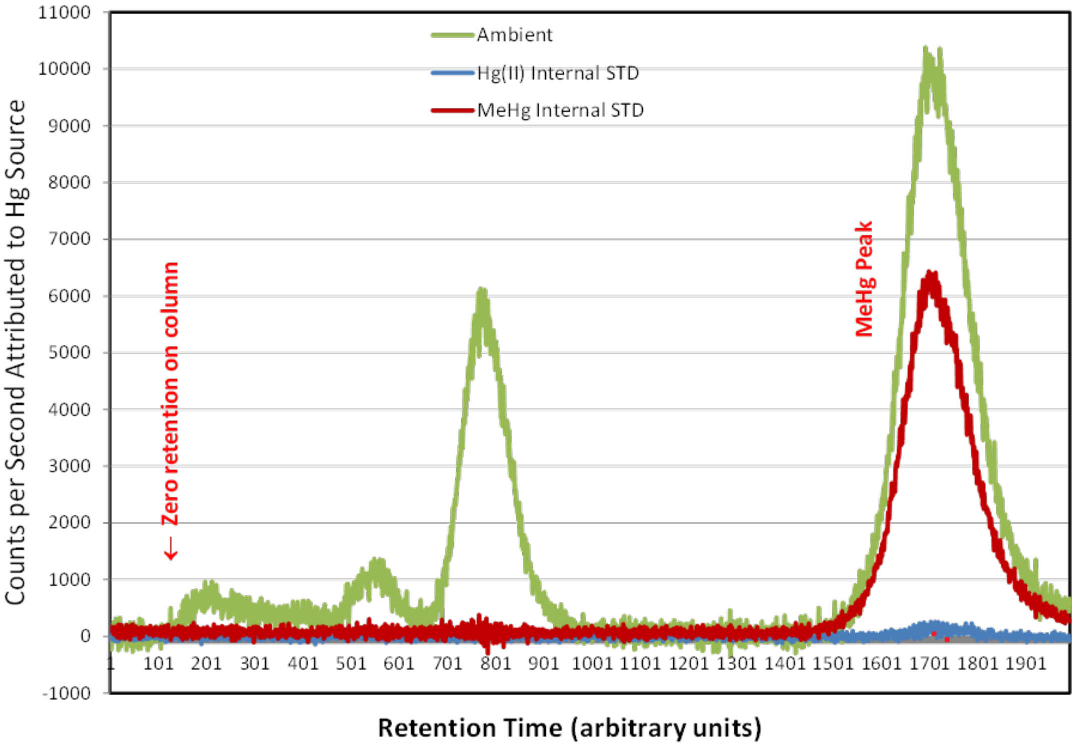

Figure 11. Chromatogram of Hg species in a fresh sample from Decatur West bioreactor outlet. Sample was analyzed using HgTU/IC-ICPMS with 0.1 M HCl in mobile phase. Contributions of Hg from each source – ambient or isotopically-labeled internal standards of MMHg and Hg^{II} – were derived from raw ion counts. The true MMHg peak is located to the right. The peaks to the left are referred to herein as "low charge Hg species", or LCHg, because they are retained less than MMHg on the ion column and hence must have a lower charge. The absence of detectable Hg^{II} internal standard in both peaks demonstrates that the method does not mistake Hg^{II} for either MMHg or LCHg.

Of course, this discovery has important implications for interpreting the results reported in this study. First, it means that the data obtained prior to 2012 using HgTU/IC-CVAFS report a combination of MMHg plus the unknown LCHg species, which is referred to herein as [MMHg+LCHg], rather than MMHg alone. Second, note that since the method completely recovers MMHg from surface water, [MMHg+LCHg] is never (in the absence of analytical error) less than the true MMHg. Third, in some samples, [MMHg+LCHg] was measured in the 0-0.3 ng/L range, but MMHg as measured by distillation/ethylation was undetectable. While the lab performing the conventional analysis did report having some problems near their detection limit at the time the samples were analyzed, the reader should not rule out the possibility that MMHg might not have been present in some cases when LCHg species were present.

Taken alone, the results shown in Section 5 – all obtained using HgTU/IC-CVAFS with 1.0 M HCl in the mobile phase – cannot prove that methylation of Hg occurs within bioreactors.

However, fresh bioreactor outlet samples were subsequently analyzed, which revealed that both MMHg and LCHg were present (Figure 11). In addition, significant levels of MMHg are almost always detected in environmental samples where oxygen becomes depleted enough to permit iron or sulfate reduction to occur, and the results of the present study agree very closely with the standard method in anaerobic water samples (Figure 10) (Hintelmann, 2010). Since the results presented in Section 5 also exhibit the highest values of [MMHg+LCHg] in samples associated with hypo- and anoxic conditions in bioreactors, this study confirms the initial hypothesis that Hg methylation does occur in bioreactors under such conditions, albeit with less quantitative certainty than expected at the outset.

5. Bioreactor Monitoring: Results and Discussion

In all, samples were obtained from nine different bioreactors in the study area (Table 1). The Amenia and Decatur West bioreactors were sampled most frequently during 2008 and 2009. The original plan to sample Deland East at a similar frequency was abandoned after discovering that water in the outlet control structure was affected by backwater from the stream. The other reactors in Decatur and Deland were sampled sporadically during 2008-2009. Finally, a survey of all the reactors at the three main sites plus two additional ones was conducted during the summer of 2010.

Because of the differences in dates and in sites monitored, the results from this study are presented in two parts: (a) intensive monitoring of Decatur West and Amenia reactors for the June 2008-September 2009 period and (b) a survey of reactors, mainly from the summer of 2010.

5.1 Frequently-Sampled Sites

The two frequently-sampled reactors at Decatur West and Amenia exhibited markedly different biogeochemical behaviors, both of which provide an understanding of the conditions under which Hg may become methylated in bioreactors (Table 2). Overall, the Amenia reactor did not efficiently remove nitrate (Figure 12A). There was very little evidence of sulfate reduction (Figure 12B), and Hg methylation was almost always minimal except at the first two sampling times (Figure 12D). On the other hand, the Decatur West reactor had lower nitrate levels in the inlet water and very efficiently denitrified what entered it (Figure 13A). Therefore, it exhibited significant sulfate reduction and Hg methylation (Figures 13B and D, respectively). Tile water pH, a variable known to strongly affect Hg biogeochemistry, was not notably different between the two reactors.

Table 2. Summary of ancillary chemical parameters in the primary reactors during 2008-2010.

Variable	Decatur West	Amenia
Nitrate		
Mean inlet (mg-N/L)	3.0	9.3
Mean depletion (%)	84	16
Sulfate		
Mean inlet sulfate (mg-S/L)	16.2	3.1
Mean depletion (%)	34	0
Dissolved organic carbon		
Mean inlet (mg-C/L)	2.2	2.6
Mean outlet (%)	11	14
pH (June-July 2008 only)		
Inlet	7.2	7.4
Outlet	6.7	6.8

The evidence that Hg methylation occurred within the reactors rather than in the fields was derived from the fact that the inlet samples consistently contained very low to non-detectable levels of MMHg+LCHg at both sites, while high levels were observed in the outlets on multiple dates. Because the inlet [MMHg+LCHg] was always at or near the detection limit of 0.1 ng/L, it was concluded that the drainage water did not contain significant [MMHg+LCHg], a conclusion confirmed by measurements in free-flowing tiles and drainage ditches.

5.1.1. Amenia

On most sample dates, nitrate in the inlet and outlet tracked one another closely at Amenia (Figure 12A) and strong depletion of nitrate was observed on only two dates. On average, the outlet nitrate was depleted by 16% relative to inlet water samples collected on the same date. Sulfate in the outlet was slightly higher than in the inlet on some dates, although 50% depletion was evident on one date in 2010 when nitrate was completely consumed (Figure 12B). Almost no production of MMHg+LCHg was observed after the initial months of the study except on the one aforementioned date in summer of 2010 when nitrate was depleted (Figure 12D).

5.1.2. Decatur West

Extensive denitrification in the Decatur West reactor (Figure 13A) was evident from the high frequency with which nitrate in the outlet water was lower than in the inlet water samples obtained at the same time (one exception is noted on Figure 12). On dates sampled for this study, nitrate in the outlet was depleted by 84% relative to the inlet. Strong denitrification was also apparent in the samples collected and analyzed by Dr. R. Cooke (Department of Agricultural and Biological Engineering, UIUC). In this reactor, sulfate levels in the outlet averaged 70% of the inlet samples collected on the same day, indicating that significant sulfate reduction also frequently occurred (Figure 13B). Finally, elevated MMHg+LCHg concentrations in outlet water were observed during the summer of each year (Figure 13D).

5.1.3. Stagnant Conditions in June/July 2008

Stagnant water can build up in bioreactors if flow slows while the stop blocks in the control structures are kept high or if the reactors contain depressions that retain water in contact with the wood chips. Such conditions developed during the summer of 2008 in the three Decatur and Amenia reactors. Samples obtained on three or four dates in June and July all contained very high DOC (20 to 50 mg-C/L) in the outlets and depleted nitrate concentrations, which indicate exceptionally long contact times with the wood chips under warm conditions (Table 3).

Evidence of Hg methylation was found in all reactors. The inlet contained little or non-detectable [MMHg+LCHg] (<0.1 ng/L) while all reactors had high levels in the outlet (over 1.5 ng/L) on one or more of the sample dates. All three of the Decatur reactors reached values of 6 ng/L or higher. We note that 2.0±0.5 ng/L MMHg is fairly typical of highly anaerobic waters in lakes or wetlands (Figure 10), so it is reasonable to suggest on the basis of these observations that MMHg was in this range in the bioreactors under anaerobic conditions. The very high MMHg values should be regarded as uncertain until a new set of samples collected under similar conditions are analyzed.

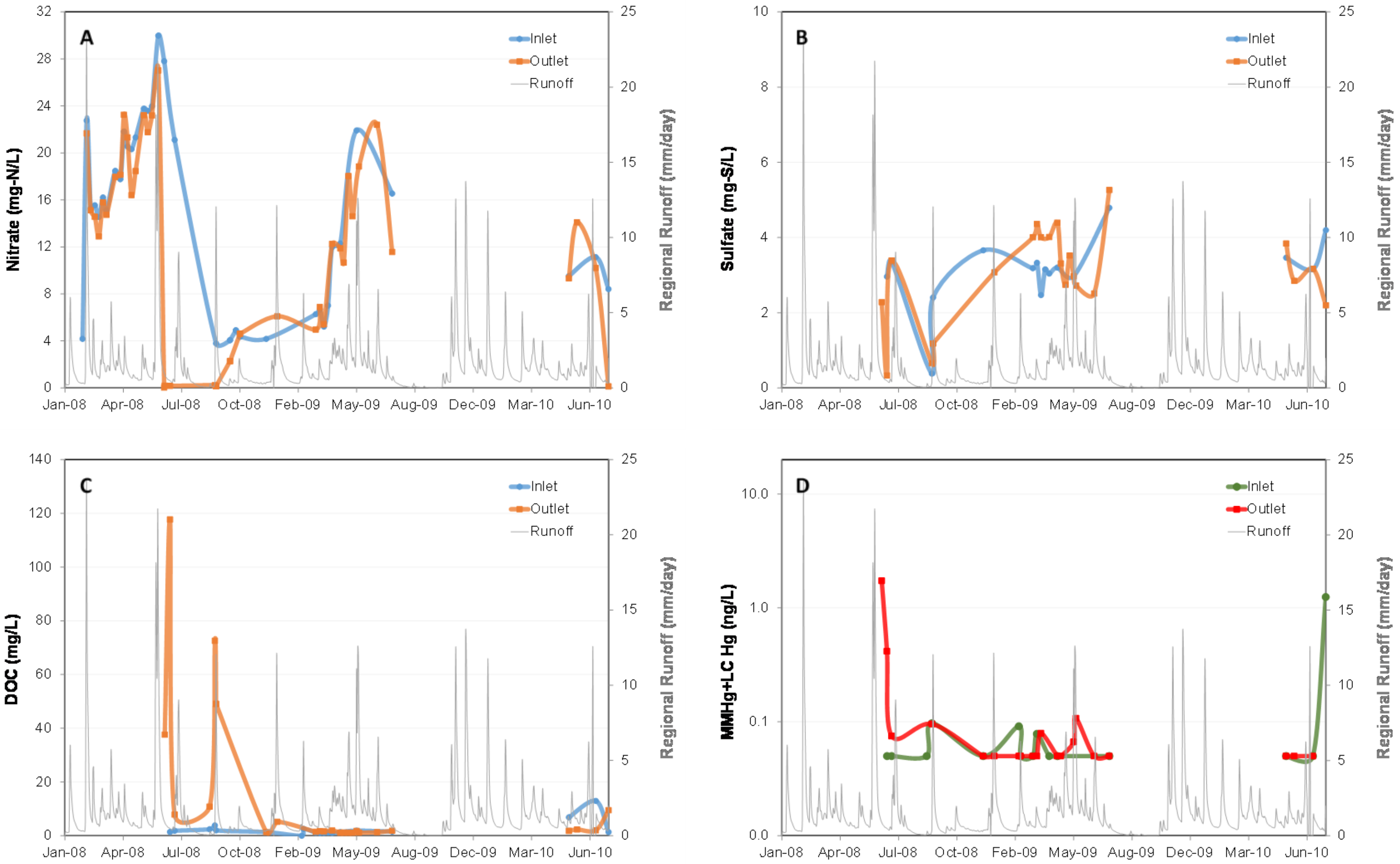

Figure 12. Monitoring data from the Amenia bioreactor. (A) Nitrate, (B) sulfate, (C) dissolved organic carbon, and (D) [MMHg+LCHg]. “Regional runoff” refers to area-normalized, USGS streamflow data (see Figure 6).

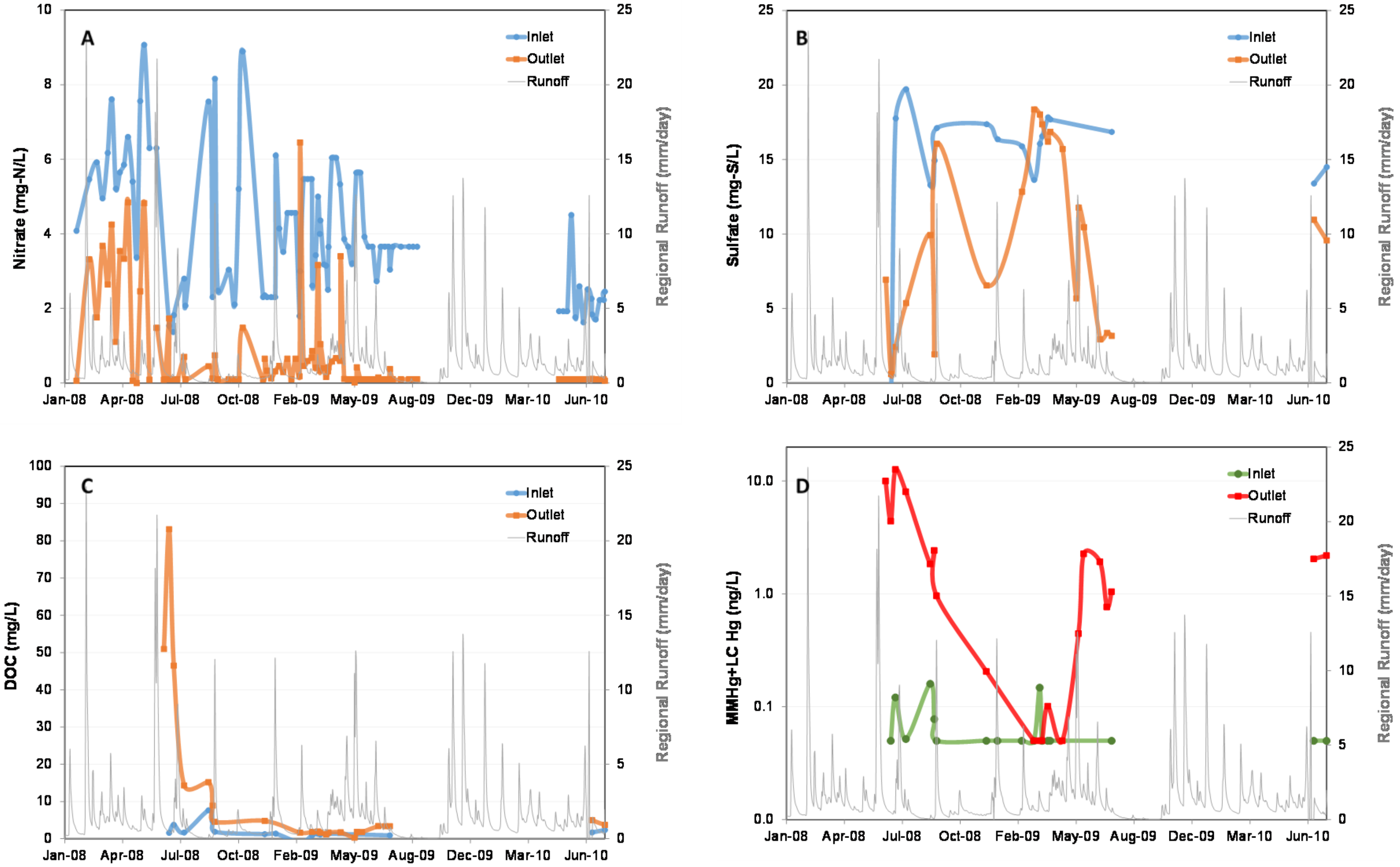

Figure 13. Monitoring data from the Decatur West bioreactor. (A) Nitrate, (B) sulfate, (C) dissolved organic carbon, and (D) [MMHg+LCHg]. "Regional runoff" refers to area-normalized, USGS streamflow data (see Figure 6).

Table 3. Buildup of [MMHg+LCHg] under stagnant water conditions.

	[MMHg+LCHg] (ng-Hg/L)			[DOC] (mg-C/L)
	Average	Max	Min	Average
Decatur 1				
In	0.06	0.09	0.05	1.56
Out	9.34	11.66	6.34	24.90
Decatur East				
In	0.09	0.16	0.05	2.48
Out	4.21	5.97	2.34	19.36
Decatur West				
In	0.07	0.12	0.05	2.32
Out	8.78	12.66	4.42	48.71
Amenia				
In	0.05	0.05	0.05	1.61
Out	0.74	1.73	0.07	54.39

The exact reasons that all of the bioreactors had stagnant water at the start of the project are not known. However, contemporaneous field observations indicate that no flow was observed at Decatur West during June and July 2008. In addition, the recorded water depths in the control structure during this period increased in a way that is consistent with water slowly building up within the bioreactor rather than rising and falling with stormflow from the tile. This behavior is more consistent with the practice of water table management rather than the typical operation of bioreactors.

Due in large part to these observations, a third generation reactor design (Figure 2B) that lacks a depression in the bottom of the reactor where stagnant water can accumulate was used in the construction of all subsequent bioreactors by Dr. Cooke.

5.1.4. September 2008

The first two weeks of September 2008 were exceptional hydrologically speaking, as the study area experienced heavy rainfall. During the week of September 2-9, about 100 mm of precipitation fell and then the remnants of Hurricane Ike swept through the study area on September 14, 2008, depositing an additional 100-120 mm of precipitation in Decatur and Champaign in a few hours. Hours after the storm passed, samples were collected from several reactors in an attempt to observe the effects of the storm. The Decatur West site was completely flooded on that day and the high water level in the ditch at Amenia caused water to back up in the reactor. Some reactors were also sampled before and after the storm on September 5 and 16.

Consistent with the 0.2-0.7 mg-S/L levels of sulfate in wet deposition from that week (reported by NADP), levels of sulfate in the reactor inlet water dropped to the ~2 mg-S/L range, compared to the typical 5-15 mg-S/L in the reactor inlet water (Figures 12B and 13B). Concentrations of Hg also declined to 0.2-2 ng/L the week before the storm and then further to 0.1-1.0 ng/L on the 16th (Figures 12D and 13D).

5.1.5. Seasonality of MMHg Production

In nearly every aquatic ecosystem, the production of MMHg exhibits marked seasonality, with higher rates under warm conditions (Hintelmann, 2010). The data from Decatur West and Amenia confirm this trend in the bioreactors. In all study years, [MMHg+LCHg] was highest in the summer months, but in a manner that was closely tied to temperature and nitrate depletion. During winter and spring high flow conditions, very little [MMHg+LCHg] was produced (Figures 12D and 13D). This result is consistent with the combined effects of cool temperatures (Figure 6B) on denitrification (Cooke, 2009) and the low residence times of water in the reactor under high flow conditions, which mainly occur between late fall and the end of spring.

5.2. Survey of All Bioreactors

From the study of seasonality described in Section 5.1.5, it is clear that detecting methylation in bioreactors depends on sampling at a time when the bioreactors have depleted nitrate. Because this may not occur at the same time in all reactors, the highest [MMHg+LCHg] observations should be compared across all sites during each summer (Figure 14A, Table 4). When doing this comparison with all sites, the maximum [MMHg+LCHg] values appear to be constrained by bioreactor drainage density in a fashion similar to that of the average nitrate removal efficacy curve for bioreactors in the area (Figure 14B). The removal efficacy is the mean annual reduction in nitrate load exported by a bioreactor. Note that essentially no methylation occurred in reactors whose mean nitrate load reduction was 30%. This observation is consistent with the necessity for nitrate to be removed at least in microsites within the bioreactors before sulfate reduction and Hg methylation can occur. High [MMHg+LCHg] values in excess of 1 ng/L all occurred in bioreactors with mean nitrate removals of over 50%. These results suggest that the same design parameter – drainage density – that is used in sizing reactors in the central Illinois region can also be used to guide estimates of Hg methylation rates, at least for reactors similar to these.

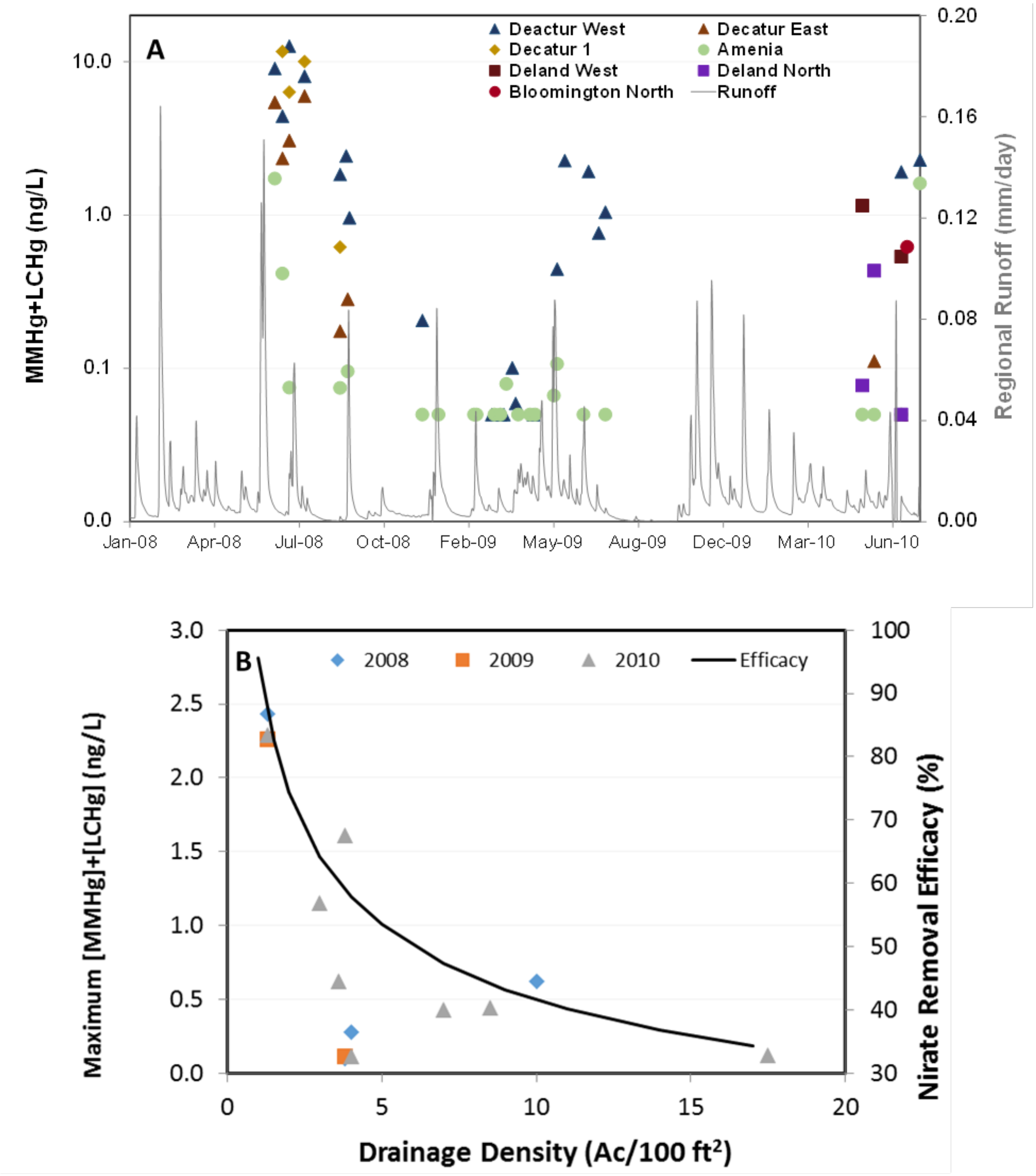

Figure 14. Effect of drainage density on MMHg production. (A) All observations of [MMHg+LCHg] from the bioreactors. (B) Control of [MMHg+LCHg] production by drainage density of bioreactor (area of field drained per 100 ft^2 of bioreactor area). Curve for nitrate removal efficacy was developed by Cooke (2009).

Table 4. Maximum annual [MMHg+LCHg] in bioreactors sampled in this study.*

Site	Drainage Density (Ac/100 ft^2)	Maximum [MMHg+LCHg] (ng/L)		
		2008	2009	2010
Decatur West	1.3	2.43	2.26	2.29
Decatur East	4.0	0.28		0.11
Decatur 1	10.0	0.62		
Amenia	3.8	0.1	0.11	1.61
Deland East	8.5			0.44
Deland West	3.0			1.15
Deland North	7.0			0.43
Mt. Zion	17.5			0.12
Bloomington North	3.6			0.62

* Data from June-July 2008 were excluded because there was stagnant water in the Decatur reactors at the time and the samples were therefore not representative of conditions in normally operated bioreactors.

6. Conclusions

In no season was MMHg ever detected in inlet samples at concentrations at much above the detection limit of ~0.1 ng/L, but levels of MMHg+LCHg over 2 ng/L were observed in the outlets during warm periods when nitrate had become depleted within the bioreactor. Such results are consistent with measured MMHg concentrations in highly anaerobic environments and with nitrate inhibition of iron and sulfate reduction. It is worth noting that sulfate depletion was observed in most samples with elevated [MMHg+LCHg].

The maximum [MMHg+LCHg] observed in any given bioreactor followed an inverse function of the bioreactor loading density, i.e., the ratio of the area drained to the area of the bioreactor pit. The function has a form similar to that observed for bioreactor denitrification efficacy and suggests that optimal bioreactor designs that permit substantial denitrification while minimizing Hg methylation are feasible. Note that the areal loading density parameter was used within a region where all fields received similar average rainfall depths and where the bioreactors were typically operated within a small range of water depths. Thus, this design parameter is roughly proportional to the ratio of the volume of water entering each reactor to the volume of woodchips in contact with the water in the bioreactors.

Finally, extremely high MMHg+LCHg levels were observed when stagnant water conditions occurred within the bioreactors. Thus, it is recommended that bioreactors not be built with bottom depresssional areas where stagnant water can reside, in order to avoid developing anoxic conditions where methylation can occur. For the same reasons, bioreactors should not be used simultaneously with controlled drainage (water table management) if restricting the drainage results in keeping the bioreactors flooded for long periods of time. The first of these recommendations has already been incorporated into the Natural Resources Conservation Service’s Denitrifying Bioreactor Code (USDA, 2015) on the basis of pre-publication access to these results.

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